FH AACHEN
UNIVERSITY OF APPLIED SCIENCES

EBS 2023

4th European Biosensor Symposium 2023

Aachen, Germany

August 27 – 30, 2023

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Welcome to EBS 2023 in Aachen

Dear colleagues, dear friends,

We would like to warmly welcome you to University of Applied Sciences here in the city of Aachen for the 4th European BioSensor Symposium – 4th EBS !!!

After the years of the pandemic, we are happy that we can finally meet again in person, which gives a new boost for scientific exchange that is important for all of us and especially for Ph.D. students and junior scientists.

The imperial city of Aachen, charmingly located on the border of Germany, Belgium and the Netherlands, is a popular tourist destination, partly because of its convenient geographical location but also thanks to its long history, artistic treasures and elegant stores. However, the waters have most affected the city's privileged location; the Volcanic Eifel heats the water, which springs from the ground at up to 74 °C in many places. This makes the Aachen thermal springs some of the warmest in Central Europe, and they have been used by both the Celts and Romans. Eventually, these healing springs prompted Emperor Charlemagne to build his palace in Aachen, making it the center of the great Frankish Empire.

The city of Aachen has developed into a technology hub, hosting one of the leading universities of technology in Germany with the RWTH Aachen (Rheinisch-Westfälische Technische Hochschule), the academic hospital Klinikum Aachen, one of the largest medical faculties in Europe, as well as the FH Aachen (Aachen University of Applied Sciences), which is one of the strongest universities of applied sciences in Germany in terms of technology-oriented research.

The European BioSensor Symposium (EBS) originally emerges from the German BioSensor Symposium (DBS), first held in Munich 1999 and subsequently at nine different locations in Germany, which are well known hot spots for biosensor research and development. EBS is a bi-annual conference organized first in Potsdam, Germany, in 2017, and after that in Florence, Italy in 2019. The 3rd EBS in 2021 took place for the first time online due to the corona pandemic and was organized by TH Wildau, Germany. The aim of EBS is to stimulate and enhance exchange among researchers working in Europe in the field of biosensors.

In 2023, the city of Aachen and FH Aachen will be venue and host of the 4th EBS. 189 contributions with 3 plenary speakers, 14 keynote lectures, 54 oral talks and 117 posters, partly with short poster presentation, will provide an exhaustive view of the research activity in the following scientific topics:

- Technologies for innovative formats such as implantable, non-invasive, single-use biosensors, and paper-based diagnostics;
- bioengineered and biomimetic recognition elements (MIPs, aptamers, receptors, modified enzymes with increasing catalytic activity, sensor-actuator molecules etc.);
- nanotechnology, surface engineering and bioelectronics (single-molecules and molecular sensors, DNA-Origami, nanoparticles and nanostructures enhancing biosensor performance etc.);
- cell-based biosensors (e.g., pharma screening, diagnostics, cell toxicity, REACH), single cell-based diagnostics (patch-clamp and electrodes, sensors for regenerative therapies);
- advances in applications (clinics and „traditional“ areas such as environment and food safety, infection diagnostics including alternative strategies, cultural heritage);

- microfluidics and actuators for biosensing and integration;
- theory, modelling and software development (e.g., multivalence in bio-sensing).

The European BioSensor Symposium should bring together young and experienced scientists to discuss new insights in all aspects of biosensor research and related areas, to push forward cutting-edge ideas and interesting approaches, and to stimulate the scientific exchange between all groups and countries. Young researchers at the beginning of their career are invited to present their most recent results, while seniors give introductions on special advanced aspects of biosensors, technology and upcoming trends. An industry exhibition with selected companies rounds off the comprehensive picture of the state of biosensor research, development and applications in Europe.

We would like to thank the Local Organizing Committee for their countless hours of work and incredibly strong dedication (you did a fantastic job!), the Scientific Board for their helpful suggestions and evaluation of submitted contributions, and the sponsors and exhibitors, who provided their support to EBS. Each conference is only as good as their scientific contributions: a great “hug” to all plenary / keynote speakers sharing their experience with us, and also thanks to all scientists offering their oral- and poster presentations. A special thanks goes to FH Aachen for hosting this event!

Prof. Dr. Michael J. Schöning
Symposium Chair, FH Aachen

Prof. Dr. Patrick Wagner
Symposium Co-Chair, KU Leuven

On behalf of the organizing committee

Dear participants of the 4th European Biosensor Symposium,

Welcome at FH Aachen, welcome at EBS!

With more than 15,000 students, almost 2,000 graduates per year, 10 faculties and 12 in-house institutes, Aachen University of Applied Sciences (FH Aachen) is one of the largest and most important universities of applied sciences in Germany. Around 240 professors and about 900 employees work here in teaching, research and administration.

FH Aachen aims to be one of the strongest research universities of applied sciences in Germany, too. Its competencies lie primarily in the future fields of energy, mobility and life sciences, as well as digitization and industrial production. Current research results flow directly into teaching.

FH Aachen considers itself as a research university with regional, national and international networks. We promote and conduct research and development that is oriented towards regional, national and international needs and is available to industry, science and society as an innovative research and development partner. FH Aachen promotes the mutual transfer of knowledge between research and application and thus makes a significant contribution to strengthening the competitiveness of science, business and culture, particularly in the Aachen region and the border triangle of Germany, Belgium and the Netherlands.

Our research is based on the principles of transparency and good scientific practice. Aware of its responsibility as part of society, the research and development activities of FH Aachen are guided by the principles of ecologically, socially and economically sustainable development.

At the Institute of Nano- and Biotechnologies (INB) – one of the leading institutes of FH Aachen – researchers deal with interdisciplinary subjects in biology and microelectronics, mainly in the areas of chemo- and biosensor technology, DNA sensor technology and nanostructures, optical micro- and nanosystems technology, semiconductor technology and nanoelectronics, cell culture technology, applied immunology, industrial microbiology, enzyme technology as well as biocatalysis.

I am especially pleased that the 4th EBS has received a very strong in-house support by the complete staff of the Institute of Nano- and Biotechnologies, who have cleared numerous practical and organizational tasks.

I wish you all a fruitful exchange of ideas during the forthcoming days, stimulating discussions, and learning about the latest concepts and developments in your field of research. Of course, a symposium like the EBS is also the ideal place for building up new contacts and to boost our networks: not in the least, research is a team sport.

Please enjoy the days at our university and get to know our beautiful City of Aachen.

Prof. Dr. T. Ritz, Vice-Rector of Research, Innovation and Technology Transfer

Committees

Organizing Committee

- Prof. Dr. Michael J. Schöning (Symposium Chair, FH Aachen)
- Prof. Dr. Patrick Wagner (Symposium Co-Chair, KU Leuven)
- Prof. Dr. Fred Lisdat (TH Wildau)
- Prof. Dr. Ulla Wollenberger (Potsdam University)
- Prof. Dr. Maria Minunni (University of Florence)

Local Organizing Committee

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- Prof. Dr. Till Bachmann (University of Edinburgh)
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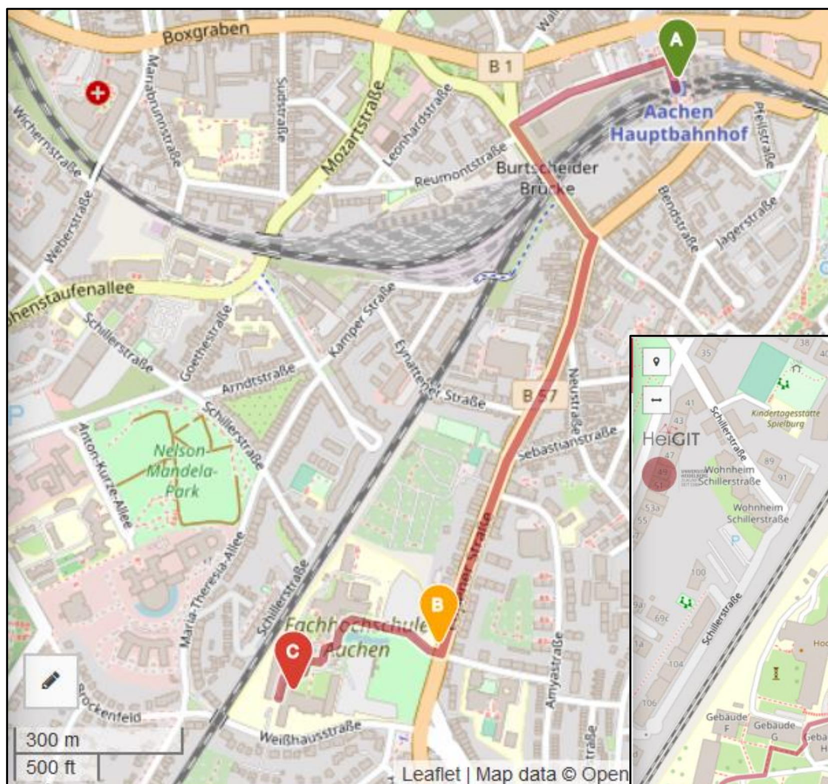
- Dr. Michael Steinwand (Innovendia Consulting Services)
- Prof. Dr. Gerald Urban (University of Freiburg)
- Prof. Dr. Patrick Wagner (KU Leuven)
- Prof. Dr. Joachim Wegener (University of Regensburg)
- Prof. Dr. Ulla Wollenberger (Potsdam University)

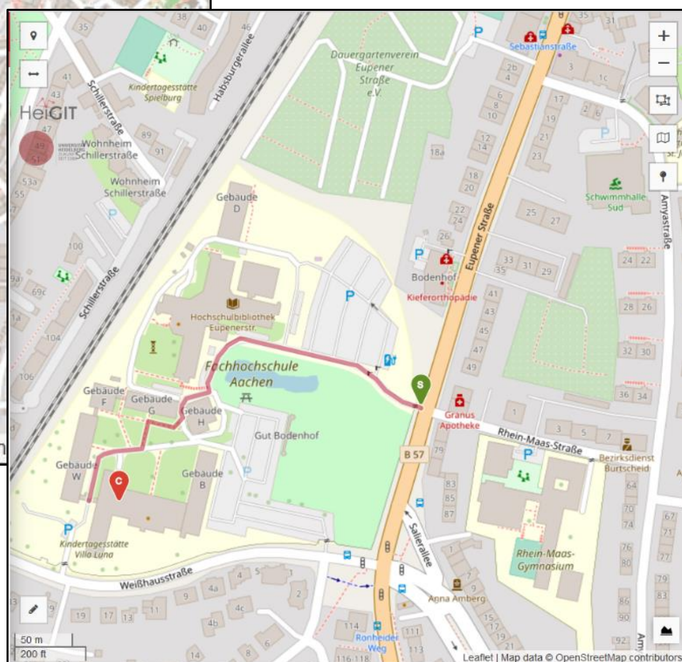
Symposium venue and general information

The symposium will take place at:

FH Aachen | University of Applied Sciences,
Campus Eupener Straße
Eupener Straße 70
52066 Aachen, Building C.

It is about 1.5 km (20 minutes walking distance) from the central station of Aachen (Aachen Hauptbahnhof, A).





Walking information from central station

Leave Aachen Hauptbahnhof (A) to “Bahnhofstraße”, then turn left into “Zollamtstraße”. Follow this street for 250 m and then turn left to “Burtscheider Straße”. After another 250 m turn right to “Krugenofen” (B 57). Following this street for 780 m in south direction leads you to the entry of FH Aachen (B). Signs will guide you to the building (C).

Arriving by public transport

The following bus stops are close to FH Aachen, Campus Eupener Straße:

- Ronheider Weg (in the south of entry to Campus Eupener Straße (B), Lines: 14, 30, 34, 53, 54)
- Burtscheid Sebastianstraße (in the north of entry to Campus Eupener Straße (B), Lines: 14, 34, 54)

Social events

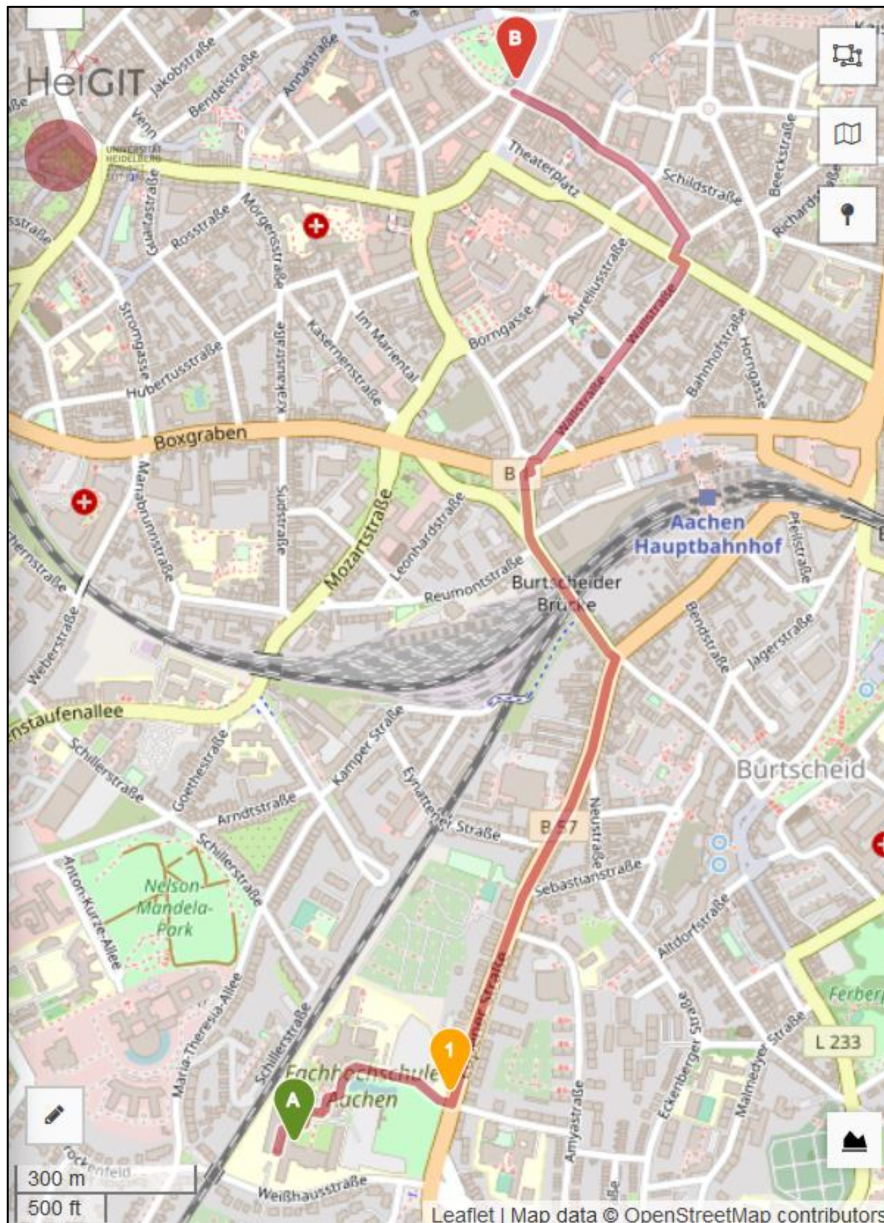
Get together

On Sunday evening (27th August) a get together is scheduled from 17:00 till 20:00 at the symposium venue (FH Aachen, Campus Eupener Straße, Eupener Straße 70, Building C).

Guided city tour

A guided city tour is organized on Monday evening, 28th August. The 90 minutes tour starts 18:30 in the center of Aachen at the "Tourist Info Elisenbrunnen" located at "Friedrich-Wilhelm-Platz, 52062 Aachen" see B on the map below. The starting point is about 30 minutes walking distance (2.5 km) from the symposium location.

By Sascha Faber - Own work, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=126442106



Walking information

(A) exit Eupener Straße (1) turn left → follow road for 780 meter in north direction → turn left to Burtscheider Straße (240 m) → keep right to Burtscheider Straße (100 m) → turn right to Lagerhausstraße (30 m, B 1) → turn right to Wallstraße (410 m) → turn left to Theaterstraße (24 m) → turn right to Schützenstraße (40 m) → turn left to Wirichsbongardstraße. After about 370 m you will reach the meeting point “Tourist Info Elisenbrunnen”.

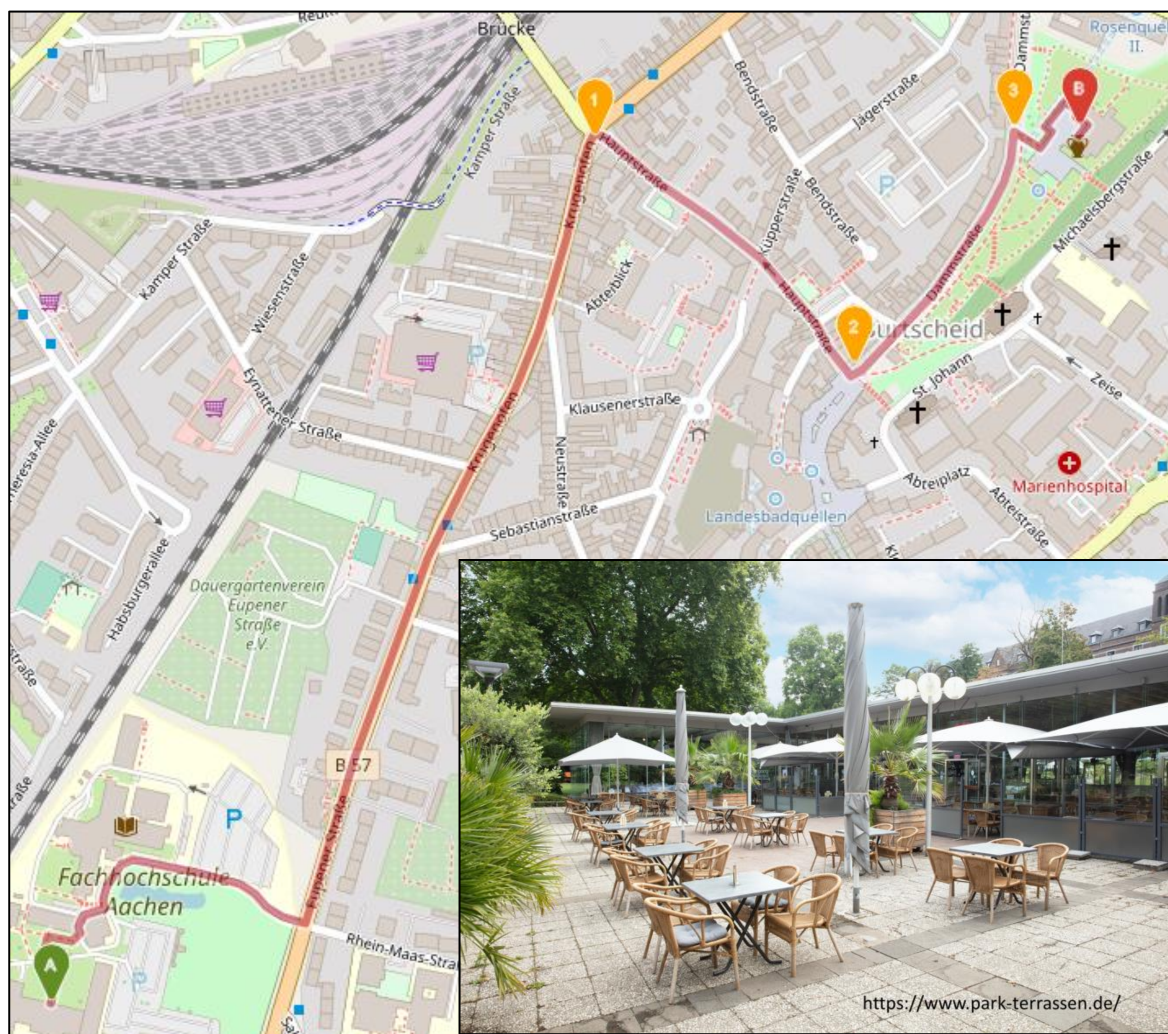
During this tour, professional guides will show you the alleys and historical places along 2.000 years of Aachen’s history. You will get to know Aachen with all its facets, such as beautiful historic town houses in a modern city with many old and new fountains and countless stories around the cathedral and city hall.

Conference dinner

The conference dinner is scheduled on Tuesday evening, 29th August, starting at 20:00. It will be served at the “Kurparkterrassen” - Dammstraße 40, 52066 Aachen - (https://www.park-terrassen.de/).

Walking information

(A) exit to Eupener Straße → follow road for 1.2 km in north direction (the street changes its name to Krugenofen) → turn right into Hauptstraße (1) → follow road for 330 m → turn left into Burtscheider Markt (2). Following this street, you will find the conference dinner location (B) on your right hand side after about 270 m (3).



Guidelines for presenters and chairs

Information for presenters

- Please check the time and lecture room of your presentation in the daily program and on the info boards as there might have been changes.
- Technical staff is assigned to each lecture room for help with technical equipment.
- Each lecture room is equipped with a computer (PowerPoint 2019, Acrobat Reader DC) and a beamer. You will also find a laser pointer and a microphone.
- Your slides shall be prepared in the format of 16:9, the 4:3 format is also possible.
- Please upload your slides the latest in the morning before your talk between 8:00 – 8:30 at the "presentation upload" room. The technical staff will take care that it is available at the computer in your presentation room.
- For previewing your presentation, please be at the lecture room before session. Please also inform the chairperson that you are there.
- Please make sure to stay in your session to ensure smooth changes between the individual presentations.
- The time allotted for the presentations is:
    - 12 min. (plus 3 min. discussion) for oral presentations,
    - 25 min. (plus 5 min. discussion) for keynote lecture, and
    - 40 min. (plus 5 min. discussion) for plenary lectures.
- The chairpersons are requested to stop presentations after the allotted time has passed.

Information for poster

- Poster size: The maximum poster size is DIN A0 portrait (1189 mm x 841 mm).
- Poster mounting: Please mount your poster on Sunday, August 27, between 17:00 and 20:00 during the "Get Together", or on Monday, August 28, from 8:00 – 9:15. Mounting material will be provided at the poster boards. Posters can be removed after the last poster session on Wednesday, August 30, and at the latest after the closing ceremony. Please note that all posters remaining after 16:00 will be removed and disposed.
- If your contribution has been selected as <u>short poster presentation</u>, in addition to the poster please prepare a short presentation (PPTX/PDF) of 3 minutes (maximum 3 - 4 slides including title, authors, affiliations). The presentations need to be uploaded on Sunday, August 27, during the get together or latest on Monday, August 28, from 8:00 – 9:15 at the "short poster presentation upload" room.

Information for chairs

- You are kindly asked to switch between presentations by simply announcing the name of the next presenter and the title of the presentation. Due to the tight schedule, there will be no sufficient time for introducing individual lecturers in a more detailed manner.
- Please do your best to limit the duration of each presentation and discussion to the allotted time.
- If a lecturer is missing, please stick to the original program, i.e., extend the discussion time of the preceding presentation or allow a break for the duration of the missing lecture. This enables participants to move in between sessions and to listen to chosen individual lectures according to the announced sequence.

Awards

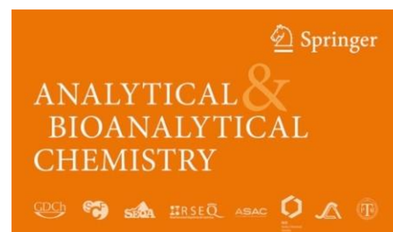
In total 8 poster awards will be given during the EBS symposium. They are split into best posters with and without short presentation.

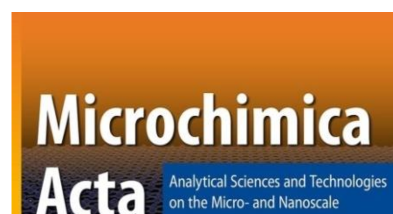
4 * Best poster with short presentation award

The “Best poster with short presentation awards” will be sponsored by the Open Access Journal sensors from MDPI. The award will consist in

- a certificate,
- a monetary prize of 250 € each.

4 * Best poster award





The “Best poster awards” will be sponsored by the Springer journals “Analytical and Bioanalytical Chemistry” and “Microchimica Acta” as well as from “Applied Research” from the Wiley publishing company.

The award will consist in

- a certificate,
- a monetary prize of 300 € sponsored by “Applied Research”,
  or
  a voucher for springer books worth 300 € sponsored by “Analytical and Bioanalytical Chemistry” and “Microchimica Acta” each.

Conference program

Sunday 27th – Wednesday 30th, August

Sunday, 27th August

17:00 - 21:00	Registration / Get together

PL: plenary lecture

KN: keynote lecture

OC: oral contribution

Monday, 28th August

08:00 - 09:00	Registration			
09:00 - 09:30	Opening Ceremony (Room C103)			
	Room C101		Room C103	
Session	Potentiometric sensors		Optical biosensors – SERS	
09:30 - 10:00	KN1	**Prof. Dr. Sabine Szunerits (University of Lille, France)** *Refining cardiovascular risk stratification in patients using graphene-based bioFETs*	KN2	**Prof. Dr. Ilko Bald (University of Potsdam, Germany)** *The lab-on-DNA origami approach to optical sensing*
10:00 - 10:15	OC1	G. García-Molina, P. Natale, L. Valenzuela, J. Alvarez-Malmagro, C. Gutiérrez-Sánchez, A. Iglesias, I. López-Montero, M. Vélez, M. Pita, A.L. De Lacey *Development of a potentiometric ATP biosensor based on E. coli F1Fo ATPase activity reconstituted on gold electrodes*	OC2	N. Bellassai, R. D'Agata, A. Marti, A. Rozzi, S. Volpi, M. Allegretti, R. Corradini, P. Giacomini, J. Huskens, G. Spoto *Detection of circulating tumor DNA in real samples with dual-functional polymer on plasmonic biosensor*
10:15 - 10:30	OC3	S. Papp, J. Kozma, R.E. Gyurcsányi *Solid-contact ion-selective electrodes based on redox-functionalized hydrophobic polymers for calibration-free sensing*	OC4	M. Majdinasab, A. Azziz, Q. Liu, M. Edely, M. Lamy de la Chapelle *Label-free surface-enhanced Raman scattering for interleukin 6 detection*
10:30 - 10:45	OC5	T. Karschuck, J. Ser, S. Achtsnicht, S. Schmidt, A. Poghossian, P. Wagner, M.J. Schöning *Impact of enzyme coverage on performance of a capacitive field-effect biosensor*	OC6	G. Klös, L.S. Peña, V. Pavlov, A.L. Cortajarena *Multi-functional ssDNA for DNA origami biosensor application*
10:45 - 11:00	OC7	N. Dau, V.T. Thu, G. Mattana, B. Piro *A flexible multi-sensing platform for chronic wound monitoring*	OC8	M. Toma, S. Namihara, Y. Itakura, K. Kajikawa *Sensitive label-free biosensing by colors of plasmonic metal nanostructure arrays*
11:00 - 11:25	Coffee Break			
11:25 - 12:50	Short Poster Presentations (Room C103)			
12:50 - 15:10	Lunch, Exhibition and Poster Market A			
15:10 - 15:55	PL1	**Prof. Dr. Evgeny Katz (Clarkson University Potsdam, USA)** *Integration of biosensing and bioactuation using signal-responsive materials – general concept and experimental examples*		
15:55 - 16:15	Coffee Break			
Session	Impedance biosensors		Optics and materials	
16:15 - 16:45	KN3	**Prof. Dr. Jan Vacek (Palacky University Olomouc, Czech Republic)** *Electrochemistry and chiral surface-supported films*	KN4	**Prof. Dr. Patricia Losada-Pérez (Université libre de Bruxelles, Belgium)** *How do solid surface properties affect the formation and stability of supported lipid membranes?*
16:45 - 17:00	OC9	M. Khorshid, S.B. Sichani, I. Wilhelm, A. Clement, J. Hürttlen, M. Menger, G. Pohlmann, P. Wagner *An impedimetric-based biosensor for monitoring COPD and CF biomarkers in patients' exhaled breath condensate (EBC)*	OC10	S. Vasudevan, A.-I. Bunea, J. Kaitez, H. Gürbüz, B. Rezaei, M. Perreira, A. Martínez-Serrano, A. Heiskanen, S.S. Keller, J. Emnéus *Optoelectrical carbon devices as enabling tools for neuroscience*
17:00 - 17:15	OC11	H. Schulze, A. Arnott, A. Libori, E.A. Obaje, T.T. Bachmann *Temperature-enhanced mcr 1 colistin resistance gene detection with electrochemical impedance spectroscopy biosensors*	OC12	E. Schmälzlin, K. Madhav, G. Dähne, M. Olszyna, L. Dähne *Miniaturized, label-free surface nanosensing, based on whispering gallery modes emerging from fluorescent microbeads*
17:15 - 17:30	OC13	M. Liu, N. Jiang, J. Wu, C. Chen, L. Zhuang, P. Wang *Dual-functional biomimetic organoid chip of real-time imaging and synchronous electrical impedance monitoring*	OC14	M. Eickhoff, S. Hölzel, G. Steingelb, M. Kumar, P. Hille, J. Schörmann, R. Hötzel, S. Figge *InGaN/GaN nanowires as selective biosensors with dual optical readout*
17:30 - 17:45	OC15	J. Li, Y. Ding, Y. Shi, Z. Liu, R. Cao, Y. Chen, S. Liu, C. Wu *A zinc oxide nanorod-based biosensor for the detection of tumor markers in saliva*	OC16	H. Yilmaz, A. Ramoji, A. Pistiki, A. Silge, U. Neugebauer, I. Schie, O. Ryabchykov, K. Weber, J. Popp *Raman spectroscopic sensing of the host response*
18:30 - 20:00	Guided City Tour Aachen			

Tuesday, 29th August

Time		Room C101		Room C103
09:00 - 9:45	PL2	**Prof. Dr. Tatsuo Yoshinobu (Tohoku University Sendai, Japan)** *Light-addressable potentiometric sensors and related technologies for biological and electrochemical systems*		
		Room C101		Room C103
Session		Electrochemical biosensors		Optical biosensors – SPR
9:45 - 10:15	KN5	**Prof. Dr. Filip Meysman (University of Antwerp, Belgium)** *How microbes discovered electricity way before Alessandro Volta*	KN6	**Dr. Thomas Hirsch (University of Regensburg, Germany)** A bright match: high lanthanide content meets surface protection via bilayer strategy – small, efficient, NIR-excitable upconversion probes for bioapplications
10:15 - 10:30	OC17	S. Hoffmann, T. Dotan, Y. Shacham-Diamand *Flexible electrochemical biosensors for direct monitoring of heat stress in Nicotiana tabacum plants*	OC18	F. Battaglia, F. Torrini, P. Palladino, S. Scarano, M. Minunni *Polyserotonin as new functional monomer for molecular imprinting: an applicative study for TNF-α detection by surface plasmon resonance*
10:30 - 10:45	OC19	E.V. Daboss, A.A. Karyakin *Simultaneous noninvasive monitoring of diabetes and hypoxia using ‘artificial peroxidase’ nanozyme – enzyme based biosensors*	OC20	A. Dillen, C. Scarpellini, A. Mohrbacher, W. Daenen, S. Driesen, W. Vandezande, D. Daems, P. Zijlstra, D. Spasic, J. Lammertyn *FO-SPR sensors and duplexed aptamers: an innovative approach for continuous biosensing*
10:45 - 11:00	OC21	A. Tricase, V. Marchianò, N. Ditaranto, E. Macchi, C. Di Franco, R. Kidayaveettil, D. Leech, M. Piscitelli, G. Scamarcio, G. Perchiazzi, L. Torsia, P. Bollella *Printing enzymes on electrodes: a new frontier to fabricate enzyme-based amperometric biosensors*	OC22	K. Toma, Y. Satomura, K. Iitani, T. Arakawa, K. Mitsubayashi *Real-time and label-free monitoring of vancomycin concentration using a long-range surface plasmon resonance aptasensor*
11:00 - 11:15	OC23	G. Göbel, F. Müller, A. Talke, U. Ahnert, F. Lisdat *Determination of protease activities at different protein modified 3D structures*	OC24	J. Bednár, V. Maybeck, A. Offenhäusser *Measuring cell-substrate separation with surface plasmon resonance microscopy to assess biosensor performance*
11:15 - 11:45		Coffee Break and Group Photo		
Session		Aptamer-type biosensors		Particles and biosensing
11:45 - 12:15	KN7	**Dr. Marcus Menger (Fraunhofer Institute for Cell Therapy and Immunology, Postdam, Germany)** *Aptamers and aptasensors for highly specific recognition*	KN8	**Prof. Dr. Hans-Joachim Krause (Forschungszentrum Jülich, Germany)** *Immunoassays based on frequency mixing magnetic detection*
12:15 - 12:30	OC25	N. Bagheri, A. Idili, F. Ricci, A. Porchetta *PAM-engineered toehold switch DNA as target-responsive activators of CRISPR/Cas12a for sensing applications*	OC26	S. Streif, A.J. Baeumner *Liposome-based high-throughput and point-of-care neutralization tests for SARS-CoV-2*
12:30 - 12:45	OC27	C.A. Schrage, P. Dippner, N. Labetzki, S. Kruss *Fluorescent nanosensors for allergy diagnostics*	OC28	I. Tavernaro, P. Srivastava, L. Scholtz, N. Nirmalananthan-Budaua, U. Resch-Genger *Multicolored sensors based on silica and polymeric particles for ratiometric monitoring of pH, oxygen and saccharides*
12:45 - 13:00	OC29	P. Ivaskovic, A. Florent, H. Debreyne, J.-J. Toulmé *Engineering optical aptasensors for the detection of fungicides*	OC30	X. Liu, F. Schulz, W.J. Parak *An intriguing polymeric modification strategy for solid particles to investigate endosomal escape of cargos from polyelectrolyte microcapsule*
13:00 - 13:15	OC31	S. Ranallo, S. Bracaglia, F. Ricci *DNA nanotechnology meets synthetic biology: new perspective of cell-free biosensors for antibody detection*	OC32	L. Zeininger *Ratiometric determination of morphology-dependent emulsion droplet luminescence for in-situ tracking of exoenzyme activity*
13:15 - 15:45		Lunch, Exhibition and Poster Market B		

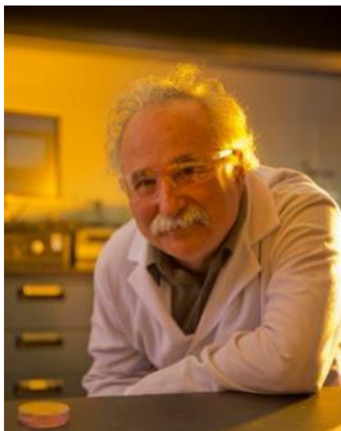
Session		Materials and fabrication		Cantilever sensors and QCM
15:45 - 16:15	KN9	**Prof. Dr. Bernhard Wolfrum (Technical University Munich, Germany)** *Sensing and stimulation at small peripheral nerves using 4D-printed cuff electrodes*	KN10	**Prof. Dr. Alberto Escarpa (University of Alcala, Spain)** *Catalytic micromotors in action for (bio)sensing applications*
16:15 - 16:30	OC33	H. Gliemann, M. Franzreb, C. Wöll *Surface-anchored metal-organic frameworks as highly potential material platform for biosensing application*	OC34	C. Kranz, A. Hellmann, S. Daboss, E. Daboss *From microbiosensors to AFM probe-integrated (bio)sensors: towards single cell measurements*
16:30 - 16:45	OC35	D. Murugan, H. Jiang, R.R.G. Soares, N. Madaboosi, S. Ingebrandt, V. Pachauri *Studying the metal-organic framework bio-interface for novel biosensing applications*	OC36	C.F. Werner, Y. Takahashi, K. Miyaoka, R. Mitobe, M. Sohgawa, M. Noda *Liposome-immobilized microcantilever array sensor for the simultaneous detection of alpha-synuclein in multiple analytes*
16:45 - 17:00	OC37	A. Scroccarello, F.D. Pelle, S. Fiori, D. Compagnone *Laser-induced metal nanostructures on cellulosic substrates for colorimetric devices development*	OC38	D. Özsoylu, F.Aliazizi, P. Wagner, M.J. Schöning *First step towards "template cell-free" fabrication of a surface imprinted polymer-based biosensor for pathogenic bacteria detection*
17:00 - 17:15	OC39	F.P. Angelov, L.N. Quang, G.G. Zavaleta, J.Y. Pan, S.I. Bisgaard, K.L. Bøgh, A. Heiskanen, J. Emnéus, Y. Sun, S.S. Keller *Microfabrication of pyrolytic carbon microneedles for electrochemical biosensing in the skin*	OC40	S. Spagnolo, K. Davoudian, S. Ahmadi, E. Chan, J. Süle, R. Kocsis, T. Hianik, M. Thompson *A novel thiol-based linker with antifouling properties for detection of P. aeruginosa by QCM-D aptasensor in milk*
17:15 - 18:15	(Scientific Committee Meeting)			
20:00 - 22:30	Conference Dinner			

Wednesday, 30th August

Time		Room C101		Room C103
9:00 - 9:45	PL3	**Prof. Dr. Fabiana Arduini (Universita di Roma Tor Vergata, Italy)** *Paper as smart material to deliver ecodesigned lab on a chip with improved analytical features and unprecedented applications*		
		Room C101		Room C103
Session		Lab-on-Chip systems		Immunosensors
9:45 - 10:15	KN11	**Prof. Dr. Christina Wege (University of Stuttgart, Germany)** *Plant virus-based enzyme nanocarriers for robust biosensor layouts*	KN12	**Prof. Dr. Fabio Biscarini (University of Modena and Reggio Emilia, Italy)** *Electrolyte-gated transistors based on ambipolar reduced graphene oxide: the mechanism of transduction of biorecognition events*
10:15 - 10:30	OC41	D. Vloemans, H. Ordutowski, J. Qu, L. Van Hileghem, W. Verbist, S. Santos, C. Van Tricht, D. Spasic, F. Dal Dosso, J. Lammertyn *(i)SIMPLE: next-generation self-powered microfluidic platform for point-of-care diagnostic applications*	OC42	J. Raya, N. Pascual, M.-T. Martin-Gómez, E. Padilla, J.P. Horcajada, M.-P. Marco *Quorum sensing profiling for P. aeruginosa infection samples from clinical patients*
10:30 - 10:45	OC43	C. Warmt *Loop-mediated isothermal amplification in the field of biosensors and point of care: can isothermal amplification outperform PCR?*	OC44	S. Paßreiter, J. Klüpfel, H.-P. Holthoff, M. Ungerer, M. Lohse, P. Knolle, U. Protzer, M. Elsner, M. Seidel *A competitive chemiluminescence immunoassay for the automated detection of surrogate neutralizing SARS-CoV-2 antibodies*
10:45 - 11:00	OC45	S. Agarwal, C. Warmt, M. Hamidizadeh, J. Henkel, F.F. Bier *Lateral flow assay based POCT for Loop mediated isothermally amplified nucleic acid with enzymatic incorporation of biotin labelled dUTP and hybridization of DNA probes to detect N-gene of SARS-CoV-2*	OC46	M. García-Cortés, F. Pradanas-González, B. Glahn-Martínez, A. Luque-Uría, M. del Barrio, T.K. Nevanen, R. Barderas, C.M. Maragos, G. Orellana, E. Benito-Peña, M.C. Moreno-Bondi *Epitope-mimicking peptides as versatile biological tools for the development of mycotoxin immunosensing*
11:00 - 11:15	OC47	C. Qin, Q. Yuan, M. Liu, L. Xu, P. Wang *Biohybrid tongue for real-time glucose-sensing based on hypothalamic neuronal network*	OC48	V. Serafín, M. Blázquez-García, V. Ruiz-Valdepeñas Montiel, S. Benedé, E. Molina, M. Gamella, B. Arévalo, L. Mata, P. Galán-Malo, I. Segura-Gil, J.M. Pingarrón, S. Campuzano *Advancing food allergy with multiomics molecular-level electrochemical biosensing*
11:15 - 13:15		Lunch, Exhibition and Poster Market B		
Session		Biosensors & IoT		MIP-based biosensors
13:15 - 13:45	KN13	**Dr. Ulrich Rant (CEO Dynamic Biosensors, Germany)** *Taking novel challenges in molecular interaction analysis – from small molecules to cells*	KN14	**Dr. Ljiljana Fruk (University of Cambridge, England)** *Nanostructured probes for early detection of cancer*
13:45 - 14:00	OC49	M. Birkholz, M. Kögler *Sustainable design of online biosensors*	OC50	P.A. Lieberzeit, S.S. Alzahrani, K. Sirivibulkovit, C.L. Onorat *Can polymers replace proteins in assays? Chances and challenges*
14:00 - 14:15	OC51	F. Kleiser, A. Weltin, S.J. Rupitsch, J. Kieninger *Biosensors in the cloud – Freiburg's embedded potentiostat (FreiStat) provides the missing link for IoT and AI applications*	OC52	N. Cennamo, D. Maniglio, L. Zeni, A.M. Bossi *Soft molecularly imprinted nanoparticles for the optical sensing of protein biomarkers*
14:15 - 14:30	OC53	E. Macchia, C. Sarcina, M. Scandurra, M. Caputo, M. Catacchio, C. Di Franco, P. Bollella, M. Chironna, F. Torricelli, I. Esposito, R. Österbacka, G. Scamarcio, L. Torsi *Single-molecule bioelectronic sensor: improving reliability with machine learning approaches*	OC54	F.A. Tabar, J.W. Lowdon, M. Caldara, T.J. Cleij, P. Wagner, K. Eersels, H. Dilien, B. van Grinsven *Thermal determination of PFOA in environmental samples*
14:30 - 15:00		Poster Awards, Closing Ceremony		

Plenary speakers

PL1



Prof. Dr. Evgeny Katz
(Clarkson University Potsdam, USA)

PL2

Prof. Dr. Tatsuo Yoshinobu
(Tohoku University Sendai, Japan)

PL3

Prof. Dr. Fabiana Arduini
(Universita di Roma Tor Vergata, Italy)

Integration of Biosensing and Bioactuation Using Signal - Responsive Materials – General Concept and Experimental Examples

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Keywords: bioelectronics, biosensors, signal-responsive material

Electrochemically-triggered payload release as a potential method for signal-responsive "smart drug" development has been increasingly utilized in the field of modern pharmaceuticals. Among the various approaches to design electrochemically-triggered payload release systems, the basis of using electrochemical reactions to develop sensing and releasing electrodes allows for increased diversity and adaptability with regards to signal, payload, and release conditions. In this lecture, an overview of the two main categories (pH-independent and pH-dependent) of electrochemically-triggered release and their most commonly used subsets will be summarized. These variations provided the foundational basis from which an electrochemically-triggered payload release system can be designed and adapted to meet the demands of potential treatment requirements. Moreover, different practically demonstrated adaptions of electrochemically-triggered payload release sensing and releasing electrodes were evaluated comprehensively and the advantages and disadvantages will be discussed. Finally, some overarching recommendations for optimized use of these electrochemical biosensing and bioactuating systems are proposed.

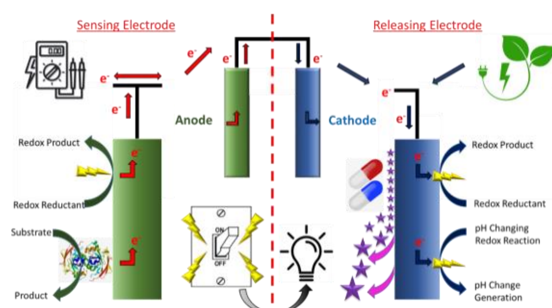

Figure 1: *Integration of biosensing and bioactuation shown schematically.*

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Acknowledgements

This work was supported by Human Frontier Science Program (HFSP), project grant RGP0002/2018.

Light-addressable potentiometric sensors and related technologies for biological and electrochemical systems

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Keywords: light-addressable potentiometric sensor, LAPS, chemical imaging sensor

The light-addressable potentiometric sensor (LAPS) [1] belongs, together with the ion-sensitive field-effect transistor (ISFET), to the family of electrochemical sensors based on the field effect in semiconductors. The LAPS has a unique feature of light-addressability; the concentration of the analyte at an arbitrary position on the sensing surface is read out in the form of a photocurrent signal induced by a light beam, which makes it possible to integrate a plurality of sensing functionalities on a single sensor plate. In addition, a two-dimensional map of the concentration of the analyte can be obtained by scanning the sensor plate with a light beam.

Numerous efforts have been made by many groups to enhance a LAPS's performance and extend its applicability to various targets [2]. The former and the latter goals have been pursued by considering the physical and chemical aspects of the sensor. The performances of a LAPS as a transducer, namely, the spatial resolution, the signal-to-noise ratio, and the measurement speed were improved by optimizing the design and the parameters of the sensor plate and the light source as well as sophisticating the measurement electronics. At the same time, various chemistry and biochemistry based on ionophores, enzymes, antibodies, DNA probes, aptamers, and cells were brought onto the sensing surface to make it sensitive to different analytes.

As a result of these efforts, different designs of sensor systems based on LAPS have been applied to a wide range of specimens. As an example of non-biological applications, it was applied to study the mechanism of corrosion on a metal surface, where a pH change in the course of local corrosion was detected in close proximity to the metal surface. Many biological applications of a LAPS have been reported [3], including quantifying ions and molecules in biological samples, detecting DNAs, cells, and viruses, and measuring metabolic activities. The application of an optical-fiber-based LAPS to in vivo measurement of pH in the brain was also reported [4].

In parallel to the development of the LAPS, several technologies closely related to a LAPS but beyond its principle have also been proposed. Scanning photo-induced impedance microscopy (SPIM) [5] can obtain a map of impedance distribution on the sensor surface with a measurement setup similar to that of a LAPS. The light-addressable electrode (also called light-activated electrochemistry; LAE) [6] allows a DC current to flow and activate reactions at the illuminated position on the surface of a semiconductor electrode, whereas a LAPS surface is insulated and blocks a DC current. The photoelectrochemical imaging system (PEIS) [7] allows AC photocurrent imaging at an electrolyte–semiconductor interface and was applied to the mapping of cell surface charges. Furthermore, a light-controlled volume change of a hydrogel was applied to flow control in a microfluidic channel [8]. Some of these technologies may be combined to construct an analytical system with light-addressing technology as a common basis.

In this talk, I would like to overview the development of the LAPS and related technologies and discuss their future possibilities.

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Paper as smart material to deliver ecodesigned lab on a chip with improved analytical features and unprecedented applications

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Keywords: electrochemical (bio)sensors, smartphone-assisted devices, lab on a chip on paper .

As reported in my recent review entitled "Electrochemical paper-based devices: When the simple replacement of the support to print ecodesigned electrodes radically improves the features of the electrochemical devices" published in Current Opinion in Electrochemistry SI: Emerging Opinions (2022) [1]: "*Paper-based electrochemical (bio)sensors have emerged as highly attractive analytical devices for their superior sustainable features, such as avoiding the use of polyester as support and the reduction of waste, being incinerated after use. However, paper-based electrochemical (bio)sensors have recently demonstrated further advantages, including the simple combination with vertical microfluidics and their use as a reservoir to deliver smart electrochemical (bio)sensors able to i) contain the reagents, ii) preconcentrate the target analyte, and iii) synthesize the nanomaterials inside the paper network. Furthermore, these devices have demonstrated their ability to overcome the limitations of the other printed electrochemical sensors in the measurement of entirely liquid samples by detecting the target analyte in the aerosol phase or solid sample, without the additional sampling system. These achievements highlight their valuable and varied advantages in the sensing sector*". In this plenary lecture, I will report on the roadmap research activity carried out in the last 8 years related to the development of paper-based electrochemical devices for delivering lab-on-a-chip on paper as well as sustainable tools for overcoming the limitation of polyester-ceramic based printed sensors [2-9]. Furthermore, following the approach recently selected for printed electronics in which the hybrid systems have been the most useful for market entry, we recently demonstrated this approach for a market entry device for virus detection in saliva (patent filled), a wearable paper-based immunosensor washing-free for cortisol detection in sweat [10], and a paper-based device combined with polyvinyl chloride electrochemical system in which a paper layer loaded with reagents is inserted into this device, working as a new concept of paper card-like for a reagent-free and easy measurement of target analyte (i.e. glucose) in solution (i.e. tears) [11].

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Acknowledgements

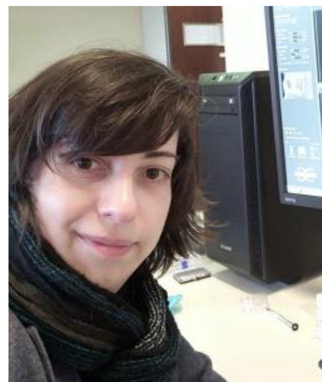
Horizon Europe Reliance Project GA N. 101058570 and Lazio Innova E-CROME Project Grant ID: A0375-2020- 36563.

Keynote speakers

KN1: Prof. Dr. Sabine Szunerits
(University of Lille, France)

KN2: Prof. Dr. Ilko Bald
(University of Potsdam, Germany)

KN3: Prof. Dr. Jan Vacek
(Palacky University Olomouc, Czech Republic)



KN4: Prof. Dr. Patricia Losada-Pérez
(Université libre de Bruxelles, Belgium)

KN5: Prof. Dr. Filip Meysman
(University of Antwerp, Belgium)

KN6: Dr. Thomas Hirsch
(University of Regensburg, Germany

KN7: Dr. Marcus Menger
(Fraunhofer Institute for Cell Therapy and Immunology, Postdam, Germany

KN8: Prof. Dr. Hans-Joachim Krause
(Forschungszentrum Jülich, Germany)

KN9: Prof. Dr. Bernhard Wolfrum
(Technical University Munich, Germany)

KN10: Prof. Dr. Alberto Escarpa
(University of Alcala, Spain)

KN11: Prof. Dr. Christina Wege
(University of Stuttgart, Germany

KN12: Prof. Dr. Fabio Biscarini
(University of Modena and Reggio Emilia, Italy)

KN13: Dr. Ulrich Rant
(CEO Dynamic Biosensors, Germany)

KN14: Dr. Ljiljana Fruk
(University of Cambridge, England)

Refining Cardiovascular Risk Stratification in Patients using Graphene-based bioFETs

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Keywords: graphene, troponin, breath analysis

Introduction

Cardiovascular diseases (CVDs) remain one of the leading causes of death worldwide. To improve therapeutic outcomes and reduce health care costs, a better understanding of disease-specific variation across cardiac patients is needed with emerging technologies providing innovative opportunities in the form of home diagnosis. Electrical biosensors are especially promising when interconnected with 2D materials such as graphene. Despite impressive achievements in the electrical performance of graphene-based field effect transistors (gFET), some key bottlenecks need to be tackled: reproducibility of gFET preparation, maintaining high mobility after surface modification and sensing in high ionic strength medium.

Results and Discussion

We have recently demonstrated the validity and sensitivity of graphene-based field-effect transistors (gFET) (**Figure 1**) as point-of-care (POC) options for the rapid and highly sensitive detection of troponin (cTnI) as well as brain natriuretic peptide (NT-proBNP) in serum samples of different patient groups. The importance of adapted surface chemistry approaches to anchor cardiac specific aptamers (DNA and PNA based) on the gFET was one of the reasons for achieving this performance.

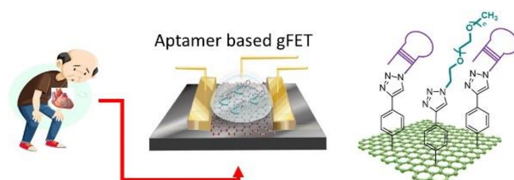

Figure 1: *Sensing of cardiac biomarkers on aptamer-based gFETs*

Conclusions

The question might be raised if it is likely that an assay, such as the one described here, that can detect troponin levels below the reference range, will produce clinical benefits. In our mind, quantifying cardiac troponin in blood samples from patients with very low, and presently undetectable levels, will allow to refine cardiovascular risk stratification in patients with cardiovascular risk factors such as tobacco use, type 2 diabetes, dyslipidaemia and obesity, even in the absence of cardiovascular medical history or symptoms.. Moreover, we strongly believe that the implementation of a breathing rather than blood based gFET sensor will be beneficial for patients.

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Acknowledgements

Financial supports from the Centre National de la Recherche Scientifique (CNRS), the University of Lille are acknowledged.

The Lab-on-a-DNA origami approach to optical sensing

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DNA origami nanostructures are self-assembled into almost arbitrary two- and three-dimensional shapes from a long, single-stranded viral scaffold strand and a set of short artificial oligonucleotides. Each DNA strand can be functionalized individually using well-established DNA chemistry, representing addressable sites that allow for the nanometre precise placement of various chemical entities such as proteins, molecular chromophores, nanoparticles, or simply DNA motifs. By means of microscopic and spectroscopic techniques, these entities can be visualized or detected, and either their mutual interaction or their interaction with external stimuli such as radiation can be studied. This gives rise to the Lab-on-a-DNA origami approach.[1]

Chromophores can be arranged on DNA origami into arrays with well-defined inter-dye distances thereby creating artificial light-harvesting antennas,[2] which can be further exploited as self-referenced sensors based on Förster resonance energy transfer (FRET).[3] By attaching gold or silver nanoparticles to the DNA origami nanostructures novel plasmonic nanostructures are formed in which light is focused into nanoscale cavities creating intense hot spots for surface-enhanced Raman scattering (SERS).[4] In addition to the precise arrangement of nanoparticles resulting in different forms of nanolenses,[5] the DNA origami nanostructures also allow for a specific binding of analyte molecules exactly in the SERS hot spots.[6] Recently, we have created a novel DNA origami nanofork that is dedicated for single-molecule detection using SERS.[7] This structure has been used to detect chemical changes in the molecule hemin.[8] Further developments such as the implementation of DNA origami structures within optical fibers will be shown.

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Electrochemistry and Chiral Surface-Supported Films

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Keywords: helicene, electrodeposition, EPR, anodic reaction

Introduction

The phenomenon of chiral resolution has been intensively applied in the field of electrochemical research, with the aim of developing chiral surfaces and electrodes that will then be used in the enantioselective analysis, with potential applications in circularly polarized light detectors, molecular switches and (opto)electronic devices.

Results and Discussion

Helicenes are chiral polyaromatics that can be resolved into two (*P* and *M*) enantiomers (Figure 1). It has been shown that helicenes can be deposited (polymerized) onto carbon electrode surfaces under suitable experimental conditions. Pilot experiments were conducted with the heptahelicene thiophene derivative, 3-([7]helicen-9-yl)thiophene [1]. Polymers obtained by electrosynthesis were deposited on the surfaces of glassy carbon and carbon fiber microelectrodes [2]. After anodizing the electrodes in the helicene monomer solution, an overoxidized film formed on the electrode surfaces [1]. Lowering the potential limit for anodic deposition, a conductive (redox-active) version of the polymer was also prepared for the same helicene derivative [3]. Pilot experiments performed with racemic 3-([7]helicen-9-yl)thiophene have been further extended to include other helicenes and their derivatives, and it has been shown that thiophene or other substitution is not necessary for the preparation (electrosynthesis) of polymer films. The electrosynthesis process can be optimized to provide polymer layers *via* the direct oxidation of helicenes, whereby the presumed cation radicals associate and form a deposit on the electrode surface. This way, carbohelicenes can be used on their own to prepare polymer layers, and compact chiral layers can be prepared by electrosynthesis starting from their pure *P* and *M* enantiomeric versions [4]. When prepared on the surfaces of transparent ITO (indium tin oxide) electrodes (Figure 1), their enantiopurities can be easily demonstrated by CD spectroscopy.

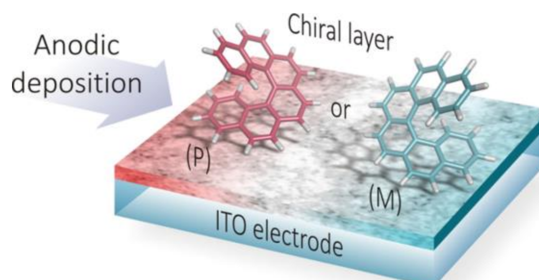

Figure 1: *Schematic representation of anodic deposition of (P)- and (M)-enantiomers of [6]helicene onto ITO electrode. For more details, see ref. [6].*

Conclusions

In this contribution, new directions in the field of chiral helical polyaromatic molecules (helicenes) are highlighted that are useful for the preparation of optically and redox-active thin layers and functional electrode surfaces (reviewed in [5]). Recently, a process based on potentiodynamic electropolymerization has been developed, by means of which anodic deposition can be used to prepare chiral layers from [6]helicene monomers in both conductive (redox-active) and non-conductive versions [6]. This procedure led to the preparation of an inherently chiral polymer, which was used as a chiral electrode in its conductive version and as an optic/optoelectronic elements in general [7].

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Acknowledgements

The author gratefully acknowledges the financial support of RVO61989592, MEYS CZ.

How do solid surface properties affect the formation and stability of supported lipid membranes?

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Biointerfaces encompass natural interfaces between biomolecules, their assemblies and organic or inorganic surfaces they interact with. Their study is motivated by the need to monitor and understand biomolecular adsorption at interfaces to develop materials for biosensors, diagnostics, and therapeutics. Artificial lipid membranes are useful model systems for many processes taking place at the cell membrane. Solid supported lipid bilayers (SLBs) are excellent platforms for studying the biophysical properties of cell membranes, as well as versatile biomimetic films for biotechnology applications. [1]. Moreover, lipid-coated nanostructured materials can be used for controlled drug delivery purposes giving the high drug loading capacity of the nanostructured system, whose release could be modulated by the supported lipid bilayer (SLBs) [2].
Among the existing approaches used to form SLBs, vesicle fusion and rupture onto solid supports is the most commonly employed owing to its straightforward procedure [3]. SLBs are typically formed on atomically flat and very hydrophilic surfaces, overlooking the influence of roughness and topography on membrane formation and organization. As a matter of fact, lipid bilayers in vivo are corrugated at the nanoscale level, as a result from interactions with proteins, fibrils and other components within the intracellular and extracellular environment. Fundamental studies of the effect of surface roughness on SLBs are scarce and restricted to few contributions, where nanoroughness has shown to affect lipid mobility by a 5-fold decrease and inhibit domain growth in phase-separated membranes [4,5]. In this talk, we will explain how solid surface properties affect the formation and stability of SLBs [6,7]. We will review the effect of surface energy on vesicle adsorption and rupture and assess the impact of nanoroughness on the formation of SLBs onto hydrophilic surfaces. Combining quartz crystal microbalance with dissipation (QCM-D) and atomic force microscopy force spectroscopy (AFM-FS), we show that nanoroughness impacts the formation of SLBs by increasing the activation energy of vesicle fusion, rupture and spreading and affects the stability and lateral organization of the formed SLBs.

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How microbes discovered electricity way before Alessandro Volta

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Keywords: long-range electron transport, cable bacteria, protein conductivity, bio-electronics

A quantum leap in biological conduction

The ability to create electricity, that is to send a stream of electrons through a thin wire, lies at the heart of modern technology. Most people believe that we (humans) were the first to exploit the benefits of electricity, after Alessandro Volta invented the first battery in 1799. Recent findings however show that long before Volta, microbes already evolved highly conductive wiring systems that enable electrical currents over extremely long (i.e., centimeter scale) distances.

Electron transport in biological systems is classically thought to occur over nanometre to micrometre distances. Yet, in 2012, a game-changing discovery was made: multicellular bacteria were found in the seafloor that can channel electrons from cell to cell along the longitudinal axis of centimeter-long filaments (Pfeffer et al., 2012; Meysman, 2018). These so-called cable bacteria mediate electrical currents over centimeter distances (i.e. 10^7 nm!). This finding extended the known distance of biological electron transport at once by four orders of magnitude, and hence resulted in a quantum leap in the emerging field of electromicrobiology.

Highly conductive wiring

Cable bacteria internally conduct electrons along their cm-long body via a network of highly conductive fibres embedded in the cell envelope (Meysman et al., 2019). Direct measurements of the conductivity of these fibers show extraordinary high conductivity (~100 S cm^{-1}; Meysman et al., 2019), which is eight orders of magnitude higher than that of the "conductive" membrane proteins in mitochondria and chloroplasts, and even exceeds that of most semiconductors used in electronics.

These protein fibers hence demonstrate that supramolecular protein structures can attain an extremely high conductivity. This observation profoundly challenges our understanding of protein biochemistry and bio-physics. Moreover, recent results demonstrate that the extreme protein conductivity in cable bacteria is conferred through a nickel-based conduction mechanism that is fundamentally different from currently known electron transport mechanisms (Boschker et al., 2021). These findings greatly expand the paradigm of biological charge transport and could enable new bio-electronic applications.

Future applications

Our ongoing research aims to demonstrate the controlled, recombinant production of these conductive protein nanofibers, thus achieving a major breakthrough that creates a window of opportunity for radically new technological applications such as bio-electronics. The resulting "proteonic" fiber materials could allow more sustainable production and recycling pathways, thus assisting with the path towards a circular and carbon-neutral economy (e.g. by reducing e-waste).

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Acknowledgements

This research received support by University of Antwerp via the TopBOF program, Research Foundation Flanders (FWO), Netherlands Organization for Scientific Research (VICI grant 016.VICI.170.072) and the EIC (Pathfinder project 101046719 PRINGLE).

A Bright Match: High Lanthanide Content Meets Surface Protection via Bilayer Strategy – Small, Efficient, NIR-excitable Upconversion Probes for Bioapplications

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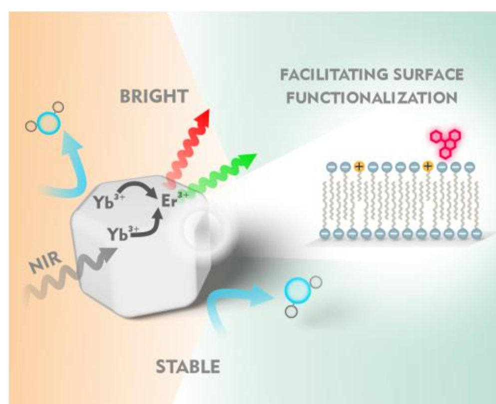
Abstract:

NIR-excitable lanthanide-doped upconversion nanoparticles (UCNPs) of the type $NaYF_4$:Yb,Er are promising probes for biomedical applications due to their remarkable advantages like low scattering and therefore deep tissue penetration of the excitation light, outstanding photostability and sharp emission bands. On the contrary, such particles still suffering from limited brightness, mainly attributed to the low absorption coefficient of the lanthanide ions and a variety of luminescence quenching pathways [1]. Here, the design of 10 nm small but bright UCNPs is presented. The luminescence was increased by rethinking the doping concentrations of sensitizer and activator ions. A detailed photophysical study revealed that in core-shell systems of the composition $NaYbF_4$:Er@$NaYF_4$ the absorption cross-section can be significantly increased by factor six without affecting the total particle size [2]. The influence of composition and architecture on the photophysical properties was studied by absorbance, luminescence, and lifetime measurements. The particles with high lanthanide content were further modified with a novel bilayer strategy resulting in colloidal and chemically stable particles in biological media [3]. Due to efficient shielding from the aqueous environment, water-quenching effects are be reduced to almost the same level as it is in organic solutions. Those bright bilayer-modified particles have been functionalized with organic dyes, without losing colloidal stability of the particles, suitable for FRET applications intended for singlet oxygen detection and for sensing of reactive oxygen species. Hence, that combination of particles engineering together with the novel surface modification strategy is beneficial for the design of small but stable NIR excitable probes for biosensing and bioimaging.



Alloyed $NaYbF_4$:Er nanoparticles with bilayer coating as efficient and colloidal stable probes for photon upconversion.

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Aptamers and Aptasensors for Highly Specific Recognition

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Keywords: aptamer, aptasensor, biosensor, nucleic acid, point-of-care, SELEX

Introduction

Aptamers are short, single-stranded nucleic acids (DNA or RNA) and can use as high specific recognition elements for a wide range of target molecules. Numerous aptamer properties, but particularly the reproducible production of aptamers including chemical modifications and the ability of refolding in an active binding form, enable the use of aptamers in many different biosensor systems. The results are a new class of biosensors called aptasensors.

Results and Discussion

Aptamers are generated by multi-step process (figure 1) involving an iterative in vitro selection procedure (SELEX - Systematic Evolution of Ligands by EXponential enrichment). The selection of high-affinity and specific aptamers from resulting enriched nucleic acid pools are supported by sequence analysis, aptamer binding characterisation methods, and aptamer optimisation. Various selection strategies (Capture-SELEX, magnetic bead-based SELEX, CE-SELEX, Cell-SELEX, etc.) have enabled the development of aptamers against targets such as small molecules, peptides and proteins or whole cells [1].

A typical biosensor consists of a bio-receptor, a transducer and an electronic signal amplifier. The transducer or detector element can use different physicochemical ways (optical, electrochemical, piezoelectric, etc.) which in the case of an aptasensor result from the interaction of a target molecule (analyte) with its specific aptamer (bioreceptor) [2]. Alternative competitive or sandwich assay formats can improve the specificity and sensitivity of analytical devices [3].

Furthermore, lateral flow assays (LFA) based aptasensors provide a convenient, low-cost and easy-to-use format for point-of-care (POC) devices (figure 2).

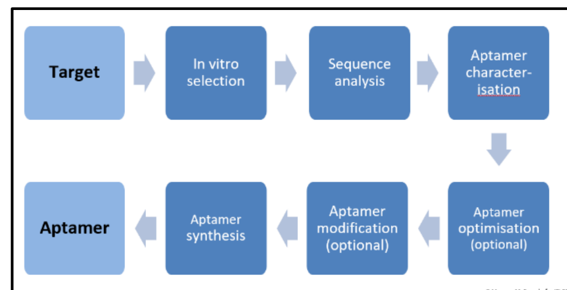

Figure 1: *Multi-step process of aptamer generation.*

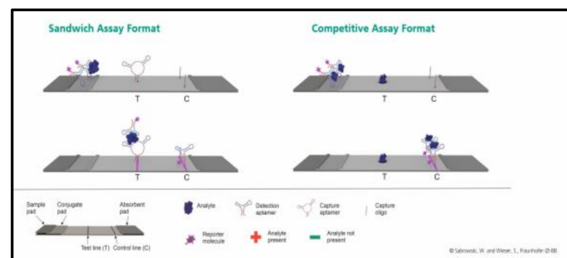

Figure 2: *Alternative LFA formats.*

Conclusions

The application spectrum of aptamer-based biosensors is almost unlimited and ranges from environmental and food analysis to diagnostics. The use of modified aptamers (SOMAmers, Clickmers, etc.) will significantly improve the detection limit and increase the specificity and sensitivity of aptasensors in the future.

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Acknowledgements

This work was supported by the German Federal Ministry of Education and Research BMBF within the funding program RUBIN [grant number 03RU1U111D and 03RU1U115D to M.M.M.]

Immunoassays based on Frequency Mixing Magnetic Detection

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Keywords: frequency mixing magnetic detection, magnetic nanoparticles, magnetic immunoassay

Introduction

For selective detection and quantification of biomolecular targets, we use magnetic label-based immunoassays employing the highly specific interaction between antigens and antibodies bound to magnetic nanoparticle (MNP) markers, which are measured by frequency mixing magnetic detection (FMMD) [1].

Results and Discussion

Superparamagnetic MNP exhibit a nonlinear magnetization. Upon magnetic excitation at two distinct frequencies f_1 and f_2 incident on the sample, the nonlinearity gives rise to the generation of intermodulation frequencies. Coaxial coils provide magnetic excitation at, e.g., f_1 = 40.5 kHz while the MNP are driven near magnetic saturation with a driving frequency f_2 = 63 Hz incident on the sample (see Fig. 1a). Response signals generated at linear combination frequencies $f_1 \pm n{\cdot}f_2$ are picked up by a detection coil, differentially connected to a reference coil, and are demodulated in the magnetic reader device (Fig. 1b). The appearance of the mixing components is highly specific to the nonlinearity of the magnetization curve of the particles. Preferably, the response signal at frequency $f_1 + 2{\cdot}f_2$ is detected. The method yields a very large dynamic range of detection, extending to more than 4 orders of magnitude in the number of particles. Analysis of the phase of the response gives information on the magnetic relaxation of the MNP, and thus on their hydrodynamic size and binding state. Variation of excitation amplitudes or a static magnetic offset field enables determining the size distribution of the magnetic cores of the MNP.

With the FMMD technique, magnetic sandwich immunoassays for various biomolecular targets have been successfully realized. The sample liquid is washed through a Polyethylene (PE) filter by gravity flow, where the target entities are sandwiched between immobilized primary antibodies (Ab) and secondary Ab binding to the MNP markers (Fig. 1c). Examples include detection of Cholera toxin B in water [2], antibiotics like penicillin and kanamycin in milk, and SARS-CoV-2-specific antibodies in blood [3].

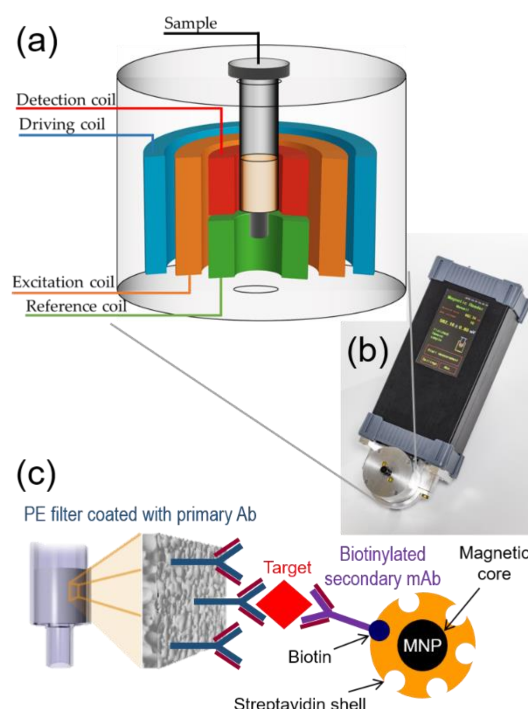

Figure 1: *(a) Schematic of the measurement head of the (b) Magnetic Reader. (c) The sample is washed through a PE filter coated with primary antibodies. MNP decorated with secondary antibodies binding to the targets serve as markers, they are detected with FMMD.*

Conclusions

The magnetic immunoassays exhibited improved detection limits as compared to conventional techniques like Enzyme linked immuno sorbent assays (ELISA).

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Acknowledgements

Support by German BMBF (project no. 13N15253), BMEL (2818710C19 and 281C406C21), EFRE-NRW (0801299) and DFG (KR3864/8-1) is gratefully acknowledged.

Sensing and stimulation at small peripheral nerves using 4D printed cuff electrodes

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Abstract

We report a new concept for nerve cuff electrodes that simplify the implantation process on nerves as small as 100 µm. Cuff electrodes are a type of peripheral nerve interface used for neural stimulation or recording. Applications include the treatment of chronic pain, sleep apnea or high blood pressure. Most existing methods for securing cuff electrodes on the nerve rely on surgical threading, zip tie-like closing mechanisms, or prefolded spiral cuff geometries. These approaches work well for large nerves in the mm range, which contain many fibers branching out to different target regions. A more selective recording and stimulation interface could be realized by targeting smaller nerves with diameters in the range of 100 µm or below. However, typical cuff electrode systems are challenging to interface with such nerves due to their fragile nature and the difficulties associated with handling small probes.

Our approach utilizes 3D and 4D printing techniques, along with flexible and superabsorbent materials, which conform to the nerve's shape upon contact with body fluid. This allows the electrodes to wrap around the nerve without the need for manual deformation, reducing the risk of nerve damage. We have successfully implanted these devices in a locust and demonstrated their effectiveness for both, recording and stimulating neural activity and associated muscle activation. A geometric design with electrodes distributed along the axial as well as the circumferential directions further increase the possibility for spatially selective sensing and stimulation.

Catalytic micromotors in action for (bio)sensing applications

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Keywords: micromotors, diagnostics, non-invasive, motion

Introduction

Micromotors represent one of the most exciting horizons in micro and nanotechnologies. The utilization of self-propelled micromotors in (bio)chemical assays has led to a fundamentally new approach where their continuous movement around the sample and the mixing associated effect, all this as a collective behavior, greatly enhances the target-receptor interactions and hence the performance of the assay [1-3].

Results and Discussion

In this Keynote, formally speaking, the analytical possibilities of micro motors for on-the-fly (bio)sensing will be discussed in clinical diagnostics and food safety environments. Micromotors are constituted by a few microscale layers that confer them self-propulsion (catalytic layer, e.g., platinum nanoparticles, PtNPs), (bio)functionalization capabilities (sensing layer, e.g., graphene oxide, rGO), and magnetic guidance (magnetic layer, e.g., Ni). In addition to integrating nanomaterials, micromotors technology can also incorporate molecular recognition-based functionalization (e.g., aptamers) and they are highly compatible with electrochemical and optical detection approaches, and even with microfluidics. As a representative example, **Figure 1** illustrates a schematic of magneto-catalytic rGO-based micromotors for simultaneous aptassay of mycotoxins using fluorescence detection. Point-of-care technology can also benefit from the inherent advantages of micromotors, so they are an attractive alternative to perform fast, sensitive, and reliable diagnostic testing, even when an extremely low volume of samples is available.

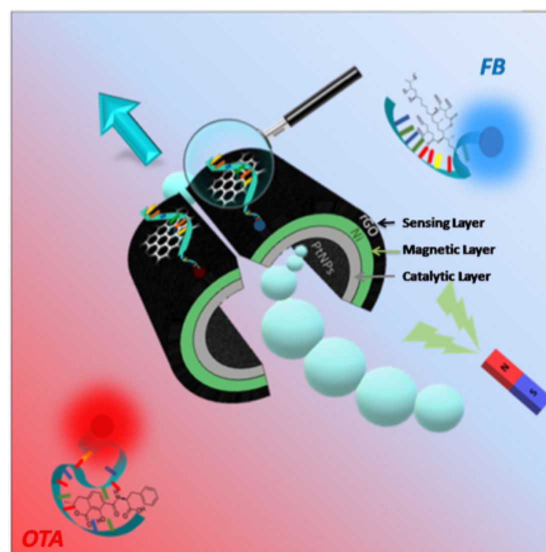

Figure 1: *Magneto-catalytic rGO-based micromotors for fluorescence apt-assay of mycotoxins*

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The financial support of grant PID2020-118154GB-I00 funded by MCIN/AEI/ 10.13039/501100011033 and the Community of Madrid, grant number S2018/NMT-4349 (TRANSNANOAVANSENS) are gratefully acknowledged.

Plant virus-based enzyme nanocarriers for robust biosensor layouts

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Soft-matter bionanoparticles of plant viral origin have emerged as advantageous scaffolds for immobilizing biomolecules at high surface densities on their outer protein shells, and are applied as adapter coatings for the display of sensor enzymes for colorimetric read-out in microtiter plates, or for direct electrochemical analyte detection, e.g., in enzyme-based field-effect devices (EnFEDs). In both layouts, engineered tobacco mosaic virus (TMV) nanorods were able to improve the characteristics of glucose, penicillin and diacetyl/acetoin sensors considerably. Every TMV particle of 300 nm length and 18 nm diameter offers more than 2,100 selectively addressable coupling sites with regular ≈3 nm spacing, which can be equipped with flexible biotin-terminated PEG linkers/spacers and bioaffinity-coupled enzyme-streptavidin conjugates efficiently. Such TMV support can stabilize the bioreceptor layer over many repeated uses, in the case of penicillin EnFEDs for at least a year with near-constant performance. TMV-assisted sensors have also outcompeted convential configurations with regard to sensitivity, noise, response time and linear detection range, as demonstrated in collaborative studies. Recent work on standardized sensor fabrication has revealed the beneficial effect of a polyelectrolyte 'glue' applied for the layer-by-layer deposition of stacked polymer-TMV-enzyme sheaths.

Plant viral adapter scaffolds seem highly promising also for other biomolecule-assisted detection layouts, aiming at long-term stable, easily calibratable point-of-care virus sensors and antibody-mediated target capture, as currently under investigation by use of an immunoglobulin-binding protein A-fashioned virus variant. Covalent, selective enzyme immobilization directly from bacterial raw homogenates has been realized with coenzyme A-coated TMV, through phosphopantetheinyl transferase Sfp-mediated protein ligation. For future sensor formats including single particle-based reporters, kinked and branched as well as length-adapted nanorods are accessible through RNA-guided *in vitro* self-assembly of TMV coat proteins (CPs). Distinct CP types combined in single TLPs allow installing cooperating molecules or ensembles for multitasking, either in blends or on longitudinal rod domains. These versatile opportunities point at many potential uses of TMV-based hybrid structures in novel 'smart' sensors and in fundamental research on collaborating biomolecules, due to the high availability, easy handling and long-time durability of plant viral building blocks.

Major co-authors/collaborators:
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Electrolyte-gated transistors based on ambipolar reduced graphene oxide: the mechanism of transduction of biorecognition events

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Abstract: Electrolyte-gated transistors EGTs based on reduced graphene oxide rGO were demonstrated as ultra-sensitive and highly specific biosensors and immunosensors. We focus on anti-drug antibodies (ADAs) towards Infliximab (IFX), a drug used to treat rheumatoid arthritis and other auto-immune diseases. The drug IFX is bound to the gate electrode as the specific probe. Our rGO-EGTs exhibit low voltage operations (≤ 0.3 V), a robust response within 15 min, and ultra-high sensitivity (10 aM limit of detection). By a multiparametric analysis of the whole rGO-EGT transfer curves we quantify ADAs also in the co-presence of its antagonist tumour necrosis factor alpha (TNF-a), the natural circulating target of IFX.
Then we explain how biorecognition at the gate electrode affects the peculiar physical properties of rGO and how then is transduced into large current variation. From the analysis of the electrochemical potential profile across the rGO-EGT, we derive the equation describing the rGO-EGT transfer curve as a function of the analyte concentration. The multiparametric rGO-EGT ambipolar response is analysed to show that the effects of recognition events affect the charge neutrality point and interfacial capacitance, whereas the curvature of the transfer curve and charge carrier mobility of holes and electrons in the rGO channel remain unaffected.

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This work was funded by ERA-NET EuroNanoMedIII project AMI. Co-financing of European Union - FSE-REACT-EU, PON research and Innovation 2014-2020 DM1062/2021 is also acknowledged.

Tackling novel challenges in molecular interaction analysis –

from small molecules to cells

Ulrich Rant

Dynamic Biosensors GmbH, Munich, DE

The quest for better drugs necessitates improved analytical methods to characterize molecular interactions of highly engineered binders and complex molecular targets.

I will present our recent efforts to develop novel methods for (i) the analysis of ternary interactions involving bifunctional molecules, such as bispecific antibodies and PROTACs, and (ii) the analysis of binding kinetics on living cells in microfluidic traps, i.e., real-time interaction cytometry (RT-IC).

Nanostructured Probes for Early Detection of Cancer

Author

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ABSTRACT

Modern molecular science is merging our knowledge of biochemistry, molecular biology, physics and artificial intelligence so we can come up with materials that have a potential to take us into the era of precision medicine. Particular advances have been made in the design of bio-nano hybrids, which combine the biomolecules and man-made nanostructures and overcome intrinsic differences between individual elements. [1]

In this talk, emphasis will be put on development of hybrid materials that can aid early detection of cancer and, in such way, enable timely treatment and increase the success of existing therapeutic protocols. In particular, development of *in vivo* probes for ageing (senescent) cells and biosensors for early detection of hard-to-treat cancers [2] will be discussed, and some views on what the future holds for the field of nanomaterials in biosensing given.

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Abstracts - oral contributions

Development of a Potentiometric ATP Biosensor Based on *E. coli* F_1F_o ATPase Activity Reconstituted on Gold Electrodes

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Keywords: ATP-synthase, Biomimetic membrane, Potentiometric determination, Microbial detection.

Introduction

Modification of electrode surfaces with supported phospholipid bilayers as biomimetic membranes allows membrane-bound enzymes to be arranged in a controlled manner for electrochemical applications. One of the main applications is the development of highly specific and robust biosensors.

Adenosine triphosphate (ATP) is a good indicator of cellular metabolism. Therefore, its reliable detection is desirable to help monitor the presence of pathological events [1] or to correlate it with microbial contamination on surfaces, which is very important in the food industry and in healthcare [2, 3]. With this in mind, it is of interest to develop biosensors for the sensitive, direct and reliable detection of ATP.

Results and Discussion

An electrochemical ATP biosensor has been developed based on the reconstitution of a membrane enzyme, ATPase F_1-F_o from *Escherichia coli*, in a biomimetic membrane on a gold electrode, whose surface is modified with a monolayer of 4-aminothiophenol (4-APh) that acts as a pH-dependent redox probe.

The biosensor works by correlating two variables: the potentiometric signal of 4-APh and the ATP concentration. The ATPase activity hydrolyzes the ATP present in the medium and acidifies the electrode/bilayer interphase with the translocated protons, producing a displacement of the electrochemical signal of 4-APh, which depends on pH.

The potentiometric biosensor can provide a quantified value of ATP concentration within 5–10 min ranging from 1 μM to 1 mM ATP.

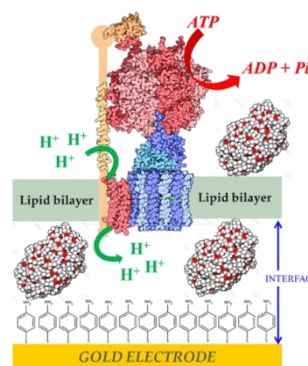

Figure 1: *Schematic representation of the ATP potentiometric biosensor system.*

Conclusions

This broad range ATP biosensor can offer an alternative way of measuring in few minutes the existence of microbial contamination without needing big expensive laboratory facilities.

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Acknowledgements

CTQ2015-71290-R Project and BES-2016-078815 Contract

Detection of circulating tumour DNA in real samples with dual-functional polymer on plasmonic biosensor

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Keywords: surface plasmon resonance, cancer diagnosis, plasmonics, antifouling polymer

Introduction

Standard protocols for circulating tumor DNA (ctDNA) analysis include complex sample handling and time-consuming procedures, which represent critical issues in pre-analytical steps **[1]**. A nanoparticle-enhanced surface plasmon resonance imaging-based assay to simplify the direct detection of tumor DNA in the patient's plasma has been recently developed **[2]**. To further streamline the workflow analysis, we herein propose a new dual-functional low-fouling poly-L-lysine (PLL)-based polymer with nanoparticle-enhanced surface plasmon resonance biosensing for the detection of ctDNA point mutation in colorectal cancer **[3]**.

Results and Discussion

The PLL-based polymer contains densely immobilized anionic oligopeptide side-chains to create a charge-balanced layer for non-specific adsorption of undesired biomolecules. At the same time, sparsely attached peptide nucleic acid probes capture complementary DNA sequences directly in human plasma. First, we thoroughly explored the role of each component of the dual-functional polymer to the antifouling capability. Then, the low-fouling activity of the new surface layer assured to detect KRAS p.G12D mutated DNA in human plasma at the attomolar level (~ 2.5 aM), and KRAS p.G13D mutated DNA in a liquid biopsy from a colorectal cancer patient.

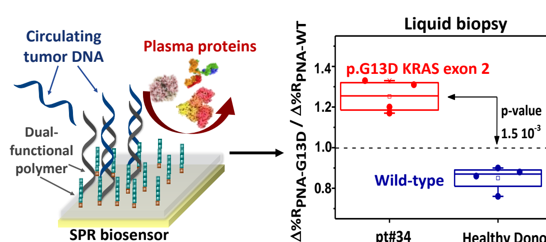

Figure 1: *Schematic representation and analytical data of ctDNA detection with dual-functional polymer on plasmonic biosensor.*

Conclusions

The work here described offers a rapid, simple, and label-free ultrasensitive detection of tumor-derived materials circulating in biological fluids with minimal pre-analytical sample treatments, making a significant improvement for early clinical diagnosis.

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Acknowledgments to Research and innovation programme 2014-2020 "PON REACT-EU project" and Horizon 2020 ULTRAPLACAD project (grant agreement no. 633937).

Solid-contact ion-selective electrodes based on redox-functionalized hydrophobic polymers for calibration-free sensing

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Keywords: ion-selective electrodes, calibration-free sensors, wearable sensors

Introduction

Ion-selective electrodes (ISEs) are indispensable analytical tools for measuring and monitoring ion concentrations in clinical and environmental applications. [1] Direct measurements in highly complex matrices such as whole blood are already routine. However, the need for reliable single-use, wearable, and field-deployed sensors with increased demands of low maintenance and calibration-free autonomous use has raised new challenges. In this respect, solid-contact ion-selective electrodes (SCISEs) benefiting from a robust miniaturizable construction offer clear advantages. [2] However, they still lag in fulfilling the elevated demands of potential (E^0) reproducibility and stability required for the cost-effective commercialization of ready-to-use non-invasive wearable ion sensors for medical and sports applications.

Results and Discussion

Potential adjustment by polarization before membrane deposition is a generic way to improve the potential reproducibility and stability [3], however, choosing the appropriate polarization potential is far from obvious. Here, we introduce the application of the galvanostatic intermittent titration technique (GITT) to find the best polarization potential [4]. We demonstrate the efficiency of the GITT through a novel solid-contact material, based on a conjugated redox polymer. To ensure proper hydrophobicity and precisely adjustable potential, we copolymerized 3,4-ethylenedioxythiophene with a perfluorinated alkyl side chain (EDOTF) and (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO) modified EDOT. We found that GITT could conveniently identify the polarization potentials to minimize the potential drift during operation (Fig. 1). We tested the utility of this method by measuring clinically relevant ions in undiluted blood serum. The potential during these measurements was the most reproducible and stable if the potential of the solid-contact was preadjusted to the value identified by the GITT.

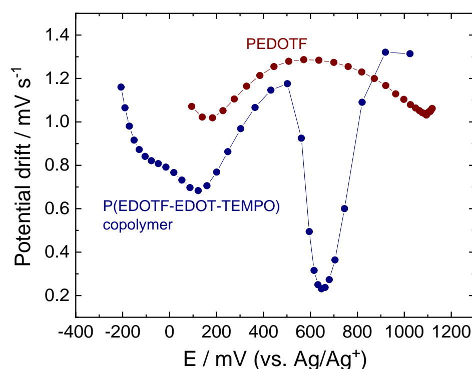

Figure 1: *Potential drift of the P(EDOTF – EDOT-TEMPO) copolymer and PEDOTF polymer calculated from GITT measurements. The GITT measurements were performed with glassy carbon electrodes coated with the respective polymer films in 0.1 M $LiClO_4$ acetonitrile solution. 32 successive cycles of polarization (1 μA for 60 s) and open circuit relaxation (300 s) were performed.*

Conclusions

We found that P(EDOTF – EDOT-TEMPO) polymer that combines the benefits of high hydrophobicity and a well-defined redox behavior results in SCISEs with excellent potential stability and reproducibility. The galvanostatic intermittent titration technique offers an unprecedently versatile mean to identify the polarization potential window of such a complex material that ensures the smallest potential drift (Fig. 1).

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Acknowledgments

This research was funded by the National Research, Development, and Innovation Fund of Hungary under Grant TKP2021-EGA-02 and NVA-02.

Label-free surface-enhanced Raman scattering for Interleukin 6 detection

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Keywords: SERS, Interleukin 6, Gold nanoparticles, Interaction, Label-free sensing

Introduction

Surface-enhanced Raman scattering (SERS) has become a widely used sensing technique in recent years, with different sensing/biosensing applications due to its high sensitivity and selectivity, easy operation, real-time detection, non-destructive and label-free analytical nature [1]. In order to the quantification and structural characterization of proteins SERS spectroscopy is considered as an excellent choice. However, direct detection of proteins by the SERS technique is very difficult due to their low Raman scattering cross section and lack the presence of the chromophore moiety in the molecular structure to generate a strong SERS signal [2]. Combination of highly specific biomolecules (i.e. antibodies and aptamers) with SERS in order to indirect sensing, can extremely improve the utilization of SERS technique in quantification and characterization of proteins [3].

Results and Discussion

In this study, an indirect label-free immunoassay was developed for highly sensitive detection of interleukin 6 (IL-6). Indirect sensing with application of antibody against IL-6 could overcome the intrinsic shortcoming of non-specificity detection of SERS. The basic principle of label-free IL-6 detection using antibody itself as the intrinsic Raman reporter is as follows: interaction of target IL-6 with antibody induces conformational changes in antibody 3D structure and in the molecular geometry around S-S bonds near the surface of SERS substrate. These structural modifications lead to the corresponding change of the output Raman signals.

In the design of SERS immunoassay, the specific antibody against IL-6 was immobilized on the surface of spherical gold nanoparticles (AuNPs, 50 nm), as sensitive SERS substrate, using electrostatic interaction. When the target IL-6 was added into the AuNPs-antibody (Ab) conjugate solution, it bound to antibody to form the antibody-antigen complex. Antibody-antigen interaction was evaluated using SERS and the recognition of IL-6 was successfully realized based on the fingerprint signal spectra of antibody and vibrational information from disulfide bonds ($\nu_{S\text{-}S}$) in antibody structure. In fact, antigen binding resulted in the increase in disulfide bond signal at ~490 cm^{-1}. Therefore, the IL-6 could be detected by analyzing the SERS intensities coming from the S-S stretching band at ~490 cm^{-1} in the antibody. The limit of detection (LOD) obtained in this study was as low as 45 fM.

Conclusions

The results obtained in this study indicated that interaction between antibody and IL-6 was not able to modify the secondary structure of antibody. However, it changed the molecular geometry around disulfide bonds by inducing a general modification of the antibody molecule structure. Label-free SERS is very simple and highly sensitive for the detection of protein-based analytes.

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Acknowledgements

This work is supported by funding from the European Union's Horizon 2020 research and innovation FET Open programme under grant agreement No 964248 (DeDNAed).

Impact of enzyme coverage on performance of a capacitive field-effect biosensor

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Keywords: penicillinase, field-effect sensing, penicillin, surface area, model

Introduction

At present, a large group of enzyme-based biosensors have been designed using field-effect pH-sensitive electrolyte-insulator-semiconductor capacitors (EISCAP) [1]. The functioning principle of these biosensors is based on the detection of hydrogen ions produced or consumed during the enzymatic reaction. Usually, it is assumed that the enzymes immobilized onto the EISCAP surface form a densely packed layer. However, detailed theoretical models for EISCAP biosensors partially covered with enzyme molecules are missing so far.

In this work, the impact of enzyme surface coverage on the EISCAP biosensor signal is theoretically and experimentally studied. Penicillinase/penicillin was used as model enzyme/substrate system.

Results and Discussion

The capacitance–voltage curve and constant–capacitance (ConCap) signal of the EISCAP biosensor was simulated as a function of the enzyme coverage (see Figure 1). Experiments were performed using a multiplexing system with a multi-cell arrangement allowing quasi-simultaneous characterization of up to sixteen EISCAP biosensors by applying one common reference electrode. The enzyme penicillinase was adsorptively immobilized onto the gate surface of the EISCAP consisting of an Al-p-Si-SiO_2-Ta_2O_5 structure. Different enzyme coverages were achieved via short-circuiting the blank EISCAPs and EISCAPs with densely packed penicillinase.

The simulations and experimental results indicate that the performance of the enzyme-based EISCAP biosensors is influenced noticeably by the surface coverage of the immobilized enzymes. As predicted by the theoretical model, the amplitude of the ConCap signal increased with increasing the enzyme coverage: for instance, at a penicillin concentration of 1 mM, an increase of the penicillinase coverage (C) from C = 0.25 to C = 1 resulted in a more than 3-fold increase of the ConCap signal. Details of the capacitive model for EISCAP biosensors with partial enzyme coverage and the obtained experimental results will be presented and discussed.

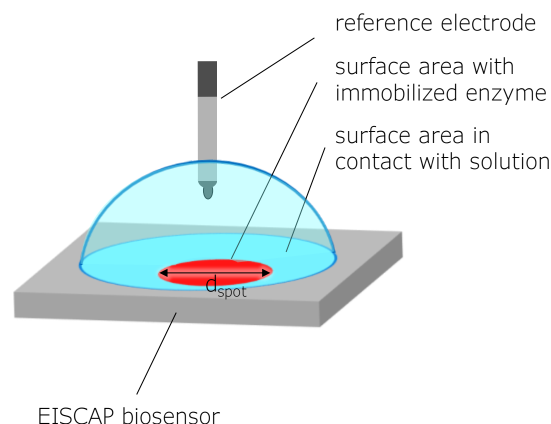

Figure 1: *Schematic setup of the EISCAP biosensor with a variable (“d_{spot}”) enzyme covered area.*

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Coupled Surface Plasmon Resonance Waveguide Based Surface Enhanced Raman Scattering

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Keywords: Waveguide, SPR, SERS, Biotin, Biosensor

Introduction

Surface Enhanced Raman Scattering (SERS) is one of the most powerful analytical methods for the rapid and non-destructive detection of biomolecules [1]. Even though the maximum enhancement of the Raman scattering of molecules is obtained by the SERS technique, one of the most important obstacles is the low reproducibility of SERS active substrates due to the random distribution of their active hotspots and lower sensing volume. The interaction of light with molecules using coupled waveguides is a complementary strategy to increase the effective volume of light-matter interaction and better reproducibility in the enhanced Raman signal [2-3]. Here we demonstrate a plasmonic waveguide-coupled surface plasmon resonance based SERS platform for the detection of biotin.

Results and Discussion

The waveguide-coupled SPR based SERS configuration constitutes a bilayer Au waveguide fabricated on a glass substrate and placed on a K9 half-cylindrical prism using refractive index matching oil (Fig. 1(a)). Detailed theoretical studies were performed by 3D finite-difference time-domain (FDTD) numerical method to optimize the waveguide structure parameters supporting strong field enhancement at the inter-layer gap and confirmed through experimental characterization studies. The Au bottom layer couples the incident light to a guided surface plasmon polariton (SPP) and causes the strong confinement of the evanescent electromagnetic field in the gap between Au layers (Fig. 1(b)). It has been observed that the bilayer Au waveguide platform exhibited a 10-fold field enhancement with respect to the single-layer Au film conventional approach. Biotin molecules were immobilized on the inner surfaces of the bilayer Au waveguide and excited with 785 nm laser at a resonance incidence angle of 55°. The enhanced evanescent field confined at the Au inter-layer space led to the excitation and SERS detection of biotin molecules with greater signal reproducibility.

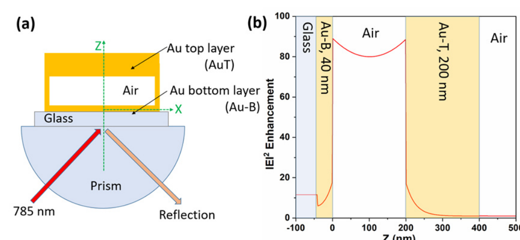

Figure 1: *(a) Sketch of the Kretschmann SPR configuration for a bilayer Au plasmonic waveguide and (b) the corresponding FDTD simulated electric field enhancement when the nanostructure was excited by 785 nm at a resonance incidence angle of 55°.*

Conclusions

A proof-of-concept Au bilayer plasmonic waveguide-based SERS biosensor has been developed and studied theoretically and experimentally. The Au waveguide showed a narrower angular SPR curve with stronger electric field enhancement compared to a single Au layer. Raman scattering was highly directional on the prism side and the SERS signal of biotin confirms the ability of the waveguide-coupled SERS biosensor to detect other biomolecules with greater signal reproducibility.

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Acknowledgements

A.K.P acknowledges the Alexander von Humboldt Foundation for the Research Fellowship.

A flexible multi-sensing platform for Chronic Wound Monitoring

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Keywords: multi-sensing platform, non-invasive, inkjet-printed device, wound monitoring

Introduction

Chronic wounds are affecting millions of people and represent a significant financial burden for patients and healthcare systems. In recent years, there has been growing interest in developing wearable flexible sensors for chronic wound monitoring to provide insights on wound status. Particularly, assessing the pressure applied to the wound can avoid ischemia and improve wound healing, while monitoring changes in pH levels provide an indication of infection. Moisture balance is critical to wound healing, so assessment of intra-dressing moisture is useful. Ascorbic acid is used in the wound treatment to promote faster healing. Because of that, monitoring the dynamic of ascorbic acid can determine the effectiveness of certain medical treatments **[1]**. In this project, our goal is to develop a low-cost and flexible multi-sensing platform for chronic wound monitoring. Four types of sensors (pressure, humidity, pH, ascorbic acid) are investigated and fabricated using inkjet-printing.

Results and Discussion

The pressure sensor has a sandwich-like structure with two parallel silver electrodes and a thin dielectric layer in-between. The sensor operates for pressures between ca. 1 Pa and 2 kPa. Its response time is 0.25 s. The sensor is included into a commercial dressing for direct measurements on the body of a volunteer. It is used to monitor how firmly a dressing is glued on the (skin) surface, the main application being the reduction of ischemia risk **[2].**

The humidity sensor was obtained by printing cellulose acetate butyrate (CAB) on silver electrodes. The humidity sensor is inserted in the dressing and the capacitance signal is recorded to follow the change of humidity with time. The sensor showed good sensitivity and good reversibility.

A flexible pH sensor was fabricated using biocompatible materials such as gold, silver, and polyaniline. These sensors are reproducible and demonstrated a sufficiently high sensitivity. During long-term continuous monitoring, the potential drift was lower than 3 mV/42h, which proved that this pH sensor is suitable for wound assessment.

A non-enzymatic ascorbic acid sensor based on the catalytic activity of gold nanoparticles (AuNPs) has been developed to monitor vitamin C levels in wound exudate and sweat. On-body evaluation of the sensor was performed on healthy consenting volunteers by placing the sensor directly on their skin. It showed good performances in real-time test, suggesting its potential on therapeutic bandages applied on chronic wounds.

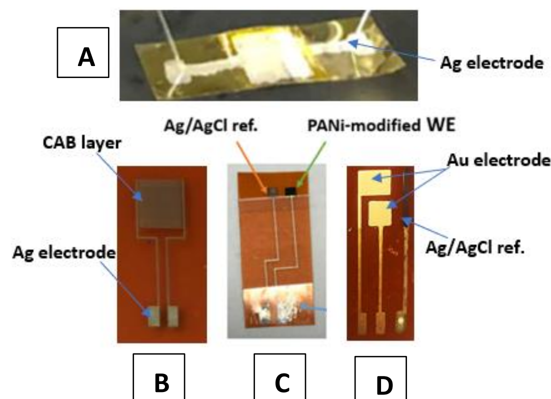

Figure 1. *(A) Pressure sensor. (B) Humidity sensor. (C) pH sensor. (D) Ascorbic acid sensor.*

Conclusions

The multisensor platform shows good performance. It is now being integrated with a microcontroller for further characterizations, including on patients.

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Acknowledgements

Nga Dau thanks the French Embassy in Hanoi, Viet Nam, for a Ph. D. scholarship.

Sensitive Label-Free Biosensing by Colors of Plasmonic Metal Nanostructure arrays

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Keywords: Biosensor, plasmonic color, metal nanostructures, immunoassay.

Introduction

Nowadays, developing portable plasmonic biosensors that can be used on-site has become an important issue. The key features of plasmonic biosensors can be designed using nanostructured metal arrays. The coloration of metal nanostructured arrays is an attractive plasmonic property for biosensor applications because colors are easily detected by a smartphone camera [1]. In this paper, we present a plasmonic biosensor that can sensitively and quantitatively detect target biomolecules from color changes of metal nanostructure arrays.

Results and Discussion

As the sensor substrate, Ag nanodome arrays consisting of polystyrene bead monolayers coated with Ag thin films are used, as shown in Fig. 1 (a). The surface colors of Ag nanodome arrays can be tuned by the diameter of the polystyrene beads. Firstly, bulk refractive index sensitivity was evaluated as the fundamental sensing performance of the colorimetric plasmonic sensor. To quantify the surface color of the Ag nanodome arrays, the hue angle in HSV color space was used. The optimal Ag nanodome arrays showing the highest bulk refractive index (RI) sensitivity exhibited purple colors at the water interface as shown in Fig. 1(a). The bulk RI sensitivity reached 590 deg/RIU (RIU: RI unit) with a refractive index resolution of 5.0 × 10^{-5} RIU. In addition, the detection of the biomolecules was demonstrated using a direct immunoassay format. To immobilize capture antibodies, the surface of the Ag nanodome arrays was functionalized with a polydopamine coating. Then rabbit IgG (rIgG) used as a target molecule was detected. A calibration curve for the detection of rIgG is shown in Figure 1(b). The limit of detection for rIgG was determined to be 134 pM [2]. Therefore, it was shown that direct detection of biomolecules at sub nM concentration was achieved by the presented biosensor scheme.

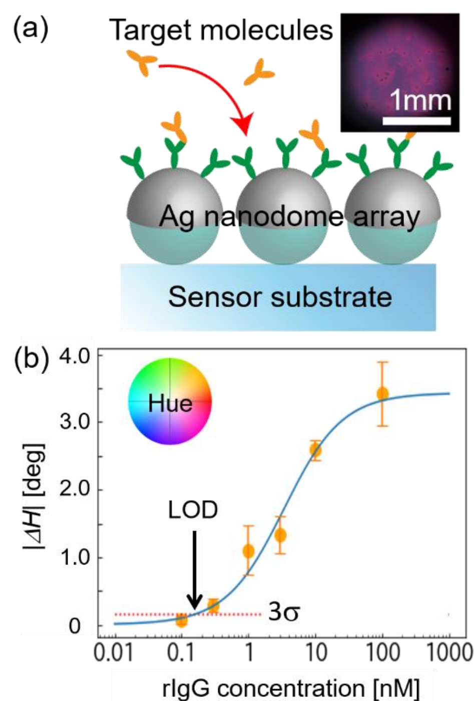

Figure 1: *(a) A Schematic drawing and a reflection image of a sensor substrate. (b) A quantitative plot of the sensor signals as a function of target molecule (rIgG) obtained by colorimetric method.*

Conclusions

In this study, a sensitive label-free biosensor was demonstrated using the unique plasmonic colors of Ag nanodome arrays. The feasibility of sensitive label-free colorimetric plasmonic biosensing will open up a way to develop highly sensitive and versatile smartphone-based biosensors.

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Acknowledgments

This work was partially supported by JSPS KAKENHI Grant Number JP 20K14748 in Grant-in-Aid for Early-Career Scientists.

An impedimetric-based biosensor for monitoring COPD and CF biomarkers in patients' exhaled breath condensate (EBC)

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Keywords: chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), exhaled breath condensate (EBC), molecularly imprinted polymer (MIP), impedimetric biosensor

Introduction

The sum of environmental influences on an individual's health from conception to death is well defined by the "exposome" concept. Chronic respiratory diseases induced by air pollution is considered the leading cause of about 7 million environment-related deaths per year. Therefore, the diagnosis of chronic obstructive pulmonary disease (COPD) and cystic fibrosis (CF) are of particular interest in human exposome research **[1]**. Literature studies indicate that the levels of 3-nitrotyrosine (3-NT), hexanal, and neutrophil elastase (NE), as oxidative stress and inflammatory biomarkers are significantly elevated in exhaled breath condensate (EBC) of COPD and CF patients **[2, 3]**.

Results and Discussion

In this research, we developed an EBC-collection unit, which collects ca. 2 ml EBC during 5 min breathing. Furthermore, molecularly imprinted polymer (MIP) layers were produced for 3-NT and hexanal using electropolymerization and sol-gel techniques, respectively. An aptamer was selected for NE. These MIPs and the aptamer were used as biorecognition elements to develop an impedimetric-based biosensor for COPD and CF. Also, a biosensing unit including an impedance analyzer was manufactured. We used home-designed gold-coated multi-electrode glass sensors. Each sensor includes 4 circular sensing spots (two electrodes per spot with equal surface area), see figure 1. Each spot was coated either with a non-imprinted polymer, or a MIP or aptamer, alternatively.

Currently, the sensitivity of the biorecognition layers is under study, and calibration measurements are being performed using different concentrations of the target biomarkers (10 pM up to 1 µM) in 0.5× PBS. Furthermore, the layer characteristics are studied by Atomic Force Microscopy (AFM).

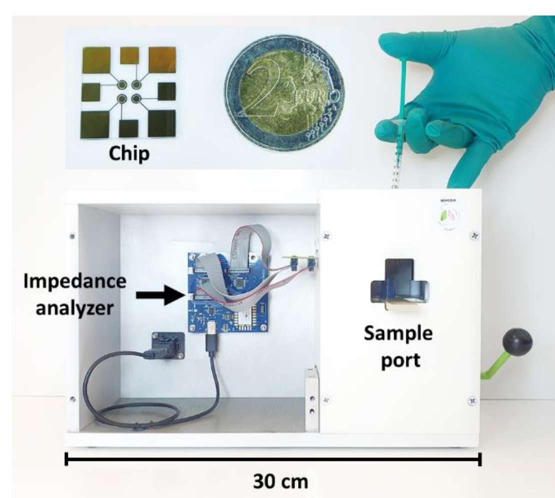

Figure 1: *The REMEDIA chip and biosensing unit. The biosensing unit includes a PalmSens EmStat Pico MUX16 impedance analyzer equipped with a 16-channel multiplexer, which is used for parallel measurements of the 8-electrode gold-coated glass sensors.*

Conclusions

In this project, we aim to develop a biosensor to monitor COPD and CF condition by detecting specific biomarkers in patients' EBC.

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Acknowledgements

The financial support by the European H2020 project REMEDIA (grant no. 874753) is gratefully acknowledged.

Optoelectrical Carbon Devices as Enabling Tools for Neuroscience

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Keywords: optoelectrical waveguides, brain probes, pyrolytic carbon, neural stem cells, optogenetics

Introduction

This talk will focus on our recent work on developing different kinds of pyrolytic carbon-based leaky optoelectrical probes (fibers (LOEF) and planar probes (LOEPs))(Fig 1A-C) for potential use as part of Bioelectronic Brain Implants for cell replacement therapy and treatment of neurodegenerative disorders. Our focus has been on Parkinson´s disease (PD), which is a disease characterized by insufficient delivery of the neurotransmitter dopamine (DA) to the region of the brain called striatum, resulting in the motor dysfunction of PD patients. We combine light controlled release of DA from optogenetically modified human stem cell-derived neurons and subsequent amperometric detection of the released dopamine. We also present a new method to create 3D pyrolytic carbon scaffolds using embedded 3D printing (Fig 1D).

Results and Discussion

We have previously reported that pyrolytic 3D carbon micropillars promote differentiation of human neural stem cells into dopaminergic neurons and that these carbon pillars simultaneously can function as sensors for detecting DA release from the neurons attached to them [1,2]. This is the basis for the present work, where we demonstrate that pyrolytic carbon based optoelectrical waveguides have multiple functions, acting as: a) a scaffold for growth and differentiation of the optogenetically modified neural stem cells, b) a waveguide for light stimulation of the stem cell-derived neurons cultured on the surface of the probes, and c) a sensor for electrochemical detection of synaptic released dopamine [3,4]. Our latest work involves using omnidirectional embedded printing of the photoresist SU-8 in an oil-silica nanoparticle bath which after pyrolysis leads to 3D carbon electrode scaffolds with graphene-type microflowers on its surface.

Conclusions

The developed leaky optoelectrical waveguides have great potential for use as part of future BBI for the treatment of PD, combining delivery of healthy dopaminergic neurons and monitoring and control of the released dopamine in striatum where it is needed.

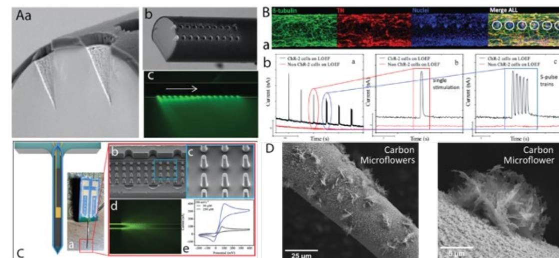

Fig. 1. Leaky optoelectrical probes: *Top panel: **A)** PoC of leaky optoelectrical fibre (LOEF) with (a) micro-optical windows (a) with light leakage through μOWs (b) populated with **B**) optogentically modified (ChR2) human stem cell-derived dopamine (DA) neurons (immunostaining of dopaminergic marker TH) (a) for optical stimulation and registration of released DA (b). Bottom panel. C) PoC planar leaky optoelectrical probe (LOEP) design with leaky optical waveguide (LOW) surrounding a central carbon pillar area (bc) and Au pseudoreference- and counter electrode, light leakage from LOW (d) and electrochemical function of the carbon pillars (e). D) Pyrolytic carbon structures with surface microflowers derived from embedded 3D printing of SU8 in an oil-silica nanoparticle bath.*

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The authors kindly acknowledge funding from the European Commission through the MSCA-ITN Training4CRM project (GA 676408) and the EIC Pathfinder Open project OpenMIND (GA 101047177).

Temperature-Enhanced *mcr-1* Colistin Resistance Gene Detection with Electrochemical Impedance Spectroscopy Biosensors

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Keywords: antimicrobial resistance diagnosis, EIS biosensor, hybridisation kinetics

Introduction

Antibiotic resistance is one of the biggest threats to public health and modern health care [1]. Especially, the growing resistance rates of Gram-negative bacteria causes increasing concerns. The occurrence of the easily transferable, plasmid-encoded *mcr-1* colistin resistance gene further worsened the situation significantly enhancing the risk of the occurrence of pan-resistant bacteria. There is therefore a strong demand for new rapid molecular diagnostic tests for the detection of *mcr-1* gene associated colistin resistance.

Results and Discussion

Electrochemical impedance spectroscopy (EIS) is a well-suited method for rapid antimicrobial resistance detection as it enables rapid, label-free target detection in a cost efficient manner [2, 3]. Here, we describe the development of an EIS-based *mcr-1* gene detection test, including the design of *mcr-1* specific peptide nucleic acid probes and assay specificity optimisation through temperature-controlled real-time kinetic EIS measurements.

A new flow cell measurement set-up enabled for the first time detailed real-time, kinetic temperature-controlled hybridisation and de-hybridisation studies of EIS-based nucleic acid biosensors. The temperature-controlled EIS set-up allowed single nucleotide polymorphism (SNP) discrimination. Target hybridisation at 60 °C enhanced the perfect match/mismatch (PM/MM) discrimination ratio from 2.1 at room temperature to 3.4. A hybridisation and washing temperature of 55 °C further increased the PM/MM discrimination ratio to 5.7 by diminishing the mismatch signal during the wash step while keeping the perfect match signal. This newly developed mcr-1 gene detection test enabled the direct, specific label and amplification-free detection of *mcr-1* gene harbouring plasmids from *Escherichia coli.*

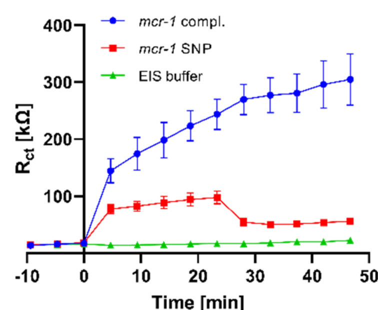

Figure 1: *Hybridization and dehybridization at 55 °C. Average charge transfer resistance (R_{CT}) values before (time interval: –10 to 0 min) and after (time point 0) addition of 1 μM mcr-1 fully complementary, SNP target, and EIS buffer alone, respectively, at 55 °C followed by replacement of the target solution with EIS buffer at time point 25 min and continued EIS measurements; n = 4.*

Conclusions

This is to the best of our knowledge the first example of a temperature controlled kinetic EIS measurement. Optimal hybridisation and washing temperatures were determined to enhance the specificity of the newly designed *mcr-1* PNA probe. This is an important step towards the development of an EIS-based rapid, point-of-care molecular diagnostic test for AMR diagnosis to optimise patient treatment and reduce the mis-use of antibiotics.

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Miniaturized, Label-Free Surface Nanosensing, based on Whispering Gallery Modes Emerging from Fluorescent Microbeads

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Keywords: whispering gallery modes, fluorescence, microspheres, microfluidic

Introduction

The increasing need for miniaturization of analytical processes requires new sensory methods. Especially interactions on surfaces, such as adsorption of (bio-)molecules, which occur in native form without labelling, are in demand. Optical label-free methods for detecting surface processes have been developed previously, e.g. surface plasmon resonance (SPR) or bio-layer interferometry (BLI). In these systems, every adsorbed molecule interacts only one time with the light wave. Thus, many molecules are required to achieve sufficient sensitivity. Furthermore, the miniaturization potential of these methods and thus, their use in microfluidic channels or small three-dimensional cell arrays is limited. Whispering gallery modes (WGM) in microresonators overcome these boundaries [1]. In these resonators, discrete wavelengths are reflected many times. The multiplication of the interacting space with the analyte generates an amplified response. Circulation numbers (Q factors) up to 10^5 are achieved in circular resonators.

Results and Discussion

We use plain microspheres as resonators (Fig. 1) [2] that are easy to introduce into small compartments.

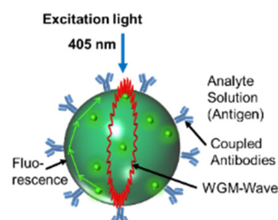

Figure 1: *WGM sensor principle using a fluorescent microsphere as resonator.*

The spheres are fluorescent and the WGMs generated inside the spheres appear as regular peaks in the emission spectra. Adsorption of molecules changes refractive index and particle diameter, resulting in wavelength-shifts of the WGM peaks (Fig. 2). 10 µm beads with Q = 10^4 achieve the same sensitivities as SPR or BLI.

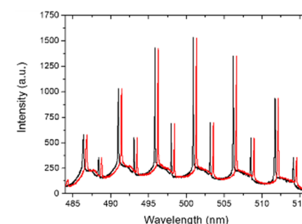

Figure 2: *Emission before (black) and after adsorption of molecules (blue).*

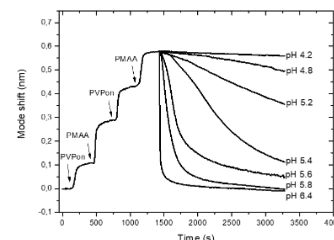

Figure 3: *Stepwise adsorption of nanometer multilayers and subsequent dissolution.*

We present applications such as monitoring coating and release of drugs from nanometer scale layer-by-layer films (Fig. 3) as well as protein binding kinetics [3, 4]. Furthermore, we shall introduce our hardware unit for measuring WGM shifts and its miniaturization with regard to future mobile unit for field use.

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Acknowledgements

The authors acknowledge support from the German Federal Ministry for Economic Affairs (BMWi), Zentrales Innovationsprogramm Mittelstand (ZIM) “WhisperCal”, Grant No. KK5056701DF0.

Dual-functional biomimetic organoid chip of real-time imaging and synchronous electrical impedance monitoring

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Keywords: biomimetic organoid chip, real-time imaging, electrical impedance monitoring, *in situ* organoid identification, olfactory organoids

Introduction

Organoids are micro-organs with 3D structure cultivated from stem cells, which has been a promising tool for biological research[1]. Identification and monitoring are important to evaluate the grow status and function of organoids. The current monitoring methods mainly focus on single mode, and cannot reflect long-term changes in real-time[2]. As organoid technology is becoming mature, biomimetic organoid chips open up new frontiers for the multifunctional platform establishment[3]. Here, a dual-functional biomimetic organoid chip was established for real-time living imaging and synchronous electrical impedance monitoring, ensuring *in situ* three-dimensional organoid growth.

Results and Discussion

In this research, we cultured olfactory organoids from C57BL/6 mice (***Figure 1a***). Then the mixture of organoids-Matrigel was re-transferred to dual-functional chips. We evaluated the growing status of organoids by dual-function detection platform, which could provide high-throughput information of impedance changes and correspondent live cell imaging. By using this, the process of gradual division and differentiation of olfactory organoids from stem cells to organoids can be continuously monitored. For impedance detection, Matrigel used in thin-layer 3D culture is non-conductive, which has a relatively maximum impedance. When the organoid-Matrigel mixture was cultured, with the growth of organoids, the blocking degree of the ionic current is significantly less, which reflects the decrease in the overall impedance of organoids-Matrigel mixture (***Figure 1b***). We found that under normal culture conditions (Organoid-N group), small organoids began to appear on the fifth day and then grew rapidly, showing a compact shape and rapid increase in size. But under abnormal culture conditions lacking necessary growth factors (Organoid-U group), no small organoids appeared (***Figure 1c***). Impedance detection results also confirmed that the organoids in Organoid-N group grew well, which was reflected by the magnitude of the impedance decrease was significantly greater (***Figure 1d***).

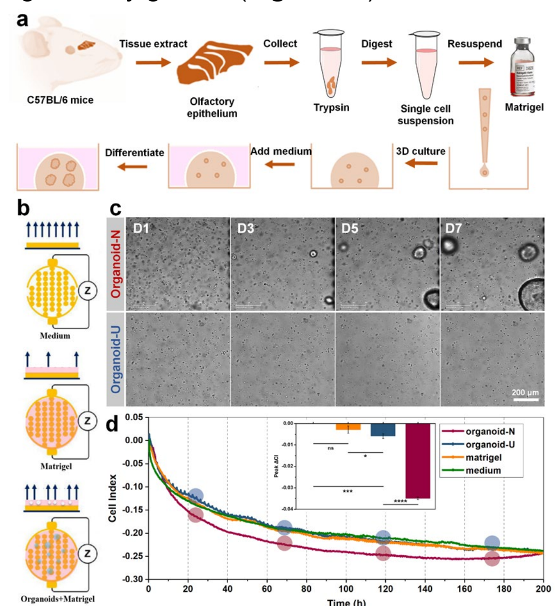

Figure 1: *(a) The process of olfactory organoids culture. (b) The electrical impedance detection principle of organoids. (c) The real-time image and (d) impedance change of olfactory organoids growth.*

Conclusions

The dual-functional biomimetic organoid chip can be applied to other types of organoids and is thus highly promising as a tool for organoid-based drug screening, toxicity assessment, and therapeutics.

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Acknowledgements

This work was supported by National Key Research and Development Program of China (No. 2021YFF1200803, 2021YFB3200801), National Natural Science Foundation of China (No. 62120106004).

InGaN/GaN Nanowires as Selective Biosensors with Dual Optical Readout

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Keywords: optical biosensors, nanowires, bias-control, enhanced selectivity

Introduction

Due to their specific electrochemical properties, group III-nitrides (III-N) have been shown to provide an excellent material platform for the application in electrochemical and biochemical sensors [1,2]. Recently, we have demonstrated that III-N nanowires (NWs) present pH-sensitive nanophotonic probes with the photoluminescence (PL) intensity responding to the proton concentration in aqueous solutions [3] and that the anodic photocurrent (PC) of InGaN NW arrays can serve as a sensitive probe for the presence of biomolecules [4].

Results and Discussion

In this contribution we demonstrate that simultaneous dual readout of InGaN/GaN NW arrays by PL and PC at different bias voltages provides optical biosensors with enhanced selectivity. We demonstrate the detection of hydrogen peroxide (H_2O_2), ascorbic acid (AA) and uric acid (UA) using both detection principles and discuss the main detection mechanisms of the employed nanostructures.

In particular, we show that the bias-dependent patterns of the PL and PC response represent fingerprints of the specific molecule and, even more important, that the operation at specific electrochemical potentials allows to unambiguously identify the presence of one of the types of molecules in a mixture of the three components. Hence, such optical probes with bias-dependent dual readout present a possible route towards label-free detection with enhanced selectivity.

Conclusions

InGaN/GaN NWs allow the optical detection of oxidation and reduction processes by simultaneous analysis of PC and PL intensity. The adjustment of a well-defined electrochemical working reveals specific sensitivity patterns for different molecules, thus allowing the selective detection of individual molecules in the presence of other species.

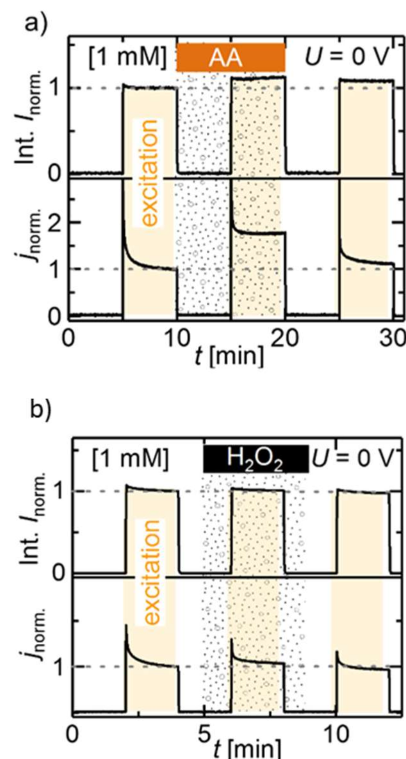

Figure 1: *Transient integrated PL intensity and current measurement normalized to the last value of the first excitation sequence in presence of 1 mM of (a) AA, (b) H_2O_2 at $U = 0$ V and pH = 7. The presence of AA, results in an increase of the PC and the PL intensity, which is not strongly altered for H_2O_2.*

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Acknowledgements

Financial support from the Deutsche Forschungsgemeinschaft (DFG 518/14-1) is gratefullly acknowledged.

A zinc oxide nanorod-based biosensor for the detection of tumor markers in saliva

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Keywords: OSCC, ZnO-NRs, CEA, saliva, biosensor

Introduction

Biosensors have emerged as a promising tool for early detection of oral squamous cell carcinoma (OSCC) due to their rapid, sensitive, and specific detection of cancer biomarkers[1]. Saliva is a non-invasive and easy-to-obtain biofluid that contains various biomarkers of OSCC, including Carcinoembryonic Antigen (CEA)[2]. Electrochemical and optical biosensors have been investigated for OSCC detection in saliva. However, further research is needed to optimize biosensor design and validate their clinical utility for OSCC detection.

Results and Discussion

We used the 3'-SH CEA aptamer to bind to the gold surface of the electrode through the Au-S bond, then blocked with 1 Mm MCH. Then, the biosensor was used to detect CEA at multiple concentrations by electrochemical measurement (*Figure 1A*). The electrodes we used were responsive to most of CEA concentrations in the saliva of OSCC patients in previous studies. Electrochemical impedance spectroscopy (EIS) and CV measurements were used to characterize the electrochemical biosensor. To measure EIS, $Fe(CN)_6]^{3-}$/$[Fe(CN)_6]^{4-}$ was employed as the redox probe, and these mi-circle diameter was used to determine the electron-transfer resistance. At 5 mM $Fe(CN)_6]^{3-}$/$[Fe(CN)_6]^{4-}$, bare electrodes exhibited minimal impedance, indicating a mass diffusion limited step in the electron-transfer process. Upon self-assembly of the aptamer onto the bare electrode, the R_{et} increased. This was attributed to the negatively charged phosphate backbone of the oligonucleotides producing an electrostatic repulsion force to $Fe(CN)_6]^{3-}$/$[Fe(CN)_6]^{4-}$. It was also observed obviously that the assembly of CEA on the aptamer-modified electrode resulted in a significant increase in Ret. These findings were in good agreement with those obtained from CV measurements. As figure 1 shows, both EIS and CV results demonstrate that the sensing interface was successfully fabricated.

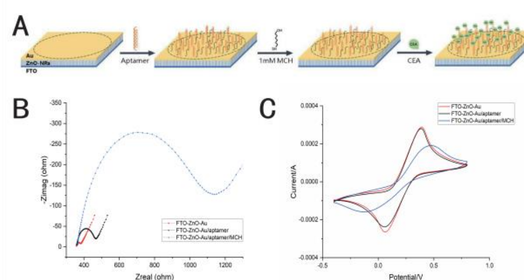

Figure 1: *Schematic diagram of the FTO-ZnO-Au electrode structure and representation of the steps of aptamer immobilization and binding to CEA (A) and EIS spectra (B), CV voltammograms (C) of immobilization steps.*

Conclusions

In this study, we selected an aptasensor with FTO-ZnO-Au structure. The sensor with this structure utilizes the three-dimensional structure constructed by ZnO-NRs to bind more aptamer molecules, and the highly specific binding of aptamers to CEA also helps to achieve a rapid and cheap detection of CEA. The biosensor realized fast detection at low cost, which provides a new method for clinical diagnosis and early screening of OSCC.

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Acknowledgements

This work was funded by the National Natural Science Foundation of China (Grant No. 32071370, and 32271427).

Raman spectroscopic sensing of the host response

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Keywords: Cell-based diagnostics, non-invasive, label-free, Raman, host response

Introduction

The host immune system is responsible for maintaining homeostasis and regulates infections caused by invading pathogens using various biochemical strategies [1]. The immune responses to infection are dominated by circulating immune cells where several effector molecules are released via the activation of various signaling pathways. Investigating the biochemical changes undertaken by the immune cells upon activation allows researchers to assess humans' responses to infectious agents in a more comprehensive way [2]. Despite the development of new clinical and laboratory tools, timely diagnosis of infection and the causative pathogen remains challenging. For this reason, new non-destructive diagnostic tools and methods are needed, in particular, for infectious disease diagnosis from biological fluids. In the last decade, label-free Raman spectroscopy is an attractive bioanalytical tool providing us with promising chemical and biological information on immune cells with a minimal labor-intensive [3]. Our research group focuses on the investigation of immune cells for diagnosis and characterization of host immune response using Raman spectroscopy [3-6].

Results and Discussion

In this study, we present the Raman spectroscopy technique as a potential diagnostic tool for the investigation of peripheral blood leukocytes. Previously we have shown the possibility to do leukocyte phenotyping using the Raman spectral signature and differentiation of leukocyte subtypes [4,5]. Further, the possibility to differentiate between fungal and bacterial infected neutrophils, monocytes, and lymphocytes has been shown [3,6]. In our recent work, Raman spectroscopy has shown promising results to identify different Raman spectral signatures of vero6 cells infected with the SARS-COV-2 virus and measles virus [7]. The study was extended to circulating blood neutrophils and it was possible to differentiate between SARS-COV-2 infected and non-infected cells.

Conclusions

Label-free Raman spectroscopy provides information on biochemical changes due to the immune response in defined in vitro infection models using leukocytes isolated directly from blood with minimum sample requirement and preparation. We present in this abstract potential of Raman spectroscopy to extract infection and inflammation-relevant information from the leukocytes. Further, the Raman spectral fingerprint of leukocytes has a high potential to influence therapeutic decisions.

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This work is supported by the BMBF, funding program Photonics Research Germany and is integrated into the Leibniz Center for Photonics in Infection Research (LPI: 13N15466, 13N15704, 13N15715). The LPI initiated by Leibniz-IPHT, Leibniz-HKI, UKJ, and FSU Jena is part of the BMBF national roadmap for research infrastructures. The author thanks the Federal Ministry of Education and Research (BMBF) for funding Project SARS-CoV-2Dx (13N15745).

Flexible electrochemical biosensors for direct monitoring of heat stress in *Nicotiana tabacum* plants

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Keywords: electrochemical biosensing, in vivo plant sensor, flexible biosensor

Introduction

In this work, flexible electrochemical biosensors are applied for in vivo stress sensing in plants for the first time. For the fast detection and long-term monitoring of heat stress, the biosensor is implanted directly into the stem of the genetically modified *Nicotiana tabacum* plant. The biosensor detects the bio-signalling enzyme β-glucuronidase (GUS), which is expressed by the plant upon triggering heat stress. Highly sensitive detection of heat stress is achieved by redox cycling with an interdigital two-working electrode setup.

Results and Discussion

Induced by heat stress, the tobacco plant produces the enzyme GUS, which cleaves 4-Nitrophenyl β-D-glucopyranoside and forms the electroactive product p-nitrophenol (PNP). PNP is detected by an electrochemical reaction on the biosensor using standard three-electrode cyclovoltammetry and redox cycling. In redox cycling, two working electrodes are individually biased at different potentials. The electrochemical signal is amplified by the cyclic movement of the electroactive species between the electrodes.[1] At the electrodes, PNP is reduced irreversibly in the first step and reacts further in a reversible redox reaction either to p-nitrosophenol or p-aminophenol.[2] The electrochemical reaction is detected by a biosensor implanted parallel to the plant stem in the vascular cambium. It consists of either a single working electrode or two working electrodes with a closely packed interdigital structure, an auxiliary electrode, and an Ag/AgCl quasi-reference electrode deposited on a thin, flexible polyimide substrate. The stem-implanted biosensor responds immediately after the heat stress events, compared to 3 days by the gold-standard method (X-gluc staining).[1] Due to its high flexibility, the biosensor adapts to the stem and is attached in a stable setup. In this setup, the heat stress of the plant was measured over a period of 12 days, which shows the high potential for long-term stress monitoring. With the two-working electrode biosensor, a five-fold signal amplification was achieved by redox cycling compared to cyclovoltammetry.

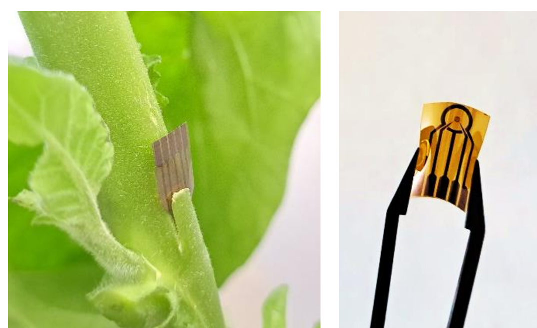

Figure 1*: Implanted flexible biosensor in the stem of a tobacco plant (left) and flexible two-working electrode biosensor (right).*

Conclusions

This work demonstrates a plant-based sensor for the detection of heat stress using flexible electrochemical biosensors for the first time. Redox cycling within the two-working electrode setup leads to signal amplification and an increased sensitivity. Flexible two-working electrode biosensors show high potential for long-term monitoring of heat stress and other types of stresses in plants.

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S.H. thanks the TAU School of Engineering and the faculty of Life Science for supporting her research.

Polyserotonin as new functional monomer for molecular imprinting: An applicative study for TNF-α detection by Surface Plasmon Resonance

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Keywords: Polyserotonin, surface plasmon resonance, imprinted Bio-polymers, TNF-α,

Introduction

Molecular imprinting and related technologies are becoming increasingly appreciated in bioanalysis and diagnostic application. Among the imprinted polymers, we have already demonstrated that endogenous neurotransmitters (NTs), such as dopamine (DA) and norepinephrine (NE), can be efficiently used as natural and sustainable monomers to straightforwardly design and synthetize a new generation of highly selective and sensitive imprinted biopolymers (IBPs) [1]. Here, we demonstrated, for the first time, the ability of another NT, *i.e.*, serotonin (SE), in forming adhesive imprinted nanofilms and compared its imprinting efficiency with those PNE and PDA. The potential ability of SE in forming the related polymer (polyserotonin, PSE) is still virtually absent in the literature, excluding very few recent works [2,3]. As a model study, tumor necrosis factor-alpha (TNF-α) was selected as a biomolecular target of interest in clinical diagnostics. The biomimetic receptor was coupled to an optical real-time and label-free Surface Plasmon Resonance (SPR) and the quantifications were performed both in buffer and biological matrices.

Results and Discussion

In the first part of the study, the potential use of PSE as IBP was evaluated by taking advantage of the direct comparison with PDA and PNE, under the same imprinting and binding conditions. The analytical performance of PSE in terms of SPR signal intensity was surprisingly higher than those of PDA and PNE. Specifically, the imprinting of PDA and PNE resulted in lower SPR response of about 3 folds and 8 folds respectively. Moreover, the best growth condition has been tailored for PSE. For this purpose, SE was polymerized with two different pH (8.5 and 9.5) and the analytical parameters of both the PSE-based IBPs were tested with SPR platform by using the whole TNF-α protein as calibrator. Both the imprinted biosensor was able to recognize the molecular of interest in a concentration-dependent manner, but the polymer developed at more basic pH, has led to a rough gain of the sensitivity of about 2 folds. After optimization in buffer conditions, TNF-α calibration was successfully achieved also in human serum, with excellent detection limit and relative standard deviation, namely 22.1 pmol L^{-1} and 1.5% respectively.

Conclusions

To expand our knowledge on NTs-derived IBPs, SE was investigated here for the first time. In this context, we demonstrated that PSE could be a new and super effective monomer for the molecular imprinting of peptides and proteins. As a case study, a PSE-based IBP biosensor for TNF-α protein was successfully prepared showing excellent sensitivity and higher binding capacity, respect to the other polymers.

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Acknowledgements

Authors thank "Fondazione Roche per la Ricerca" for the funding to the project entitled "Biocompatible Molecularly Imprinted Polymers as alternative to antibodies-based therapy for Rheumatoid Arthritis treatment (MIPRA)". The authors also thank the MIUR, for the project "Dipartimenti di eccellenza 2023-2027 (DICUS 2.0)".

Simultaneous noninvasive monitoring of diabetes and hypoxia using 'artificial peroxidase' nanozyme – enzyme based biosensors

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Keywords: non-invasive diagnostics, nanozymes, glucose, lactate

Introduction

Glucose and lactate are important blood metabolites that are commonly monitored in medical diagnostics. Glucose is a key indicator of carbohydrate metabolism and is used as a biomarker for diabetes. Lactate, though less commonly monitored, can indicate disease states and is used in sports medicine to assess endurance and fatigue in athletes. Continuous monitoring of these metabolites is becoming a trend, with the development of wearable devices and noninvasive methods of analysis **[1]**, such as sweat-based monitoring. Electrochemical biosensors show promise for simultaneous monitoring of diabetes and hypoxia.

Results and Discussion

Prussian Blue (PB) nanoparticles with an average diameter of 35 nm have been obtained by catalytic synthesis **[2]**, then nickel hexacyanoferrate (NiHCF) shells have been synthesized around the PB cores **[3]**. Dip-coating with core-shell PB – NiHCF nanozymes results in both stable and sensitive hydrogen peroxide transducers. The best performance characteristics of the resulting biosensors are provided by nanoparticles of 50 nm.

Glucose and lactate biosensors based on PB and PB-NiHCF nanoparticles have been elaborated. Replacing the transducer from PB nanoparticles to PB-NiHCF nanoparticles has almost no effect on the sensitivity and linear range of the (bio)sensors, while the operational stability is significantly improved (from 2 to 8 times). Some loss in operational stability in comparison with (bio)sensors based on PB films at similar values of sensitivity and linear range is compensated by a simpler (dropping of the transducer) and express technique for the developed biosensors fabrication.

We have successfully demonstrated biosensors use in blood and sweat analysis. Furthermore, a multibiosensor based on the four-electrode planar structure has been developed that allows for simultaneous monitoring of glucose and lactate in undiluted sweat (Figure 1). Validation of the monitor has been carried using flow-injection analysis.

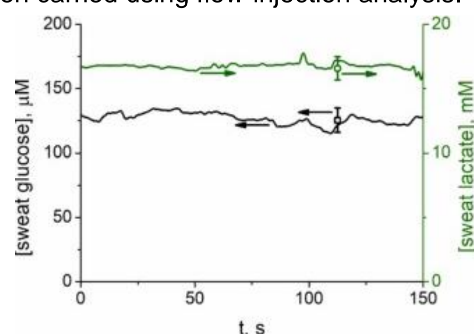

Figure 1: *Response of the multibiosensor to glucose (black line) and lactate (green line) in sweat, 0.0 V Ag|AgCl, in comparison with sweat metabolites concentration detected by FIA in diluted sweat: □ – glucose; ○ – lactate.*

Conclusions

Elaborated biosensors, based on PB-NiHCF nanoparticles and oxidases, are characterized by high sensitivity, wide linear and dynamic ranges, and improved operational stability. The stability of the transducer plays a significant role in the overall stability of the biosensor, confirming our earlier hypothesis. These biosensors have potential applications in various fields, particularly for analysing biological fluids. In line with our focus on noninvasive diagnostics, we have developed a new design of a flow-through sweat collector compatible with a four-electrode planar printed structure, enabling simultaneous glycemia and hypoxia monitoring in sweat.

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FO-SPR sensors and duplexed aptamers: An innovative approach for continuous biosensing

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Keywords: Continuous biosensing, Fiber optic surface plasmon resonance, Duplexed aptamers

Biomarker monitoring has become standard practice in various fields, as it enables diagnosis, intervention, and quality control. However, this practice currently requires tedious and time-consuming steps, thereby hindering rapid diagnosis and intervention, which is crucial in various settings, such as the ICU. This can be overcome by applying continuous biosensors, which provide real-time information about the respective biomarker concentrations, and thereby enable direct and even feedback-controlled intervention. To establish such a biosensor, several challenges need to be tackled, being that it (i) cannot rely on exogenous reagents or multistep processes, and should obtain (ii) a dynamic response and (iii) long-term stability [1]. To achieve this, here we combined fiber-optic surface plasmon resonance (FO-SPR) technology with duplexed aptamers (DAs), as depicted in Figure 1.

DAs are composed of an aptamer and a short oligonucleotide partially complementary to the aptamer (known as aptamer complementary element, ACE). As the knowledge on how the ACE affects the final performance of DA-based biosensors was limited, we successfully established and validated a thermodynamic model that describes this in equilibrium assays. This provided crucial insight on the working principles of DAs, and can help ease their development in the future [2]. Subsequently, trans-DAs (in which the aptamer and ACE were confined on separate constructs) were implemented on FO-SPR sensors. In the proposed concept, target binding induced the removal of gold nanoparticles (Au NPs) from the sensor surface, resulting in an amplified signal without multistep processes. This was demonstrated for ATP, thrombin and a single-stranded DNA (ssDNA) target, suggesting a generic approach for sensitive target detection without multistep processes [3]. Finally, cis-DAs (in which the aptamer and ACE were confined within the same construct, as shown in Figure 1) were implemented on FO-SPR sensors, which induced the spatial redistribution of Au NPs upon target binding. In this manner, target binding could be translated into a reversible signal without multistep processes or external reagents [4]. Although additional challenges remain before the proposed FO-SPR sensor can be applied for truly continuous measurements, the reported results clearly demonstrate huge potential towards achieving this goal.

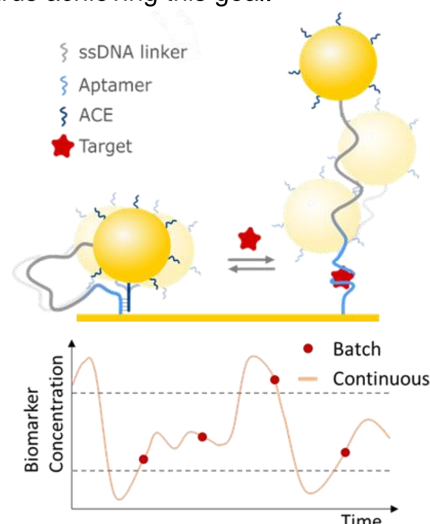

Figure 1: FO-SPR-based continuous biosensing combined with DAs. The data shown is a schematic representation.

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Acknowledgments

This work has received funding from KU Leuven (PDMt2/22/ 034), the Research Foundation Flanders (FWO SB/1S45121N, FWO SB/ 1SD5223N and FWO SBO/ S006319N), and the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No. 955623 (H2020- MSCA ITN-CONSENSE).

Printing Enzymes on Electrodes: A New Frontier to Fabricate Enzyme-based Amperometric Biosensors

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Keywords: enzymes, water-based inks, amperometric biosensors, lactate, glucose

Introduction

In the last decades, the increasing interest in developing low-cost wearable electrochemical biosensors for remote sensing pushed many researchers to look for new technological and research solutions capable to decrease manufacturing costs and enhance the reliability/reproducibility as well as stability of such biosensing platforms. [1]

This work reports on the formulation of water-based graphite inks to stencil-print enzymes (Figure 1). After preliminary characterization performed in buffer and artificial sweat, the proposed array was integrated into a wrist band to continuously monitor lactate and glucose during daily activities with the results showing promise for future applications in remote personalized medicine.

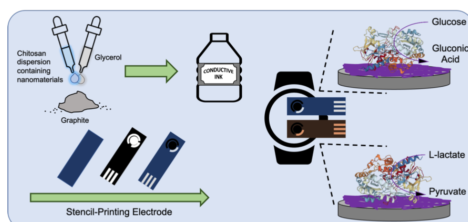

Figure 1. *Schematic representation of water-based conductive ink formulation and further integration of stencil-printed electrodes within a wristband with second-generation lactate and glucose biosensors.*

Results and Discussion

The proposed biosensor array exhibited a limit-of-detection (LOD) as low as 9.0 ± 1.0 μM for LOx/SPG-[Os(bpy)$_2$(Cl)(PVI)$_{10}$] and 3.0 ± 0.5 μM for GOx/SPG-[Os(bpy)$_2$(Cl)(PVI)$_{10}$], a sensitivityas high as 1.32 μA mM^{-1} for LOx/SPG-[Os(bpy)$_2$(Cl)(PVI)$_{10}$] and 28.4 μA mM^{-1} for GOx/SPG-[Os(bpy)$_2$(Cl)(PVI)$_{10}$]. The technology is also selectivity when tested in buffer and artificial sweat and is endowed with operational/storage stability of ~80% of the initial signal retained after 20 days. Finally, the proposed array was integrated in a wristband and successfully tested for the continuous monitoring of L-lactate and D-glucose in a healthy volunteer during daily activity.[2]

Conclusions

The proposed system shows promising features for deployment as a flexible and wearable biosensor based on biocompatible water-based inks which can be implemented for sport medicine and remote clinical care, possibly evolving towards edible biosensors for continuous metabolites monitoring.

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Real-time and label-free monitoring of vancomycin concentration using a long-range surface plasmon resonance aptasensor

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Keywords: aptamer, plasmon, real-time, monitoring, vancomycin

Introduction

Vancomycin is a crucial antibacterial agent used to treat infections caused by methicillin-resistant Staphylococcus aureus (MRSA). However, dosing and maintaining appropriate levels of vancomycin can be challenging due to its narrow therapeutic range of blood levels. Moreover, vancomycin can lead to renal dysfunction, making it essential to monitor the drug's concentration carefully. Conventional methods of monitoring vancomycin concentration, such as latex immunoturbidimetric, can be discontinuous and may not provide accurate results. In this article, we present a new plasmonic biosensor that provides an accurate method of monitoring vancomycin concentration.

Experimental

Our biosensor uses long-range surface plasmon (LRSP) resonance and peptide aptamers. To create the LRSP resonance, a glass substrate was coated with a fluoropolymer and then sputtered a thin gold film on top of it. Then a self-assembled monolayer of aptamers that contained a recognition fragment for vancomycin was formed on the gold surface. The aptamers captured vancomycin, causing a local increase in refractive index and altering the LRSP resonance condition. This change in resonance condition allowed us to measure the vancomycin concentration label-free accurately.

Results and Discussion

The Kretschmann configuration was used to measure the vancomycin concentration. This configuration allowed us to measure the changes in reflected light intensity at a fixed angle of incidence. When a vancomycin solution was applied to the LRSP aptasensor, the reflectivity increased. Then the vancomycin solution was exchanged with a buffer solution, and the reflectivity returned to its baseline level. Based on these results, a calibration curve was developed that showed the dynamic range of 0.78–100 μM, covering the therapeutic concentration range. The selectivity of the aptasensor was tested against various antibiotics and found that it could accurately detect vancomycin in the presence of other antibiotics. The biosensor also showed high reproducibility and real-time measurement capabilities. We demonstrated that it was possible to dissociate vancomycin from the aptamer by using the buffer solution, and the reflectivity increased or decreased depending on the vancomycin concentration. These results suggest that our LRSP aptasensor has the potential to become a reliable method for monitoring vancomycin concentration.

Conclusions

In conclusion, our LRSP aptasensor provides a label-free and selective method for monitoring vancomycin concentration in real-time. It is essential to note that the use of our biosensor is limited to research and is not yet approved for clinical use. Nevertheless, this novel technology could potentially address the limitations of traditional monitoring methods and provide clinicians with a more accurate way of monitoring vancomycin concentration.

Acknowledgements

We would like to acknowledge the generous support provided by JSPS for funding this research.

Determination of protease activities at different protein modified 3D structures

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Keywords: protease activity, proteinase K, silica gel, multilayer electrode

Introduction

Proteases are present in numerous metabolic processes such as protein degradation, wound healing, activation of the immune system a.o.. They are biomarkers for cancer, cardiovascular, neurodegenerative, auto-immune and other diseases **[1]**. But their significance as indication for the progression of a certain disease and the therapy efficiency depends not only on the expression of the protease but rather on its activity. Hence, the determination of protease activity is not only a valuable tool for the medical diagnosis, but also for drug development **[2]**.

Results and Discussion

Here, two different methods for the qualitative and quantitative determination of the activity from proteases are demonstrated on a model system applying proteinase K. The first semiquantitative test system is based on a protein modified and coomassie brillant blue coloured 3D-silica structure. Applying proteinase K at the casein modified silica gel the colour of the blue substrate changes at the spots of protease contact already after 1 h incubation at room temperature (fig. 1). Several substances have been studied to not influence the colour to ensure specificity of the test.

After the demonstration of the detection principle for the proteinase K the test has been adopted to the matrix metalloprotease 2 (MMP2). In this case gelatine – one substrate of the MMP2 is used for the modification of the silica structure. After 1 h of incubation a discoloration of the area where the MMP2 has been applied can be observed, but here the test is less sensitive.

The second test procedure uses the voltammetric signal of a cyt c/DNA-multilayer-electrode after incubation with a protease for the quantitative determination of its proteolytic activity.

After incubation of a 5-bilayer-cytochrome c/DNA electrode with proteinase K for 1 h a clear protease concentration dependent decrease of the voltammetric cyt c signal can be observed. This allows a better quantification of the enzymatic activity.

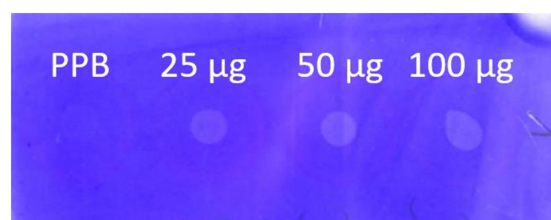

Figure 1: *Detection of the activity of proteinase K at a silica-tlc-plate modified with casein and coloured with coomassie brillant blue. Application of 20 µl of 3 different protease concentrations and 20 µl potassium phosphate buffer (PPB).*

Conclusions

Two new test systems have been established for the determination of protease activity. The first rather simple test allows a semiquantitative detection, while the second voltammetry based system can quantify the protease activity. It could be shown that both test principles can be applied for a cost-efficient activity determination of proteases within a rather short time period.

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Acknowledgements

The financial support by the BMWi (project: 16KN041859) is gratefully acknowledged.

Measuring Cell-Substrate Separation with Surface Plasmon Resonance Microscopy to Assess Biosensor Performance.

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Keywords: cell-substrate interface, label-free, non-invasive, raster-scanning microscopy

Introduction

The interaction between cells and abiotic materials governs the performance of neural implants and other biomedical applications. **[1]** In case of micro-electrode arrays (MEAs), a tight sealing between the cell membrane and an electrode for example is essential for the electronic coupling and hence to study the behavior of living neural networks. **[2]** To optimize these cell-chip interfaces with regard to the contact geometry between cell and sensor, our prototype Surface Plasmon Resonance Microscopy (SPR-M) setup is able to map the cell-substrate separation with a nanometer axial resolution and an almost diffraction limited lateral resolution, thereby being non-destructive, label-free and fast. **[3]**

Results and Discussion

Unlike other optical methods for this purpose, our SPR-M method doesn't rely on the assumption of a constant cytoplasmic refractive index, which can be modeled alongside with the cell-substrate separation. In a first study, the adhesion of HEK293 cells is evaluated for gold electrodes with a variety of coatings. High-resolution live-cell images of single cell-substrate interfaces as well as continuous recordings with decent spatial and temporal resolution were acquired. While cells on control samples were holding an average distance of about 100nm to their substrates, coated surfaces achieved a reduction to less than 50nm in average. Histograms of cell-substrate separation are compared to data from a previous transmission electron microscopy study of the same substrate- and cell-type. **[4]**

Conclusions

The measurement of cell-substrate distances is definitely one of the most demanding applications of SPR microscopy and its success in this matter, foreshadows what other biosensors may be evaluated with high spatial and temporal resolution thanks to this technique.

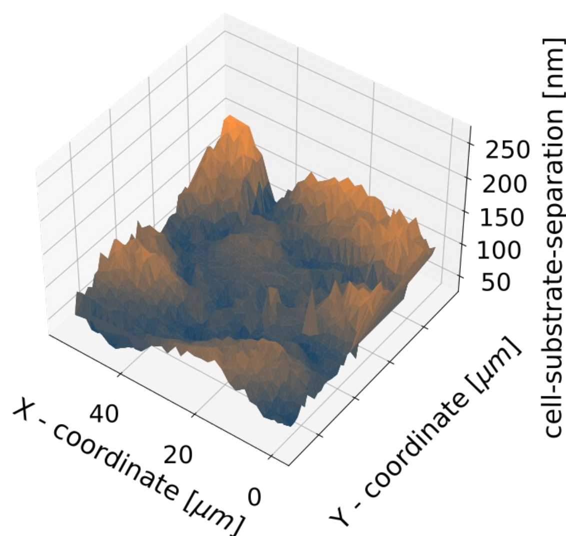

Figure 1: *Cell-substrate-separation of HEK293 cells on a Poly-L-Lysine coated SiO_2 surface in physiological conditions, assessed through a scanning SPR microscopy measurement.*

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PAM-engineered Toehold Switch DNA as Target-responsive activators of CRISPR/Cas12a for sensing applications

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Keywords: CRISPR biosensors, Cas12a, DNA Nanotechnology, Antibody, PAM

Introduction

Integrating dynamic DNA systems with protein-controlled actuation will expand our ability to convert specific protein-binding events into measurable outputs [1, 2]. Here, we report on the rational design of an antibody-responsive DNA system able to trigger the activity of the CRISPR-associated (Cas) endonuclease. Our approach combines the advantageous features of CRISPR-Cas12a detection systems for the generation of multiple turnover *trans*-cleavage activity of Cas12a and associated catalytic signal enhancement with those of DNA-based hybridization networks.

Results and Discussion

Our strategy employs a PAM-engineered toehold switch DNA hairpin and two synthetic antigen-labeled nucleic acid strands to create an artificial communication pathway between Cas12a proteins and target antibodies. Specifically, the toehold switch DNA hairpin is designed so that the complementary portion of the PAM (PAM*) is hidden in the loop, thus making PAM not accessible to Cas12a. This trick prevents efficient recognition and efficient activation of Cas12a activity. In the presence of the specific target antibody, instead, the antibody-induced colocalization of the two antigen-labeled DNA strands induces a conformational change to the toehold switch DNA hairpin and PAM complementation. Consequently, Cas12a collateral nuclease activity is switched on, and an amplified fluorescence signal transduction is generated (Figure 1). We demonstrate sensitive and specific detection of three antibodies in complex matrices (i.e. blood serum), including clinically relevant Ab as anti-HA and anti-MUC1 antibodies with a limit of detection

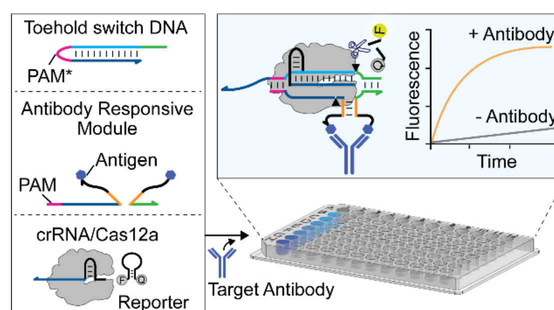

Figure 1: *Antibody-induced colocalization of two antigen-conjugated DNA strands induces a conformational change to the* DNA hairpin *leading to triggering Cas12a collateral nuclease activity.*

(LOD) of 1 and 0.1 nM, respectively.

Conclusions

Our single-step, modular sensing platform can be used to recognize any bivalent antibody for which an antigen can be conjugated to a nucleic acid strand. We believe CRISPR-based diagnostics could extend the capabilities of clinically relevant diagnostic technology for the ultrasensitive and rapid sensing of biomarkers at the point of care.

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Acknowledgments

This work received funding from the Associazione Italiana per la Ricerca sul Cancro, AIRC (A.P).

Liposome-based high-throughput and point-of-care neutralization tests for SARS-CoV-2

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Keywords: Liposomes, Point-of-care diagnostics, High-throughput screening, SARS-CoV-2

Introduction

The COVID-19 pandemic sparked interest in the assessment of the immune status of large parts of the population. Neutralizing antibodies represent a strong correlate for protection from infection or severe disease [1]. However, neutralization tests are often cell-based, making them time-consuming and costly [2]. An emerging alternative is the use of viral receptor proteins for competitive binding assays.

Results and Discussion

We developed a fluorescent high-throughput (HTS) and a colorimetric point-of-care (POC) neutralization test using dye encapsulating liposomes decorated with the receptor binding domain (RBD) of SARS-CoV-2. The liposomes are incubated with serum and subsequently captured in an angiotensin converting enzyme 2 (ACE2)-coated plate or, mixed with biotinylated ACE2, on a streptavidin test line in a lateral flow assay. The proof-of-principle was demonstrated using commercially available antibodies. Here, polyclonal neutralizing human antibodies caused complete binding inhibition, while S309 and CR3022 human monoclonal antibodies only caused the expected partial inhibition. Then, the assays were tested with real samples through a serum panel consisting of 20 sera containing varying neutralizing antibody titers, and a control panel of sera including pre-pandemic sera or reconvalescent sera from respiratory infections other than SARS-CoV-2. The assays correlated well with a standard pseudovirus neutralization test ($r = 0.847$ for HTS and $r = 0.614$ for POC format). Furthermore, excellent correlation ($r = 0.868$) between HTS and POC formats was observed. [3]

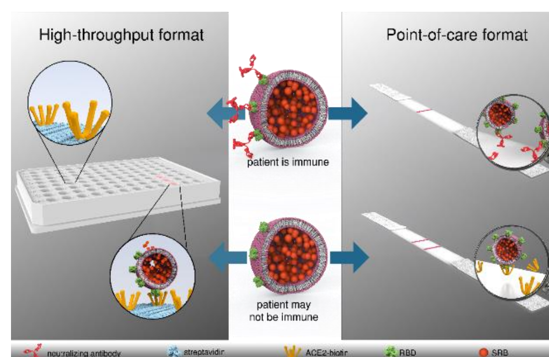

Figure 1: *Schematic of the developed HTS (i) and POC (ii) formats for the detection of neutralizing antibodies directed against the receptor binding domain of SARS-CoV-2 using RBD-conjugated liposomes encapsulating the fluorescent dye Sulforhodamine B. [3]*

Conclusions

The developed assays already correlate well with an established pseudovirus neutralization test. The use of different liposome encapsulants and sizes, as well as optimization of their surface modification, will likely further increase the sensitivity of these neutralization tests and allow for easy adaptation for other viruses.

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Thanks to Vanessa Tomanek for providing the schematics and to the Bayerische Forschungs-stiftung for funding the project.

Fluorescent Nanosensors for allergy diagnostics

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Keywords: cell-based diagnostics, non-invasive, nanoparticle, microfluidic, fluorescence

Introduction

Biogenic amines such as histamine play an important physiological role in inflammation and allergic reactions. Chromatography-based techniques are able to report histamine concentrations but they cannot capture the fast dynamics of histamine release of cells and tissue. Here, we assemble an optical sensor for fast and sensitive detection of this important signaling molecule. To detect histamine, we use near-infrared (NIR) single-walled carbon nanotubes (SWCNTs) as nonbleaching backbone that is fluorescent in the advantageous NIR tissue transparency window (800-1700 nm) and are used for a wide variety of biological sensor applications by our lab.[1,2] We modified the SWCNTs with histamine-binding aptamers, an approach previously demonstrated by us to measure serotonin release from human platelets with high spatial and temporal resolution.[3]

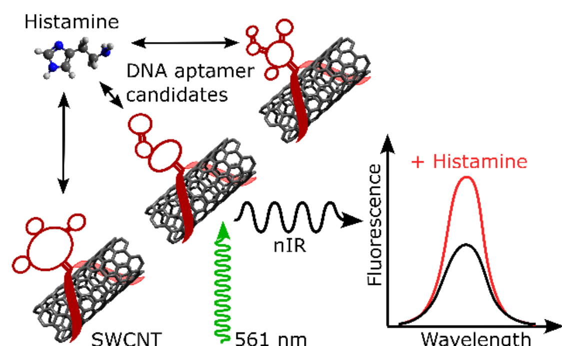

Figure 1: *Design principle of the Histamin sensor. Testing of an aptamer library for possible candidates for SWCNT surface modification. Single-walled carbon nanotubes are irradiated with visible light (561 nm) and the resulting NIR-fluorescence (~993 nm) is modulated by addition of the analyte histamine.*

Results and Discussion

The fluorescence of this novel histamine sensor increases by up to 160 % in response to 100 µM histamine (995 nm emission wavelength for (6,5)-SWCNTs). The aptamers were also compared to a library of DNA modified SWCNTs that detect other small molecules such as dopamine or epinephrine. The fluorescence change for histamine of SWCNT-Aptamer-based sensors was 3 times larger than for random oligonucleotides. We also show microfluidic surface measurements of single nanotubes that demonstrate their reversibility and capabilities to measure with analyte backgrounds. Lastly, we detect and image histamine release from cells.

Conclusions

Our work demonstrates that aptamers in combination with SWCNTs are powerful tools for histamine detection. We anticipate that this technology can be used for imaging of histamine release in complex cellular systems as well as for point-of-care diagnostics.

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Multicolored sensors based on silica and polymeric particles for ratiometric monitoring of pH, oxygen and saccharides

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In recent years, the use of functionalized micro- and nanomaterials has increased rapidly for a wide range of applications in the life and material sciences, due to their unique properties in combination with their high surface-to-volume ratio and stability. For instance, functionalized micro- and nanomaterials, that are labeled or stained with a multitude of sensor dyes can be used for monitoring, and quantification of neutral and ionic analytes. [1,2] These materials have several advantages as compared to conventional molecular probes like enhanced brightness, ease of designing ratiometric systems by combining analyte-sensitive and inert reference dyes, and increased photostability. Moreover, stained nanoparticles can enable the use of hydrophobic dyes in aqueous environments.[3]
Versatile templates and carriers for the fabrication of nanosensors by staining and/or labeling with different fluorophores and sensor molecules are biocompatible silica and polymeric particles, because they can be synthesized in large scales at low costs with different surface chemistries.
Here we present our work on multicolored sensors for the measurement of pH, oxygen and saccharides utilizing commercially available or in-house synthesized silica and polymeric particles.

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Engineering optical aptasensors for the detection of fungicides

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Keywords: fungicides, aptamers, gold nanoparticles, fluorimetry, colorimetry

Introduction

Fungicides are commonly used in agriculture for the control of various plant diseases. The presence of those substances in agricultural products is a serious threat to human health. Standard detection methods imply complicated and time-consuming analysis carried out in remote laboratories by qualified scientists on expensive instruments. Thus, development of simple, portable, rapid devices for the sensitive and selective fungicide detection is of significant importance for ensuring food safety [1],[2]. Our innovative technology exploits the numerous benefits of single-stranded DNA aptamers, in vitro evolved to specifically bind their target fungicide, as recognition elements in such biosensors. Aptamers are equivalent to monoclonal antibodies concerning their binding properties, but provide advantages, including simple, fast and cheap production, easier modification, excellent stability and reproducibility [3], in particular for the detection of small organic molecules.

Results and Discussion

Aptamers against a benzimidazole fungicide were selected from a ssDNA library containing randomly synthesized 82 nucleotides (nt) long oligomers. Their affinity and specificity were determined. The aptamer with the highest binding affinity was subjected to post-selection optimization including truncation and mutagenesis in order to identify the key-recognizing nucleotides. The optimized, 38 nt long aptamer has a Kd in the low micromolar range. The unique stem-loop structure was subsequently integrated into optical biosensors for the quantitative detection of fungicide, by fluorescence and colorimetry.

A fluorescence “turn-on” biosensing strategy based on molecular apta-beacons yielded LOD in the low ppm range. Colorimetric assays based on gold nanoparticles (NPs) were also developed. The red-to-blue color change of aptamer-grafted NPs in the presence of the fungicide was easily measured using Indigo, a simple, portable spectrophotometer, allowing the detection of fungicides in the low ppm range in the field with transmission of the results to a cell-phone (Figure 1).

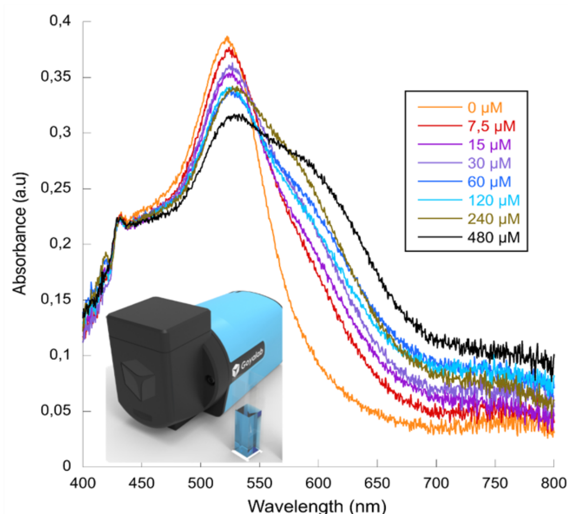

Figure 1: *Response of the colorimetric sensor to benzimidazole fungicide, measured by portable IndiGo (inset).*

Conclusions

Using aptamers as recognition elements, we developed fluorescence and colorimetric assays for the sensitive, specific and rapid detection of fungicides. Optical responses (fluorescence and absorption) were measured by a simple, affordable and portable spectrophotometer, making the assays suitable for quantitative on-site detection. The need to detect small molecules (<1000 Da) such as residues of fungicides is of utmost importance. Novaptech develops aptamers to other targets of interest for pharma, agri-food and environment.

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For this application, Indigo was developed in collaboration with Goyalab, Pessac, France (https://www.goyalab.com/fr/).

An intriguing polymeric modification strategy for solid particles to investigate endosomal escape of cargos from polyelectrolyte microcapsule

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Keywords: capsules, cargo release, endosomal escape, photothermal heating

Introduction

Polyelectrolyte microcapsule is a typical kind of carrier system used for encapsulating molecular cargo and cells delivery. Such capsules can be constructed by layer-by-layer assembly of oppositely charged polymers. Benefiting from the advantage of low leakage, high opening efficiency, and retain high cell viability after photothermal irradiation, it is well reported that they have been demonstrated to be versatile carriers for various types of molecules. By utilizing fluorescent molecular cargos, the process from carrier rupture and endosomal escape to the spread in the cytosol can be monitored with high temporal resolution.

In the previous work, quantitative analysis showed that the spreading time was found to be independent on molecular weight, diffusion coefficient, molecular volume, charge, and hydrophobicity. However, the binding of molecular cargo to certain cell components delays their intracellular spread, i.e., intracellular staining. The failure in release of some molecular cargo (solid particles such as Au nanoclusters, quantum dots and Fluospheres) is due to their interaction with the capsule walls, as it is independent from being surrounded by a lysosomal membrane. This demonstrates that while being a general approach, the encapsulation and photo-triggered release strategy failed for some types of molecular cargos. By solving the release problem of these solid particles from capsules, a more general understanding in the release process from the carrier capsules and lysosomes to the cytosol would therefore be very helpful for a more fundamental understanding of the mechanisms.

Results and Discussion

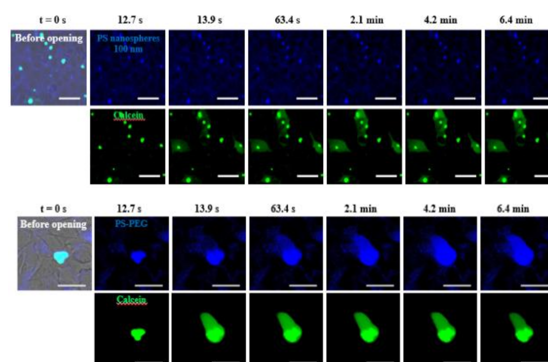

Figure 1: *Real-time imaging and analysis of the intracellular spreading of endocytosed encapsulated.*

Conclusions

Using poly (ethylene glycol) (PEG) to modify with various molecular cargos can be a versatile strategy to reduce strong interaction of the particles and inner layer of capsules for efficient delivery, thus achieving well performance of endocytosed and endosomal escape. In summary, our study will provide more insight to understand the intracellular process after genes, drugs, or proteins are delivered into cells.

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DNA nanotechnology meets synthetic biology: new perspective of cell-free biosensors for antibody detection

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Keywords: DNA Nanotechnology, cell-free biosensors, synthetic biology, analytical diagnostic tools

Introduction

Rapid and user-friendly diagnostic tests that are convenient, accessible, and well-suited for use at the point-of-care are essential for the early identification, tracking, and control of infectious diseases and other clinical needs.

In recent years, the advantages of synthetic nucleic acids (i.e., programmability of interactions, low-cost, and ease of synthesis) [1] have been coupled with the sensitivity and specificity offered by cell-free transcription/translation systems to develop innovative class of biosensors for the detection of a wide range of different targets, including nucleic acids, small molecules and proteins.[2]

Results and Discussion

Among these, we recently reported the first two examples of cell-free transcription biosensors for antibodies detection. These systems are based on synthetic genes that can be activated in the presence of a specific target antibody and trigger the *in-vitro* transcription of a signalling RNA strand.

Specifically, in a first report we have designed a programmable antigen-conjugated DNA transcriptional switch programmed so that in the absence of the target antibody the T7 RNA polymerase promoter domain is hidden into the hairpin structure and thus transcription is prevented.[3] The binding of the target antibody to a pair of antigen-conjugated strands induces a switch conformational change that results in the reconstitution of the complete promoter domain. This complex ultimately triggers the transcription of the RNA light-up aptamer leading to a fluorescence signal that informs on the presence and concentration of the target antibody.

In a second example, we have developed an electrochemical cell-free biosensor based on an antigen-conjugated synthetic gene.[4] Specifically, the antibody-induced reconstitution of the T7 RNA polymerase promoter domain triggers the transcription of a RNA output strand that will provide a measurable electrochemical signal upon the hybridization to redox-labeled oligonucleotide probe site-specifically attached to an interrogating disposable screen-printed electrode.

Using these systems, we have achieved a sensitive, specific, and multiplexed detection of different antibodies (including the influenza-relevant anti-HA antibody) directly in complex matrix samples.

Conclusions

We have demonstrated that *in-vitro* transcription processes combined with programmable responsive nucleic acid devices can be conveniently applied for the detection of clinical molecular targets, thus leading to transformative improvements in antibody detection technologies applied to clinical problems. In addition to that, we are currently investigating different strategies to further enhance the sensitivity of our systems.

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Acknowledgements

This work was supported by the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement n.843179 ("DNA-NANO-AB", S.R.), by Associazione Italiana per la Ricerca sul Cancro, AIRC (project n. 14420) (F.R.) and by the European Research Council, ERC (Consolidator Grant project n. 819160) (F.R.).

Ratiometric determination of morphology-dependant emulsion droplet luminescence for *in-situ* tracking of exoenzyme activity

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Keywords: cell-based diagnostics, non-invasive, nanoparticle, microfluidic (3-5 words, Arial 10)

Introduction

Transducing chemical information sensitively, inexpensively, and quickly into quantitative measures is of great interest to address the tangible healthcare and environmental needs of our society. Complex emulsion droplets provide a platform to transduce chemical information from antibodies, pathogens, enzymes, PFAS, or metal ions. These biphasic droplets are reconfigurable in shape characteristically to the balance of interfacial tensions at their interfaces, and present this information as a physical, motile, or optical response. While the chemo-morphological change from complex emulsions is established, deployable monitoring of this programmable droplet response compatible with existing schemes is required.

Results and Discussion

The two phases of the dynamic complex emulsion droplets present a refractive index contrast which enables total internal reflection at the internal, gravity-aligned droplet interface. When a dye is placed into the higher refractive index phase, luminescence of this dye totally internally reflects along the internal droplet interface, guided by its shape. We use a custom rotating fluorescence microscope to study the droplet emission at all angles around the droplet to understand the directional luminescence. Fluorescence measurements, spectroscopy, and ratiometric spectroscopy are verified analytically, and combined with ray tracing experiments to explore and understand the limits of the angle-dependent luminescence.

Complex emulsion droplets with a dye placed in the higher-refractive index phase have concentrated luminescence along the internal droplet interface that is angle-dependant. Incident light is collected and resulting fluorescent light concentrated at the internal droplet interface due to total internal reflection. Angle-dependant droplet emission is defined by the three-phase interface location

Conclusions

Monitoring macro-scale emission of droplet monolayers with 2 spectrometer probes enables ratiometric and reference-free detection of droplet morphology, closely matching ray tracing experiments. By controlling droplet refractive indices, droplet volume ratio, and radiative emission transfer, the signal-to-noise ratio of directional emission was further enhanced. Using this scheme, record-breaking detection of pathogenic food-borne bacteria was achieved quickly, cheaply, and with high specificity.

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Acknowledgements

Please We gratefully acknowledge funding through the Emmy Noether program of the DFG.

Surface-anchored metal-organic frameworks as highly potential material platform for biosensing application

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Keywords: sensor materials, pores, enzymes,

Introduction

Metal-organic frameworks (MOFs) are highly porous, crystalline materials consisting of metal-containing nodes and organic linker molecules. Depending on the linker molecules, the pores can be varied in terms of their size, shape, properties and functionality. As they can act as host structures for guest molecules, MOFs are classically used e.g. as gas storage materials. Here we introduce an innovative material platform, consisting of **surface-anchored** MOFs (SURMOFs), which can be grown on supporting substrates of any material in a highly oriented manner by using a layer-by-layer (LBL) liquid epitaxial growth approach (Fig.1) [1].

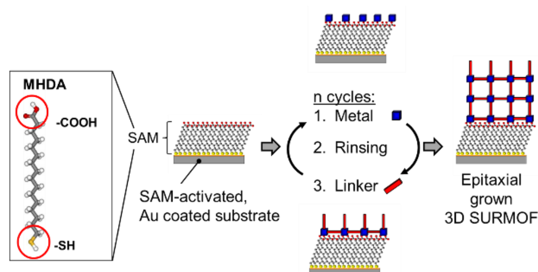

Figure 1: *Scheme of the layer-by-layer deposition of SURMOFs on a chemically functionalized substrate.*

Beside gas molecules also bio molecules like enzymes can be immobilized inside the pores of a (SUR)MOF. Here we present, which potential SURMOFs have for sensing and biosensing application.

Results and Discussion

SURMOFs per se are not necessarily water stable, but several SURMOFs were produced, which are stable even under harsh chemical conditions in aqueous solutions and under physiological conditions, which is a prerequisite for an application in the field of biosensing (Fig.2). One example is the UiO-66-NH_2 [2]. Another highly stable MOF is the NU-1000 with a pore size big enough to host enzymes.

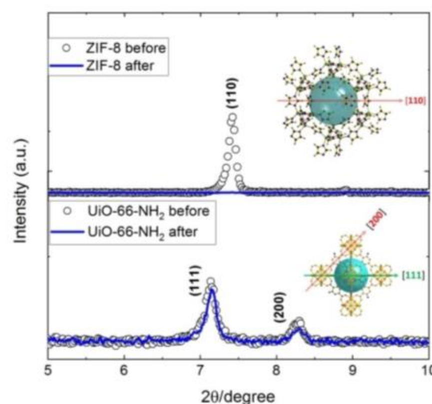

Figure 2*: XRD pattern of ZIF-8 and UiO-66-NH_2 coated substrates before (black circles) and after (blue line) boiling for one hour in water.*

In a first proof of concept, esterase EST2 was immobilized in the pores of a NU-1000 MOF powder, packed in a continuous flow reactor. The enzymatic activity has been investigated as well as the stability of the enzyme-loaded MOF material [3].

Conclusions

The results shown here will open a new perspective in implementing e.g. enzyme-loaded SURMOFs in technical biosensor environments, such as field-effect transistor-based devices to detect specific substrates for analytical purpose.

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From microbiosensors to AFM probe-integrated (bio)sensors: towards single cell measurements

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Keywords: Micro(bio)sensors, in-situ measurements adenosine triphosphate (ATP), conductive colloidal AFM probes

Introduction

Measurements of signaling molecules such as adenosine triphosphate (ATP) or hydrogen peroxide (H_2O_2) at the single cell level are still challenging but a prerequisite in understanding cell signaling. ATP is one of the most widely distributed extracellular signaling molecules, regulating physiological and pathological processes via activation of P2 purinergic receptors (metabotropic P2Y or ionotropic P2X receptors) [1].

Results and Discussion

Here, we present microbiosensors for ATP based on conductive colloidal AFM probes. ATP microbiosensors, as recently demonstrated in a collaborative effort, have been used to investigate ATP release from lung epithelial cells. For example, ATP levels at alveolar type (AT)II cells or co-cultured ATI and ATII cells [2,3] have been determined, contributing to insights into the role of ATP during strain and chemically induced release of surfactant. However, using these conventional microbiosensors impedes that ATP release can be determined at the single cell level.

We recently introduced conductive colloidal AFM probes with spherical electrodes that can be modified with polymer films like PEDOT [4] or polydopamine [5] or electrocatalytic layers for e.g., hydrogen peroxide detection. Given the large surface area but geometrical dimensions as small as 1 μm (diam.) will provide sufficient sensitivity for single cell measurements.

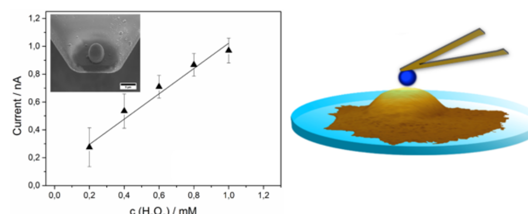

Figure 1: *Scheme of a single cell measurement using a conductive colloidal AFM probe. Calibration curve of a Prussian blue (PB) modified colloidal AFM probe in buffered solution (pH =7) for the detection of H_2O_2. Error bars reflect measurements at three different AFM probes.*

Conclusions

Using conductive colloidal AFM probes, enzymes like hexokinase and glucose oxidase can be immobilized via electrodeposition. Such AFM probe integrated ATP microbiosensors can be positioned at the single cell level (e.g., ATII cells). First measurements will be presented.

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Studying the metal-organic framework bio-interface for novel biosensing applications

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Keywords: Metal-organic frameworks, biomolecular interactions, oligonucleotides, Surface plasmon resonance, rolling circle amplification.

Introduction

MOFs are porous crystalline highly ordered periodic networks formed through co-ordination linkages between metal ions or clusters with organic ligands. Metal-organic frameworks (MOFs) are found to be promising in recent times due to their unique physicochemical properties [1]. The molecular architecture and functional capabilities of MOF are widely being explored for several applications. In this work, we study the interface of a novel iron (Fe)-based MOF, MIL 101 [2] for bimolecular interaction through surface plasmon resonance (SPR) and imaging SPR (iSPR). The MOF interface was optimized to immobilize oligo nucleotides followed by nucleic acid based rolling circle amplification (RCA) through padlock probing.

Results and Discussion

The SPR chips were fabricated in-house. Briefly, 4-inch LaSFN9 glass wafers were evaporated with ~2 nm of titanium (Ti) as an adhesive layer and ~48 nm of gold (Au) layer. Then, the diced and cleaned SPR chips were coated with Fe-MOF through automated layer-by-layer deposition method developed and optimized in-house (**Fig. 1A)**. The prepared layers were extensively characterized using atomic force microscopy (AFM) (**Fig. 1B)**. The influence of the MOF layer thickness on the SPR sensitivity was studied by obtaining the angle-based response spectrum (**Fig. 1C**). Depending on the results, 5 layers of Fe-MOF was found to be optimum and further experiments were carried out using SPR chips modified with 5 layers of Fe-MOF. The chemical stability of the prepared MOF layer was assessed at varying pH and ionic strength. In addition, iSPR was also investigated by demonstrating a nucleic acid hybridization assay (**Fig. 1D**).

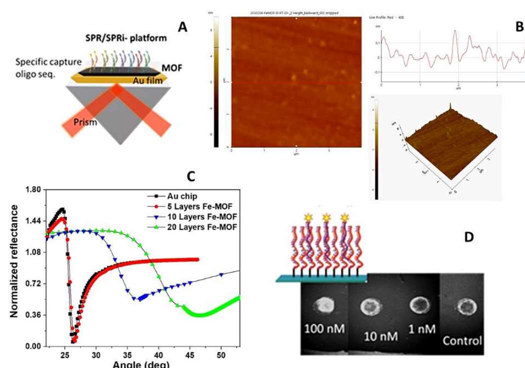

Figure 1: *(A) Schematic representation of SPR/iSPR platform. (B) SPR angle spectrum showing the influence of MOF layer thickness and RI sensitivity. (C) AFM characterization of 5 Layer Fe-MOF and (D) iSPR demonstrating the DNA hybridization assay.*

Conclusions

The obtained results showed MOF-layer depend SPR characteristics and could potentially be used as a functional interface to perform bioassays through amine chemistry. Further work is in progress to establish and demonstrate multiplexed rolling circle amplification on the developed MOF-SPR platform.

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Acknowledgements

We thank ZMNT facilities and the technicians for their assistant and support to successfully carry out the work.

Liposome-immobilized microcantilever array sensor for the simultaneous detection of alpha-synuclein in multiple analytes

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Keywords: array sensor, cantilever sensor, microfluidics, immobilization, liposome, alpha-synuclein

Introduction

Microcantilevers are very sensitive to changes on their surface and can be used to detect changes upon structural modification in a pre-absorbed layer [1]. This makes them a good candidate for the detection of biomolecules. We demonstrated the detection of amyloid beta proteins with a liposome-immobilized microcantilever sensor [2] and recently we are working on the detection of alpha-synuclein (αSync), a biomarker for the Parkinson's disease. Alpha-synuclein interacts with the lipid layer of the liposomes on the cantilever surface [3], which can be detected by the microcantilever sensor.

Results and Discussion

Figure 1 shows the 4×4 microcantilever sensor array with one attached microfluidic channel with 4 inlets, 4 sensor cells, and one outlet. The microfluidic channel is made of PDMS and was fabricated with a 3D printed mold. The 16 cantilever sensors can be read sequentially with a 24-bit load cell ADC and a 16-channel multiplexer connected to a microcontroller.

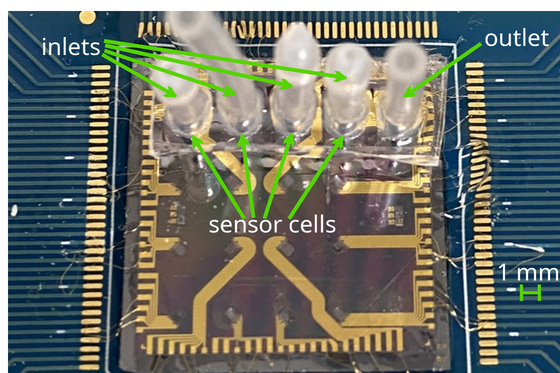

Figure 1: *Microcantilever sensor array with attached microfluidic channel for multi-analyte detection.*

Liposomes were immobilized intact on the cantilever sensor using a SAM layer, all solutions were delivered through the microfluidic channel. Figure 2 depicts the detection of αSync fibrils with a liposome-immobilized cantilever sensor.

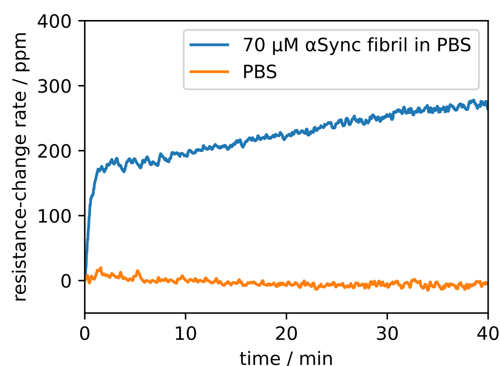

Figure 2: *Measurement of the liposome-immobilized cantilever within the microfluidic channel with 70 μM mouse-derived αSync fibrils in PBS solution and PBS solution only.*

Conclusions

A 4×4 microcantilever sensor array was combined with a PDMS microfulidic channel. Immobilization of liposomes and detection of αSync fibrils was successfully demonstrated. The sensor signals of the array can be read out with commercially available electronic components. The combination of microfluidic channel and sensor array makes it possible to record multiple parameters of multiple analytes.

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Acknowledgements

This research was supported in part by a Grant-in-Aid for Scientific Research (KAKENHI Grant No. 20H00663) from JSPS.

Laser-induced metal nanostructures on cellulosic substrates for colorimetric devices development

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Keywords: CO_2-laser, laser-patterning, one-shot nanodecoration, paper-based platform

Introduction

The widespread use of metal nanoparticle (MNP)-based devices (e.g., lateral-flow immunoassay) pushed the search for emerging approaches to easly fabricate integrated devices. This demand stimulates the search for alternative strategies to customize the nanodecoration of flexible supports (i.e., cellulosic, flexible plastics, textiles, etc.) and to assemble the same in analytical devices, with the final goal to give rise to smart (bio)sensors and (bio)sensing strategies **[1]**.

Results and Discussion

Herein, an innovative CO_2 laser writing strategy to in-situ synthesize MNP with micrometric-resolution patterns, directly onto cellulosic substrates, will be presented (**Fig. 1A**). An office-grade CO_2 laser plotter was used to achieve the direct paper nano-structuration. In brief, the CO_2 laser patterns the paper and instantly induces the MNP formation due to the photothermal energy generated; the latter triggers the metal-reduction and nucleation without the need for external reducing/stabilizing agents. Noteworthy, the MNPs-formation occurs according to tailored designs means the use of a simple design free-software, making this strategy able to give rise to customizable patterns. The proposed approach allows to scribe nacked Au, Ag, Pt, Ni, and Cu nanostructures onto cellulosic substrates; for each metal, the nanostructure formation was carefully studied and morpho-chemically characterized. The laser-nanoscribed cellulosic substrates were combined with other office-grade materials and assembled through low-cost equipment, to realize paper-based devices. Three different devices will be presented, where the laser scribed-nanostructures act as colorimetric sensing probes (**Fig.1B**), nanocatalysts (**Fig.1C**), and nanozyme (**Fig.1D**); the devices were employed in the smartphone-based analysis for the determination of oxidant agents, organic dyes, and reducing agents, respectively. The devices were challenged for the analysis of model solutions and various food and biological samples.

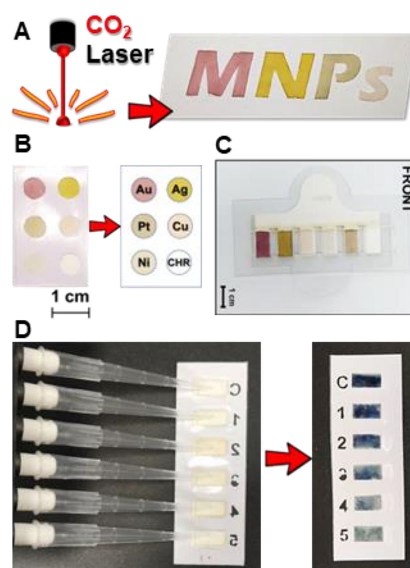

Figure 1: *MNPs laser-nanoscribing sketch (A). Paper-based devices where the laser-scribed MNPs act as colorimetric probes (B), nanocatalysts (C), and nanozyme (D).*

Conclusions

An innovative strategy for the one-shot and in-situ tailor MNP formation onto cellulosic substrates was proposed. The strategy results extremely promising to rationally develop biosensing devices and colorimetric sensor arrays.

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This research was funded by the European Union – Next Generation EU. Project Code: ECS00000041; Project CUP: C43C22000380007; Project Title: Innovation, digitalization and sustainability for the diffused economy in Central Italy – VITALITY.

First step towards "template cell-free" fabrication of a surface imprinted polymer-based biosensor for pathogenic bacteria detection

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Keywords: imprinted polymer, bacteria detection, master stamp, biosensor, QCM

Introduction

Food and water contamination by pathogenic microorganisms is one of the major concerns in food industry as well as in public health. Hence, to maintain high standards of food quality and environmental safety, rapid and accurate detection of these contaminations is essential.

Surface-imprinted polymer (SIP)-based sensors show a high potential for the detection of bacteria. The most prominent strategy to fabricate bacteria-specific SIPs is to fabricate cell-specific cavities by micro-contact imprinting using a stamp (carrying the pathogens as a template) together with a pre-polymerized oligomer solution **[1,2]**. However, each imprinting run requires fresh template cells, and the process is labor-intensive. In addition, the fabrication needs considerable experience to ensure reproducibility and high areal density of imprints.

Herein, we developed photolithographic mimics of template bacteria (such as *E. coli*) on a positive master stamp using direct laser writing lithography. Furthermore, these mimics were covered with cell-specific outer membrane components (such as isolated-membrane fragments or lipopolysaccharides) for enhanced biosensor selectivity. For electrochemical sensing, the SIP layer was created on interdigitated electrodes and integrated into a microfluidic setup.

Results and Discussion

SIP layers with cavities in a desired orientation and cell-specific geometry, density, and distribution were achieved. The cavities and the captured cells were demonstrated in Figure 1.

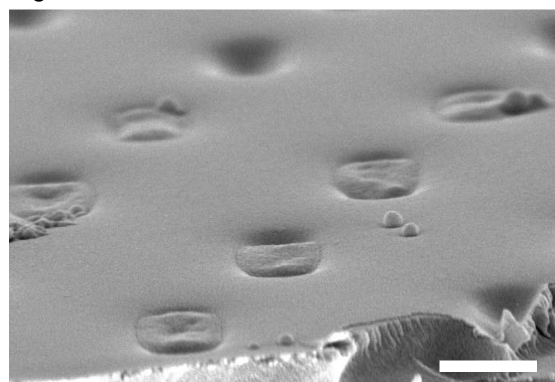

Figure 1: *Scanning electron microscopy image showing the cavities of the SIP layer and the captured E. coli cells inside these cavities. The scale bar is 1 µm.*

Conclusions

This work demonstrates the potential application of a "template cell-free" SIP layer fabrication, which could minimize or eliminate the requirement of the usage of template cells.

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Acknowledgements

The authors would like to thank the European Commission and the German Federal Ministry of Education and Research (project no.: 03F0902A) for funding in the frame of the collaborative international consortium (ARENA) financed under the 2020 AquaticPollutants Joint call of the AquaticPollutants ERA-NET Cofund (Nº 869178).

Microfabrication of pyrolytic carbon microneedles for electrochemical biosensing in the skin

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Keywords: microneedle sensors, pyrolytic carbon, electrochemical biosensing, diagnostics

Introduction

In the past decade, microneedle-based electrochemical sensors have gained increasing interest because they potentially allow for detection of bioanalytes present in the interstitial fluid (ISF) of the skin [1]. In most studies reported so far metallic or metal-coated polymeric microneedles have been used. Carbon is an excellent electrode material providing high chemical and mechanical stability, low risk of biofouling and a large potential window [2]. Here, we propose two different strategies for the fabrication of pyrolytic carbon microneedles suitable for electrochemical biosensing in the skin.

Results and Discussion

First, in-plane Si microneedles were fabricated using methods such as photolithography and reactive ion etching. The shape and dimensions of the microneedles were optimized to reduce penetration force and minimize damage in abodominal rat skin and porcine ear skin while still reaching the ISF in the dermis. Pencil-shaped microneedles with a length of 1000 µm, thickness of 180 µm and width of 200-400 µm were selected for integration of pyrolytic carbon electrodes (Fig.1A).

In parallel, out-of-plane pyrolytic carbon microneedles were developed using additive

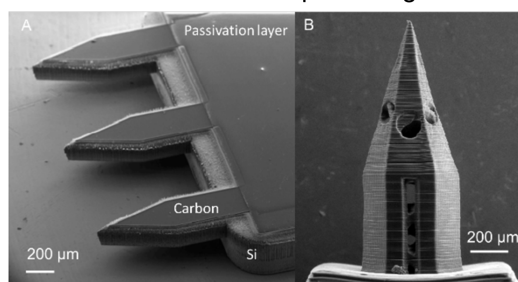

Figure 1: *A) Microfabricated in-plane Si microneedles with integrated pyrolytic carbon electrodes; B) Out-of-plane pyrolytic carbon microneedles with hexagonal gyroid design fabricated combining additive manufacturing and pyrolysis*

manufacturing and pyrolysis. 3D polymer microneedles with high structural definition and feature sizes down to 10 µm were prepared by projection microstereolithography (PµSL) and converted into carbon by pyrolysis in a quartz tube furnace at 900°C in N_2 atmosphere [3]. Pyrolytic carbon microneedles with various geometries and high electroactive surface area were fabricated (Fig.1B).

Finally, the functionality of the 3D electrodes was evaluated using cyclic voltammetry in hydrogel-based skin tissue models (Fig.1C).

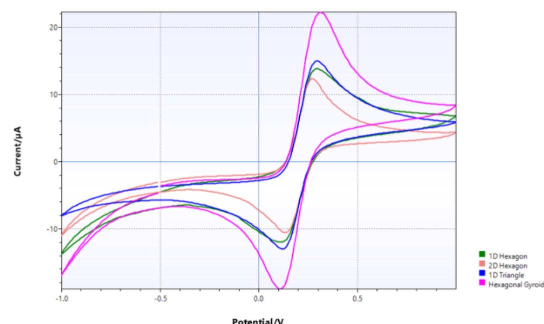

Figure 2: *Cyclic voltammograms recorded for different microneedle designs inserted in 1.5 wt.% agarose hydrogel loaded with 10 mM $[Fe(CN)_6]^{4-}$ at scan rate 50 mV/s*

Conclusions

Pyrolytic carbon microneedles are suitable for electrochemical sensing in the skin. Currently, an assay for biosensing of TNF-α is evaluated.

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The authors acknowledge funding from the Independent Research Fund Denmark (grant no. 8022-00215B), and the LEO Foundation (grant no. LF17046).

A novel thiol-based linker with antifouling properties for detection of *P. aeruginosa by QCM-D aptasensor in milk*

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Keywords: antifouling linker, aptasensor, *Pseudomonas aeruginosa*, QCM-D, gold nanoparticles

Introduction

The fouling of organic material on an interface can affect the biosensor performance **[1]**. Hence, the design of antifouling molecules can be a useful strategy to increase the sensitivity of the bacteria detection in milk **[2]**. In this work, the quartz crystal microbalance with dissipation monitoring (QCM-D) was used to prove the antifouling properties of a new thiol, SH-MEG-COOH, and its use for the design of a sensor detecting *P. aeruginosa*. Besides, the sensor surface was incubated with DNA aptamer-coated AuNPs after bacteria incubation, to increase the sensitivity.

Results and Discussion

We tested the antifouling behaviour of SH-MEG-COOH- and DNA aptamer-coated QCM crystals demonstrating a very low frequency variation compared to bare or non-antifouling coated crystals, suggesting that fouling was reduced. Then, a biosensor specific for *P. aeruginosa* was designed and tested vs. scaling bacteria concentrations in PBS (10^2 to 10^5 CFU/mL), flowing aptamer functionalized gold nanoparticle incubation. A plot of frequency changes vs. time was obtained, showing a certain proportionality between the variation of the resonance frequency of the crystal and the bacterial concentration. After the bacteria incubation, functionalized gold nanoparticles (AuNPs) suspensions were flowed. We observed a further decrease in frequency due to an increase of binding nanoparticles on the surface as a function of bacterial concentration (Figure 1). This demonstrates a specific interaction between nanoparticles and *P. aeruginosa*. The specificity of the designed aptasensors for detection of *P. aeruginosa* was tested versus high concentrations of non-specific bacteria, *E. coli* and *S. aureus* (10^5 CFU/mL) in PBS. The frequency shift was minimal in comparison of that one obtained after *P. aeruginosa* incubation.

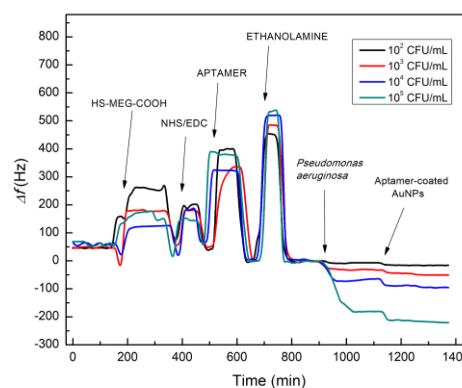

Figure 1: *Changes in the frequency following surface modification, incubation of bacteria and aptamer-coated nanoparticle suspensions.*

Conclusions

We demonstrated the antifouling properties of the new linker SH-MEG-COOH in milk, and of the aptasensor based on this linker, and its specificity to detect of *P. aeruginosa* with a limit of detection 10^2 CFU/mL. We also demonstrated the sensitivity increase by using gold nanoparticles.

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Acknowledgements

This work was funded under European Union's Horizon 2020 research and innovation program through the Marie Skłodowska-Curie grant agreement No. 101007299 (T.H. and M.T.), The Science Agency VEGA, project No. 1/0445/23 (T.H.), and by the Natural Sciences Engineering Research Council of Canada (M.T., NSERC 9522).

(i)SIMPLE: next-generation self-powered microfluidic platform for point-of-care diagnostic applications

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Keywords: Self-powered microfluidics, point-of-care, *in vitro* diagnostics

Introduction

Lab-on-a-chip technology has been a major asset for point-of-care (POC) diagnostic applications. However, most passive liquid propulsion approaches (*e.g.,* capillary microfluidics) do not meet the sample processing requirements of many laboratory tests. Therefore, we developed a self-powered microfluidic concept – (i)SIMPLE – to allow complex liquid handling while being robust, cost effective and user-friendly [1]. Here, we demonstrate its potential with 3 examples, each covering a different level of complexity and analytical performance.

Results and Discussion

First, a microfluidic chip (Fig. 1A) automating all liquid handling steps of a lateral flow immunoassay (LFA) for malarial antigen detection was developed. After applying a blood drop to the sample inlet and finger-press activation, the system carried out the sample metering, waste removal, sample loading and chasing buffer delivery within 5 min and without the need for any user intervention. A similar analytical performance as the reference method was observed (sensitivity ~100 ng/mL). Secondly, for performing therapeutic drug monitoring (TDM) of adalimumab (ADM), a clinically validated sandwich ELISA protocol was downscaled by reducing both sample and conjugate antibody volumes and incubation times. Subsequently, this was implemented in the (i)SIMPLE platform (Fig. 1B) which performed all incubation steps starting from 20 µL of plasma [2]. To further increase the time-to-result while also enabling real-time kinetic measurements, thirdly, a fibre-optic readout principle was integrated for TDM of ADM at the POC in less than 15 min. Hereto, a one-step immunoassay was developed for ADM detection within the clinically relevant range. Starting from 1 µL of plasma, sample dilution, reagent mixing and fluid propagation were all integrated into the autonomously operating (i)SIMPLE chip (Fig. 1C) [3].

Conclusions

By enabling complex multistep on-chip liquid handling, the (i)SIMPLE technology has the potential to become the next generation of high-quality POC diagnostic tests.

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This research received funding from FWO: 1S35118N, 1S33618N, 1S56423N, S003923N; KUL: C32/17/007, C32/21/014, C24/16/022, 1S54823N, IDN/20/011; VLAIO: HBC.2022.0710.

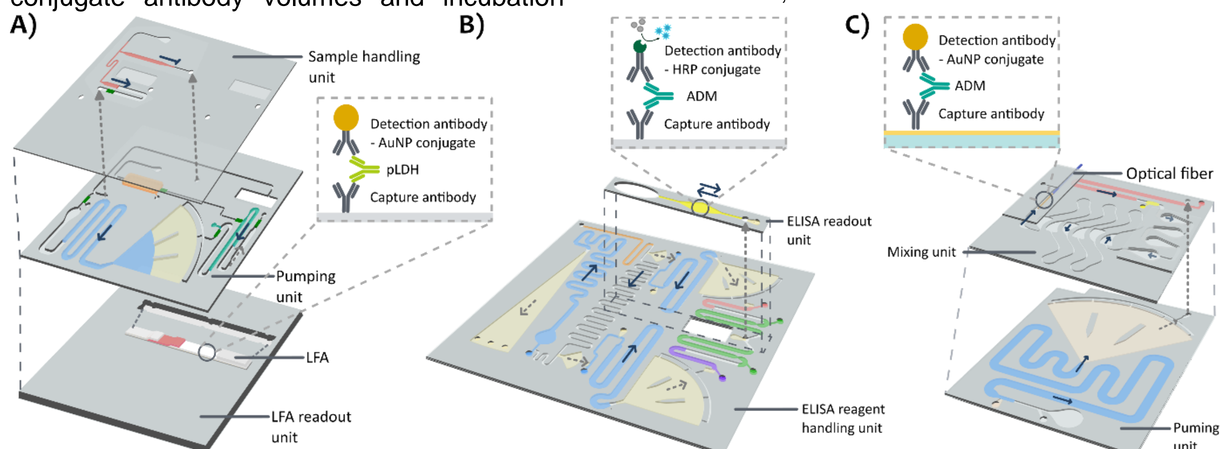

Figure 1: *Schematic illustration of the microfluidic (i)SIMPLE chips with integrated liquid handling for A) LFA, B) ELISA and C) fiber optic readout for POC diagnostic applications.*

Quorum sensing profiling for *P aeruginosa* infection samples from clinical patients

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Keywords: quorum sensing, ELISA, PQS, antibody, *P aeruginosa*

Introduction

Conventional methods for detecting *P.aeruginosa* infections can take several days to produce results, which can be too long for patients in critical condition. One promising approach is the study of Quorum Sensing (QS), a mechanism by which the bacteria communicate with each other and also production of virulence factors and biofilm formation

Materials and Methods

Immunoreagents for the detection of molecules of the Pqs QS system (2-heptyl-4-quinolone (HHQ), 2-heptyl-3-hydroxy-(1H)-4-quinolone(PQS) and 2-Heptyl-4-hydroxyquinoline-N-oxide(HQNO)) and the virulence factor pyocyanin (PYO) have been developed . Highly sensitive microplate-based ELISA have been developed and implemented to the analysis of culture media and complex respiratory samples such as sputa. The ELISAs have been used to quantify PQS, HHQ, HQNO and PYO on a small pilot clinical study.

Results and discussion

The ELISAs have been optimized to detect the analytes in complex culture media such as Müller-Hinton (MH) by a simple dilution with buffer. Clinical isolates from respiratory infections were cultured in MH medium for 16 hours, after which the four biomarkers were quantified. Clear distinctions can be observed between the isolates that come from a chronic infection versus an acute infection

Enzymatic treatment was applied to sputa samples prior to analysis in order to minimize the matrix effect. Excellent clinical sensitivity was achieved for the PQS biomarker, while HHQ provided additional specificity. Accuracy studies were performed by spiking blank sputa samples (which tested negative for *P.aeruginosa*). Good correlation was observed between the spiked values and the measured concentration. These results need to be confirmed with a larger clinical study. The graph below shows the measured levels of PQS and HHQ in positive and blank sputa samples.

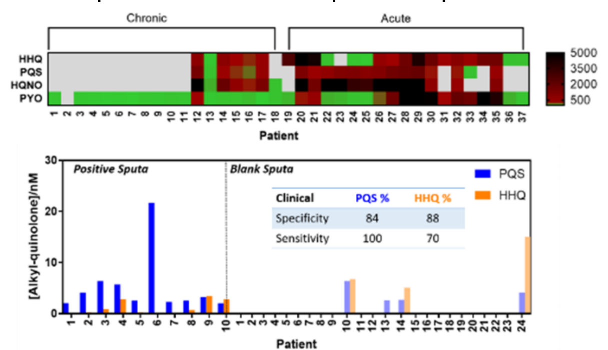

Figure 1: *Top) Clinical isolates analysis of patients from acute and chronical infection origin. Bottom) Preclinical evaluation of of sputa samples for Pqs sytem QS detection*

Conclusions

Immunochemical diagnostic tests developed for P.aeruginosa infections have the potential to improve clinical decision-making. The findings suggest that QS signatures can be used to diagnose infections, classify patients according to disease severity, and provide additional information about the pathology.

Loop-mediated isothermal amplification (LAMP) in the field of biosensors and point of care: Can isothermal amplification outperform PCR?

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Keywords: Isothermal amplification, LAMP, Point-of-Care, Lab-on-Chip, Diagnostics

Introduction

More than 4500 loop-mediated isothermal amplification (LAMP) related articles have been published since its introduction in 2000[1], including more than 1500 in the Corona years from 2020 onwards alone: LAMP has become one of the most important and most promising isothermal PCR alternatives over the past two decades. It seems to be ahead of PCR, the undisputed dogma in the field of nucleic acid amplification, in many areas. It is faster, more sensitive and higher-yielding[2], and also easier to automate or integrate into point-of-care and lab-on-chip applications. It requires only a fraction of energy and equipment and can be combined and extended with a wide variety of detection methods. But in the end, is LAMP really an alternative? Can it replace PCR in the long run, especially in the areas of on-site diagnostics and ambulatory assessment?

In our contribution, we outline current developments from research and medicine and present our own results for the rapid and simple detection of a wide variety of pathogens such as SARS-CoV-2[2] or salmonella[3] in order to answer the question of whether this form of amplification can replace PCR in the long term.

Results and Discussion

In this presentation, we show a wide variety of different applications in which we have replaced the previously used PCR with the LAMP method. In accordance with the current literature, we were able to methodically replace almost all processes. We were able to achieve sensitivities of LAMP down to the detection of only a few cells of various bacteria and viruses. In all cases, the reaction speed was much faster than PCR and detection of analytes was achieved within 15-30 minutes. Both the use as a quantitative method and as a simple, on-site endpoint detection with high sensitivity and specificity have been successfully implemented. A combination with different detection methods is not only described in the literature, but could be successfully established by us (figure 1).

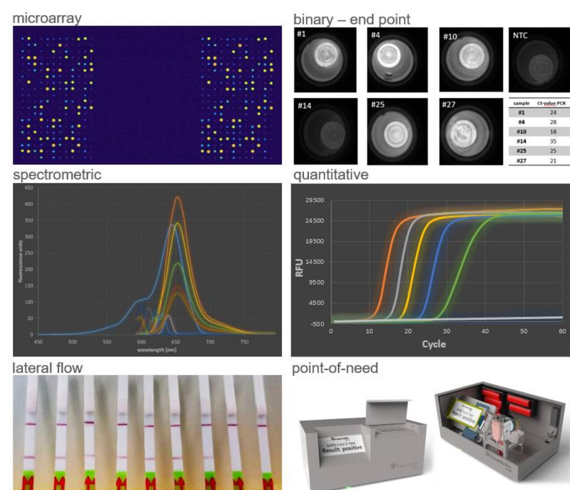

Figure 1: *Possible applications of nucleic acid amplification technologies (NAAT). LAMP can be combined with countless technologies and applied to a wide range of diagnostic questions.*

Conclusions

The LAMP method is a promising alternative to the existing gold standard.

Nevertheless, the question remains whether the isothermal amplification can only be used in addition to the conventional PCR or even has the potential to replace the gold standard in the long run?

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A competitive chemiluminescence immunoassay for the automated detection of surrogate neutralizing SARS-CoV-2 antibodies

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Keywords: SARS-CoV-2, COVID-19 serology, protein-receptor interaction, competitive chemiluminescence immunoassay, neutralizing antibodies

Introduction

The SARS-CoV-2 pandemic has underlined the importance of rapid diagnostic tools, such as serological assays for the detection of neutralizing antibodies. These tests are important for the determination of not only the amount of antibodies formed after infection or vaccination but also their neutralizing potential, preventing the cell entry of SARS-CoV-2 [1, 2]. Besides active-virus neutralization assays, which require biosafety level 3 facilities, virus-free surrogate assays are currently used but take typically several hours until results are available [3].

Results and Discussion

To overcome these drawbacks, we developed a competitive chemiluminescence (CL) immunoassay to detect surrogate neutralizing SARS-CoV-2 antibodies within 7 minutes on our analysis platform MCR-R [4]. Neutralizing antibodies bind to the viral receptor binding domain (RBD) and therefore inhibit the binding to the human angiotensin-converting enzyme 2 (ACE2) receptor. The performance of the competitive binding inhibition test was characterized with a set of 80 samples. The results were well distinguishable with positive samples giving low CL intensities and negative ones giving high intensities (Fig. 1). The assay results are in good accordance with those obtained with an ELISA-based neutralization test and a commercial surrogate neutralization assay. The developed microarray immunoassay could further be used to detect individuals with high IgG antibody titer, but only low neutralization titer, when compared to a total IgG antibody microarray immunoassay. Also, the monitoring of the neutralizing antibody levels after vaccination is possible.

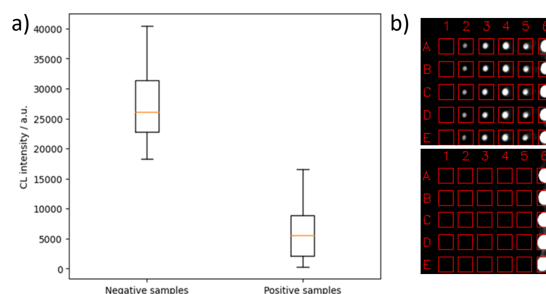

***Figure 1:** a) Neutralization measurements of 33 SARS-CoV-2 seronegative and 47 positive serum samples, b) Measurement images for a seronegative (top) and seropositive (bottom) sample at 1:2 dilution (Column 1: negative control, 2-5: ACE2 in different dilutions from 1:16 to 1:2, 6: positive control) [4].*

Conclusions

We were able to develop a rapid test for the detection of SARS-CoV-2 surrogate neutralizing antibodies in blood samples. The microarray immunoassay could further be used for the detection of individuals with a high total IgG antibody titer, but only a low neutralizing titer, as well as for monitoring the levels of surrogate neutralizing antibodies after vaccinations. The effective test performance in SARS-CoV-2 seromonitoring outlines the potential for the assay to be adapted to other diseases in the future.

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Lateral flow assay based POCT for Loop mediated isothermally amplified nucleic acid with enzymatic incorporation of biotin labelled dUTP and hybridisation of DNA probes to detect N-gene of SARS-CoV-2

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Keywords: Point-of-Care Technique, LAMP-LFA, RT-LAMP, SARS-CoV-2, IVD-device

Introduction

Covid-19 pandemic led to much research on rapid, efficient, and inexpensive alternative POCT diagnostic techniques in contrast to expensive and time consuming PCR/RT-PCR based diagnostics.[1] Isothermal amplification techniques like LAMP and RPA proved to be highly rapid for DNA/RNA amplification with similar efficacy to PCR.[2] Paper based LFA platform when applied for reading out LAMP amplified labelled products (see Figure 1), served as an inexpensive and efficient POCT diagnostic setup for result interpretation by naked eye.[1,3]. The LAMP-LFA synergism can be digitally interpreted and eventuate in giving the range of concentration of viral load in the sample tested. [3]

Results and Discussion

LAMP-LFA setup was established using cDNA and RNA of SARS-CoV-2 N-gene by introducing Biotin-dUTPs and FITC-LF primer during the LAMP amplification. The LAMP amplification of cDNA was optimised for 10 min with 96% accuracy and 15 min for RNA with 82% accuracy. To improve the efficacy of the setup, FITC was incorporated during LAMP amplification tagged to LF primer and biotin was later introduced via a specific DNA probe for the LAMP amplified region of the N-gene. This alternative strategy was tested with the heat-inactivated swab samples of positive and negative patients, and RT-LAMP-LFA was 93% accurate.

The results of LFA were readout via a smartphone based IVD-device, which recorded relative intensities of the test and control bands on the LFA. The data collected showed a linear correlation (R^2= 0.8) between the viral load and intensity of the test band.

We successfully demonstrate a rapid, efficient, inexpensive, POCT platform with smartphone based digital readout technique.

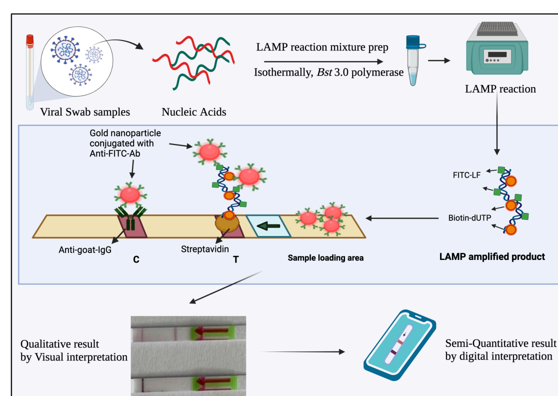

Figure 1: *The principle and experimental setup of LAMP-LFA as a POCT platform, with digitally readout out results of the LFA.*

Conclusions

The demonstrated POCT LAMP-LFA bio-sensor is a versatile diagnostic platform for nucleic-acid based diagnosis. The synergistic technique can be eventually adapted to a variety of pathogens like bacterial, fungal, viral. The digitalisation of LFA results could potentially lead to a "POCT-at home test" setup with the accuracy of a lab-based test. Nevertheless, the setup could be compacted into a paper based microfluidic for ease of use and potentially multiplexed.

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Acknowledgements

This work was supported by BMBF (Federal Ministry of Education and Research, Germany; Grant no. 03COV22B).

Epitope-mimicking peptides as versatile biological tools for the development of mycotoxin immunosensing

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Keywords: mimotopes, peptide mimetics, phage-display, biosensing, luminescence

Introduction

Detection of low molecular weight analytes, such as mycotoxins, is still challenging as direct detection of these molecules requires developing complex immunoassays or using sophisticated instrumentation.

Conventionally, the competitive immunoassay format requires the conjugation or labelling of those mycotoxins, which is challenging and potentially damaging for the user. In this sense, the exceptional ability of epitope mimics, or mimotopes, to substitute the target mycotoxin and elicit an antibody response similar to that of the analyte has been proven, overcoming the limitations of competitive immunoassays [1-3].

In this work, we present the use of phage display technology to select novel epitope-mimicking peptides and its further application in the development of biosensing strategies to detect mycotoxins in foodstuff.

Results and Discussion

Mimotopes for cyclopiazonic (CPA) acid and mycophenolic (MPA) acid were selected independently from a commercial phage display cyclic peptide library by consecutive rounds of biopanning selection [4].

Enrichment of high-affinity binding phages was observed after three panning rounds. Randomly selected monoclonal phages were amplified and screened in phage-based ELISAs. Sequencing of positive clones allowed the identification of epitope-mimicking peptide sequences.

After identifying the sequence coding for novel peptides, they have been chemically synthesized and modified with different optically detectable tags, such us fluorescent probes and nanoparticles. Alternatively, the mimopeptides have been fused to luminescent proteins by genetic engineering techniques.

Heterogeneous and homogenous assay formats have been successfully developed using our mimetic peptides in combination with optical detection providing simple and cost-effective alternatives to traditional immunoassays.

Conclusions

It has been demonstrated the great advantages of mimetic peptides to develop cost-effective immunoassays for mycotoxin sensing. In this sense, epitope-mimicking peptides have a great potential for its implementation in microsystems, lateral flow assays or other point-of-care testing devices.

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Acknowledgements

† The authors would like to dedicate this presentation to the late Prof. María C. Moreno-Bondi. Her impact on science will continue to be felt through her effect on us.

This work has been funded by the Ministry of Science, Innovation and Universities (RTI2018-096410-B-C21/22 and PID2021-127457OB-C21/22). M.G.C knowledges acknowledges financial support from European Union-NextGenerationEU, Ministry of Universities ("Maria Zambrano" postdoctoral fellowship).

Biohybrid tongue for real-time glucose-sensing based on hypothalamic neuronal network

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Keywords: cell-based biosensor, hypothalamic neuron, taste receptors, microelectrode array

Introduction

The expression of sweet receptors in the hypothalamus may play a role in the control of energy homeostasis and the development of obesity and diabetes [1, 2]. The hypothalamic glucose-sensing neurons are known to detect glucose fluctuations, but the mechanism that mediates the function of these glucose-sensing neurons remains unclear. Conventional detection methods such as patch clamp suffer from complex operation, and neuronal calcium imaging is cytotoxic and real-time detection can hardly be achieved. Therefore, hypothalamic neuron network-based bioelectronic tongue coupling with microelectrode array (MEA) was constructed for real-time glucose perception.

Results and Discussion

Primary hypothalamic neuron cells from ICR suckling mice were isolated and then cultured on MEA chips for electrophysiological signal detection (***Fig. 1A***). Immunofluorescence staining was performed using neuronal and astrocyte markers (***Fig. 1B***). The endogenous expression of sweet taste receptors (T1R2/ T1R3) in hypothalamic neuronal cells was investigated (***Fig. 1C***), laying the foundation of hypothalamic neuron network-based bioelectronic tongue for glucose perception.

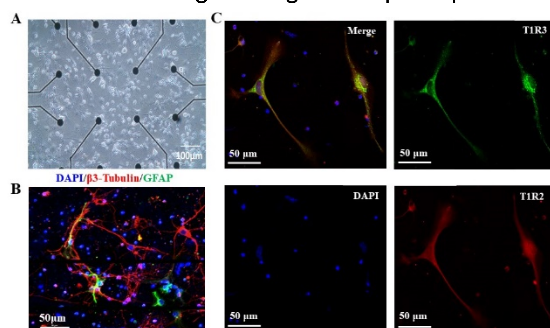

Fig. 1: *In vitro characterization of biological functions of hypothalamic neuron network.*

The spike signal response under glucose stimulation *was shown in* ***Fig. 2***. Besides, the concentration-dependent response was investigated with 10 μM~8 mM glucose. Glucose excitatory neurons (GE-Neuron), glucose inhibitory neurons (GI-Neuron) and non-glucose-sensitive neuron (GN-Neuron) was successfully detected (***Fig. 3***). But whether GE-Neuron and GI-Neuron interact with each other still needs to be further explored through cross-correlation analysis.

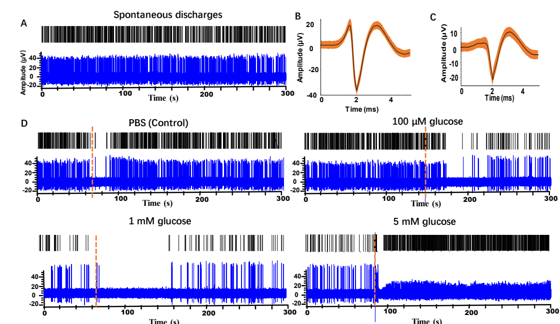

Fig. 2: *Extracellular spontaneous spike activity and the response signal to glucose.*

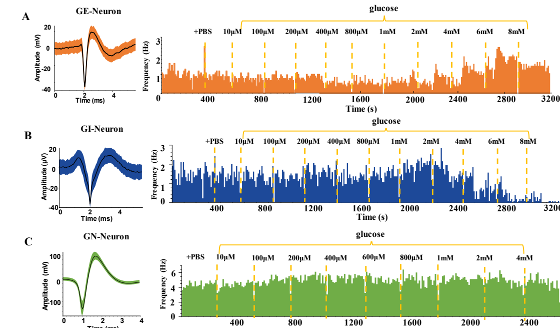

Fig. 3: *The spike waveform and peri-stimulus time histogram (PSTH) of GE-Neuron, GI-Neuron and GN-Neuron.*

Conclusions

Real-time glucose-sensing biohybrid tongue based on hypothalamic neuronal network was constructed in this study, which was important for exploring the role of sweet receptors in the brain in maintaining the balance of glucose metabolism.

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Acknowledgements

This work was supported by the UCL-ZJU Strategic Partner Funding Scheme 2021/22 and Zhejiang Provincial Natural Science Foundation of China under Grant No. LR23C130001.

Advancing food allergy with multiomics molecular-level electrochemical biosensing

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Keywords: food allergy, soybean, red meat, electrochemical immunoplatform

Introduction

The increasing number of food allergenic molecules reported in the last years is a major health concern, considering the severity of allergenic reactions in affected individuals, even with small amounts of food **[1]**. Therefore, consumer protection and food labelling require reliable methods for detection and quantification of allergens in food products capable of scanning allergens of different omic level in a fast, simple, affordable, and point-of-care manner, which can be of great help for diagnostic and therapeutic approaches to increasingly prevalent and varied food allergies.

Aware of all this, our most recent research has addressed the development of two electrochemical immunoplatforms for dual detection of the major soy allergenic protein targets (glycinin and β-conglycinin) **[2]**, and of the main mammalian oligosaccharide (α-Gal) associated with red meat allergy **[3]**.

Results and Discussion

We have developed two amperometric immunosensing platforms for the dual determination of glycinin and β-conglycinin and the individual determination of α-Gal using sandwich and competitive formats, respectively, implemented on the surface of magnetic microparticles and amperometric transduction on disposable carbon platforms.

Both immunoplatforms showed excellent analytical and operational characteristics for the amperometric determination of allergen target standards and potential for their determination in complex food matrices.

The dual bioplatform was applied to the determination of soy allergen targets in raw cookie dough and in baked cookies enriched with soy flour. The results obtained confirmed, in a pioneering manner with electrochemical biosensors, the possibility of discriminating samples incurred with as little as 0.0005 ppm of a food allergen in model cookie extracts.

The bioplatform for α-Gal offered excellent sensitivity (fM level for standards) using a simple and fast protocol (60 min) and is currently facing the analysis of different mammalian meats both raw and processed.

Conclusions

The unique features in terms of simplicity, sensitivity, disposability, affordability and compatibility with use in decentralized environments make these immunoplatforms a very attractive complement to established conventional methodologies for scanning allergens at different molecular levels (protein or carbohydrate) and origin (plant or animal). And that it is worth exploiting their full potential to ensure food safety for manufacturers, distributors and consumers and to advance research and implementation of personalized nutrition.

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Acknowledgements

Financial support of PID2019-103899RB-I00 and Grant S2018/NMT-4349.

Sustainable Design of Online Biosensors

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Keywords: Medical biosensor, sustainability, wearables, system, skin conformable, design

Introduction

The sector of information and communication technology (ICT) has seen a steady increase in global energy consumption, accounting for 5-8% of global electricity consumption in 2020 and for 3% of all greenhouse gas emissions [1]. In particular, the evolving Internet-of-Things (IoT) will become a major consumer with a projected increase from 15 × 10^9 devices in 2015 to 75 × 10^9 in 2025 [1]. Biosensors for medical monitoring or fitness trackers are mostly designed as IoT devices and the question is how to design them so that their use will not neutralize sustainability efforts.

Results and Discussion

1. The sustainability of biosensor systems under development can be assessed on the basis of the 17 UN Sustainability Goals (SDG).

2. Solutions developed by the smartphone manufacturer Fairphone and others may be used for material selection and sustainable production processes of biosensor systems.

3. MICS [2] or BTLE [3] are suitable for energy-saving and harvesting for real-time data transmission of online biosensors.

4 Medical monitoring of patients is undergoing a transition from external wearables such as wrist- and skinpatches, rings, chest straps, headbands, etc. to semi-implants or implant-table biosensors. While the first are used to monitor bodily functions, the latter apply to bio-chemical analytes and biomarkers in the body.

5. Wearable and biosensor users are currently confronted with an unsustainable structure of the Internet. An oligopoly of a few large platforms dominates almost all areas via operating systems for smartphones (Google's Android, Apple's iOS) or computers (Microsoft Windows) and via “social” media (Twitter's microblogger, Meta's Instagram, Facebook and WhatsApp, and Google's YouTube).

6. Information is tapped from users via non-transparent spying techniques in order to derive personality profiles for commercial and political advertising campaigns [4].

7. Health data are subject to special protection according to Art. 9 of the European GDPR.

8. Given the current state of the internet, it may not be expected that this protection can be guaranteed if systems or services are used from the above-mentioned oligopoly.

9. Data storage for online biosensor should be designed in edge mode, so that the data is stored at the user's site and transferred to other entities such as the treating physician only with the user's explicit consent.

10. Tools and apps developed by the FOSS community (free-and-open-source software) should best be used for data processing. A good example is the DRIP app for cycle monitoring, whose basic principles may be applied to biosensors for other parameters.

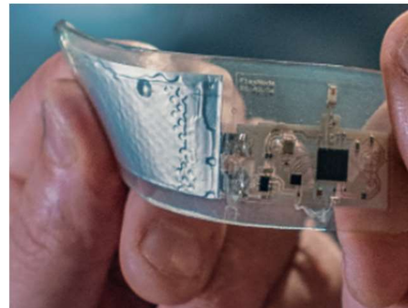

Figure 1: *Roll-to-Roll (R2R) manufactured biosensor with an elastomer integrated BLE utilizing sustainable compo-nents for real-time sensing onto e.g., skin*

Conclusions

A CO_2 footprint minimizing design of online biosensors should always be considered in order to meet SDG 7 and 13. Furthermore, the current structure of the Internet with a few Big-Data monopolists is highly unsustainable. The profits from the personal data obtained exceed the budgets of most states on earth, which is incompatible with SDG 10 that calls for reducing income inequalities within and among countries. Given the current situation, biosensor data should be stored only locally.

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Can polymers replace proteins in assays? Chances and challenges

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Keywords: molecularly imprinted polymers, nanoparticle, direct sensing, protein assays

Introduction

Even though molecularly imprinted polymers [1] have been extensively studied for many years, they have not yet made it into everyday applications, at least on a wide scale, mainly for reasons of reproducibility [2]. One way to tackle this has been the advent of solid phase synthesis of so-called "MIP-nanobodies" [3] that mimic monoclonal antibodies. Herein, we demonstrate their potential in direct and competitive assays to detect entire proteins or smaller peptides. However, this triggers the question, why thin-film MIPs fall short in that regard. In-deep characterization of film surface properties may hold a key for answering it.

Results and Discussion

Solid phase synthesis of MIP nanoparticles (NPs) towards insulin and the NS1 non-structural Dengue virus biomarker, respectively, leads to particles around 80nm in diameter. After sequential elution enriching the high-affinity fraction, they bind to their targets with appreciable selectivity: insulin-MIP NPs prefer insulin over lysozyme by a factor of almost four. Hence, this allows for designing competitive QCM assays that reach detection limits of 80nM and 2.5µM for HSA (human serum albumin) and for insulin, respectively (Figure 1).

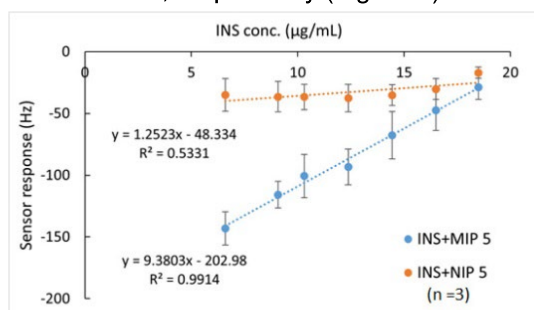

Figure 1: *Competitive QCM assay results for insulin MIP nanobodies.*

In contrast, MIP thin films suffer especially from limited batch-to-batch reproducibility. Tackling this issue is vitally important for sensing: first, it generates sensor layers on the respective transducer surface. Second, it allows for direct assays of the target protein (MIP NPs require immobilizing). However, one first needs to understand the effects of imprinting on the material surface.

Nanomechanical measurements (peak-force QNM AFM) of highly cross-linked polystyrene thin films give first insights: even though homogeneously flat, they reveal varying adhesion and rigidity of the surface. This is in line with the observation that yields of high-affinity MIP nanoparticles are low, because the process selectively enriches high-affinity fractions. Furthermore, when stamp-imprinting silica nanoparticles, particles and imprints do not distribute evenly on the MIP surface, but tend to cluster despite assembling on stamps in an ordered way. Together with Raman microscopy data on bacteria imprinting [4], this gives us the keys to addressing those issues.

Conclusions

The appreciable analytical quality of assays based on MIP NPs (literally) comes at a cost, namely comparatively low yield of high-affinity particles. Together with the inhomogeneities of MIP thin films on the micro- to nanometre level, this strongly indicates that it is necessary to develop synthetic pathways that allow for more precise control of thin-film polymerization.

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Acknowledgements

Part of this work has been funded by the Asea-Uninet University Network through the Austrian Academic Exchange Service (OeAD).

Biosensors in the cloud – Freiburg's embedded potentiostat (FreiStat) provides the missing link for IoT and AI applications

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Keywords: potentiostat, embedded instrumentation, internet-of-things (IoT), artificial intelligence (AI)

Introduction

Artificial intelligence (AI), namely deep learning, promises huge benefits for biosensor applications [1]. At the same time, biosensors play an increasing role in the internet-of-things (IoT) [2]. However, for electrochemical biosensors, we identified a lack of easily-accessible embedded instrumentation which allows for IoT or AI tools, while at the same time providing the electrochemical methods [3] with appropriate analytical performance. Therefore, we developed Freiburg's potentiostat (FreiStat) around the integrated circuit AD5941 (Analog Devices), matching the Adafruit Feather format (Figure 1). The Feather platform provides various extension boards, allowing for wireless communication, e.g., via WiFi, Bluetooth, or LoRa. The FreiStat software framework comprises firmware written in C++ for an M0 microcontroller, working jointly with a Python library for a host computer [4].

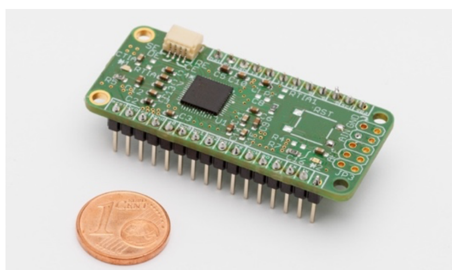

Figure 1: FreiStat main board in the Adafruit Feather format. The footprint is 51 x 23 mm^2.

In this paper, we focus on the capabilities offered by the FreiStat when connected to a single-board computer, such as the Raspberry Pi 3 Model B+, and operated through Python.

Results and Discussion

We demonstrated the FreiStat's capability for biosensor measurements using a variety of electrochemical methods with a performance comparable to analytical benchtop devices. Figure 2 shows the results of a measurement with an enzymatic lactate micro biosensor [5].

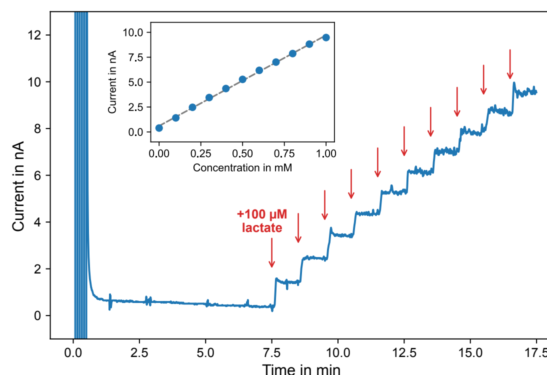

Figure 2: Chronoamperometric measurement with a lactate micro biosensor.

Additionally, voltammetric methods such as cyclic voltammetry and differential pulse voltammetry methods were tested without notable differences when compared to benchtop devices. The IBM Cloud was used to demonstrate a voltammetric analysis as an IoT example with data evaluation in the cloud.

Conclusions

The FreiStat, as a versatile embedded potentiostat, provides electrochemical methods allowing for measurements comparable to expensive analytical benchtop devices. The platform enables simple integration into a cloud for IoT applications and provides an environment for common machine learning libraries.

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Soft molecularly imprinted nanoparticles for the optical sensing of protein biomarkers

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Keywords: molecularly imprinted nanoparticles, protein imprinting, soft materials, optical sensing, plasmonic probes

Introduction

Molecularly imprinted polymers (MIPs) are biomimetics prepared by means of a template assisted synthesis [1]. MIP nanoparticles (nanoMIPs) addressed at targeting protein biomarkers are foreseen as possible alternatives to natural antibodies and receptors [2]. Entailing soft, deformable properties, to protein-recognitive nanoMIPs on plasmonic probles led to attain ultralow detection sensitivities [3].

Results and Discussion

Soft nanoMIPs were synthesized using serum proteins (human serum albumin and serum transferrin) as templates and acrylamides and its derivatives as functional monomers. The physical characterization of the nanoMIPs nanogels resulted in a hydrodynamic size of about 80 nm and a polydispersity index < 0.3. The physico-chemical characteristics of these soft, protein-selective nanoMIPs, studied by dynamic light scattering and by atomic force microscopy force-distance curves, confirmed the deformability of the nanoMIPs. The interaction between the targeted protein-analyte and the nanoMIPs, studied by isothermal titration nanocalorimetry, provided thermodynamic insights into the role of both weak bonds and desolvation in the binding, recollecting biomacromolecular interactions. The soft nanoMIPs were then chemically coupled to a variety of plasmonic probes, including gold nanogratings and unconventional plasmonic platforms, such as light-diffusing fibers (LDFs), plastic optical fibers (POFs), and spoon-shaped waveguides, showing that the analyte-binding led to significant resonance shifts, which in turn resulted in attaining ultralow sensitivities (few attomol of analyte on the sensor) [3-5].

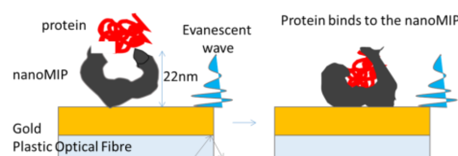

Figure 1. Interaction between the soft nanoMIP and the target protein modifies the nanogel structure.

Conclusions

Providing protein-selective nanoMIPs of responsive properties appears as a key resource to boost the detection sensitivity. This should be further investigated and that might be exploited as a key strategy for sensing development.

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Acknowledgements

AMB thanks MUR for DM 351/2022 PNRR, Missione 4, componente 1.

Single-molecule bioelectronic sensor: improving reliability with machine learning approaches

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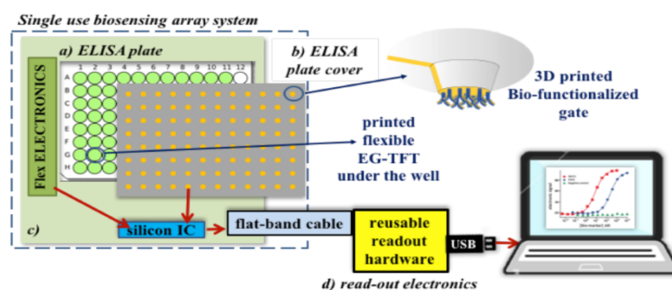
Keywords: machine learning, single molecule detection, bioelectronic devices

Digitizing biomarkers analysis by quantifying them at the single-molecule level is the new frontier for advancing the science of precision health. The enhancement of the technical capabilities of bioelectronics systems, by giving clinicians the possibility to rely on biomarkers quantifications down to the single-molecule, holds the potential to revolutionize the way healthcare is provided. Such an analytical tool will indeed enable clinicians to associate a biomarker tiniest increase to the progression of a disease, particularly at its early stage.[1] The single molecule bio-electronic smart system array for clinical testing - SiMBiT - technology has been developed within the blooming field of precision medicine, leveraging on the single molecule with large transistor (SiMoT)[2] lab-based technology that can perform single-molecule detection of both proteins and DNA bio-markers.[3,4] The SiMBiT prototype has proven its potency in early detection of pancreatic cancer, being capable to discriminate among low-grade and high-grade mucinous cyst's lesions in peripheral biofluids, such as plasma samples. In this perspective, machine learning approaches play a pivotal role in developing classifiers for a fast, reliable multiparametric biosensors output. Supervised model based on multivariate data processing has been undertaken to enable multiplexing, *i.e.* the simultaneous quantification of three biomarkers, namely MUC1 and CD55 proteins and KRAS DNA mutated sequence, in plasma and cysts' fluid samples. The main technological aspect of the SiMBiT device, with particular emphasis on the potency of machine learning approaches, will be discussed.



SiMBiT portable prototype.

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Acknowledgements

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Thermal determination of PFOA in environmental samples

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Keywords: molecularly imprinted polymer, heat transfer method, perfluorooctanoic acid

Introduction

Perfluorooctanoic acid (PFOA) is a member of the polyfluoroalkyl substances (PFAS) and has been widely used for decades in various products. These substances have the ability to bio-accumulate and are potentially carcinogenic and neurotoxic. Therefore, a sensing tool that could enable highly-sensitive, rapid, and cost-effective method for monitoring PFOA is highly desirable [1]. Recently, the heat-transfer method (HTM) has emerged, which is fast, sensitive and reliable and has been successfully employed for detection of various compounds [2]. This method can utilize molecularly imprinted polymers (MIPs) as cheap and promising alternatives to biological affinity reagents [3]. This work therefore demonstrates how the coupling of PFOA MIPs with the HTM can yield a low-cost and robust sensor platform targeted at environmental analysis.

Results and Discussion

PFOA MIPs were created by bulk free radical polymerization. The optimized MIPs (MIP3) where then immobilized on aluminium chips and PFOA rebinding to these chips was analysed using HTM.

In Fig. 1, the time-dependent temperature profile of MIP3/NIP3 to increasing PFOA concentrations (0.1 nM−0.5 μM) in phosphate buffered saline (PBS) is shown. The data demonstrate that increasing the amount of PFOA will cause the temperature registered in the flow cell to decrease. This can be explained by the fact that the target binds to the MIPs, changing the thermal conductivity of the polymer. The limit of detection (LoD) for this sample was 22 pM calculated by the 3σ method. The measurements using NIP particles demonstrate that this effect is far less pronounced, which confirms the non-specific binding of the target to the polymer matrix.

In order to examine the performance of the sensor in complex matrices and to demonstrate its practical applications, river water and soil samples were collected and spiked with increasing concentrations of PFOA to which the sensor was exposed. The LoDs for the river water and soil samples were increased due to the complexity of the matrices. However, they were acceptable as PFOA concentration tends to be higher in river water and soil and they satisfied the regulatory standards.

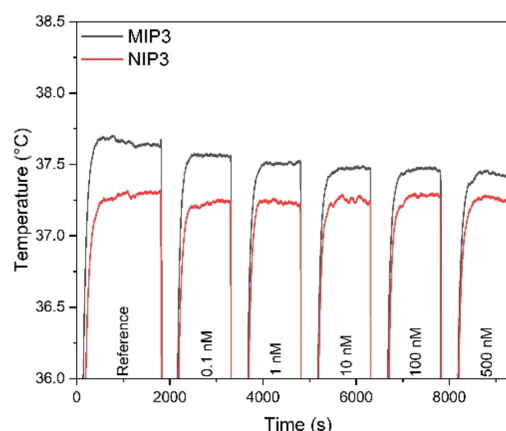

Figure 1: *Temperature response for MIP3 and NIP3.*

Conclusions

In this research, a MIP-based sensor is represented that is able to detect PFOA in complex environmental samples, illustrating the potential applicability of the sensor as a low-cost tool for the fast on-site screening of PFAS contamination.

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Acknowledgements

The work presented in this article is funded by Maastricht University and KU Leuven through the Global PhD Partnership.

Abstracts - short poster presentations

Plasmon enhanced fluorescence and electrochemical aptasensor for SARS-CoV-2 spike protein detection

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Keywords: gold nanohole array, plasmon enhanced fluorescence, aptamer sensor

Introduction

A new dual transducer aptamer-based biosensor is introduced for SARS-CoV-2 spike protein detection, which is the main cause of the recent COVID-19 pandemic [1]. The biosensor combines the recording of plasmon enhanced fluorescence and electrochemical signals associated to binding events between spike protein and aptamer modified gold nanohole array electrodes. These two signals provide redundant information of the same binding process with different sensitivities, and thus extended detection range. The proposed biosensor features a rapid detection of the spike protein of SARS-CoV-2 virus with enhanced signal reliability.

Aptamers are synthetic, single-stranded oligonucleotides that bind to a specific target molecule with high affinity and specificity, alike antibodies. Aptamers can be designed to bind to a wide range of targets, including proteins, small molecules, and even whole cells [2]. Due to their robustness, versatility, and reproducibility, aptamers have emerged as promising alternatives to antibodies in various applications, including biosensing and diagnostics.

The gold nanoholes are fabricated by direct nanoimprint lithography, and their plasmonic properties are utilized to enhance the fluorescence signal of the fluorophore Cy5.5 tagged to the aptamer. The surface plasmon resonance effect in the gold nanohole array creates a high electric field enhancement, which amplifies the fluorescence signal [3]. The biosensor also utilizes aptamer-bound ferrocene as redox-active probe for monitoring of voltametric redox signals simultaneously with the fluorescence. The redox currents emerge from the formation of stem-loop structures after the binding of the spike protein to the aptamer.

The proposed biosensor provides a rapid and reliable detection of the spike protein. The monitoring of surface associated binding processes with two independent transducer systems facilitates an enhanced sensor performance and a better understanding of physicochemical processes at the solid-liquid interface.

Figure 1: *Schematic representation of the fluorescence and electrochemical aptasensor setup.*

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Acknowledgements

We gratefully acknowledge the financial support from the China Scholarship Council (No.201904910560).

Microbeads for Immobilisation of Amphiphilic Biomarkers

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Keywords: hydrophobic, amphiphilic, microbeads, immobilisation, phospholipids

Introduction

Lipids and amphiphilic molecules are ubiquitous and play a central role in the human body. Lipid alterations occur in diseases such as cardiovascular disease [1], cancer and autoimmune diseases. Therefore, detection systems for lipids and amphiphilic molecules are needed. Microbeads are powerful for simultaneous quantitive multiplex detection of different lipophilic biomarkers [2].

Results and Discussion

Fluorescence-encoded and solvent-resistant microbeads for immobilising amphiphilic molecules did not exist until now. We succeeded in producing such microbeads. By passive adsorption, the phospholipids A, B and C could be bound to the microbead surfaces. Directional binding of these was confirmed by enzymatic reaction. Microbeads with a hydrophilic surface bind phospholipids non-directionally and are no longer reactively accessible. Thus, a hydrophobic microbead surface is mandatory. Exemplary for the application of this microbeads, anti-IgG cardiolipin antibodies were detected with the hydrophobic microbeads from human serum.

Conclusions

With the newly developed hydrophobic, dual-encoded and solvent-resistant microbeads, it is possible to bind amphiphilic biomolecules to the microbead surfaces in a directed manner. One application of the platform is the detection of anti-phospholipid antibodies from human serum.

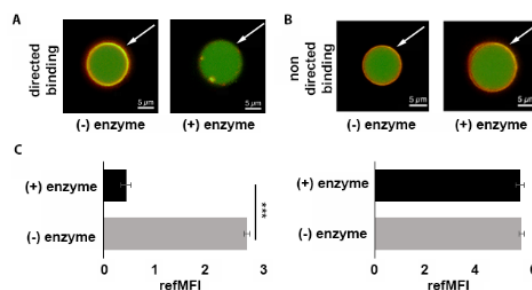

Figure 1: *Directional binding of phospholipids. The directional binding of the phospholipids on the microbead surface with the help of phospholipase C. (A) directed binding on the hydrophobic surface. (B) non-directed binding on a hydrophilic surface. (C) comparison of refMFI values from hydrophobic and hydrophilic microbead surface.*

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Acknowledgements

The authors received funding from the Federal Ministry of Education and Research – Unternehmen Region – Wachstumskern PRAEMED.BIO (03WKDB2C).

Towards a Digital CRISPR-Powered Biosensing Platform for Nucleic Acid Detection Using Single-Impact Electrochemistry

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Keywords: CRISPR-based diagnostic, nucleic acids quantification, single-impact electrochemistry, silver nanoparticles, microfluidics.

Introduction

An emerging approach for the detection and quantification of nucleic acids is clustered regularly interspaced short palindromic repeats (CRISPR)/Cas-based diagnostics, which relies on the specificity, programmability, and ease of use of the CRISPR technology [1]. Class 2 systems, such as Cas12 and Cas13, have been particularly utilized for the detection of nucleic acids due to their non-specific collateral cleavage activity upon target recognition. Current methods primarily employ standard fluorometric, colorimetric, or electrochemical readouts [2]. Here, we present our results towards establishing a nanoparticle-based CRISPR-powered biosensor concept for reliable and highly selective quantification of nucleic acids using a digital readout through single-impact electrochemical detection. The detection scheme is shown in Figure 1.

Results & Work in Progress

In a first step, commercial silver nanoparticles (AgNPs) were functionalized with mono-thiolated single-stranded DNA using a freeze-thaw cycle similar to the protocol of Liu et al. [3]. Successful functionalization was confirmed by UV/Vis spectroscopy and dynamic light scattering, indicated by a slight red-shift and an increased hydrodynamic radius, respectively. In a next step, the DNA-AgNP conjugates are immobilized on a microfluidic chip and released by the collateral cleavage activity of the Cas enzyme. Released AgNPs are oxidized on a microelectrode array, resulting in a current transient. The concentration of the target sequence can then be determined by simply counting the number of observed spikes in a given time frame [4].

Conclusions

Our proposed sensor concept will combine the collateral cleavage activity of CRISPR/Cas Class 2 systems with the detection of AgNPs using single-impact electrochemistry as a novel digital readout strategy. This enables an amplification-free, ultrasensitive, and reliable quantification of diagnostic, prognostic, and monitoring nucleic acid-based biomarkers for point-of-care applications.

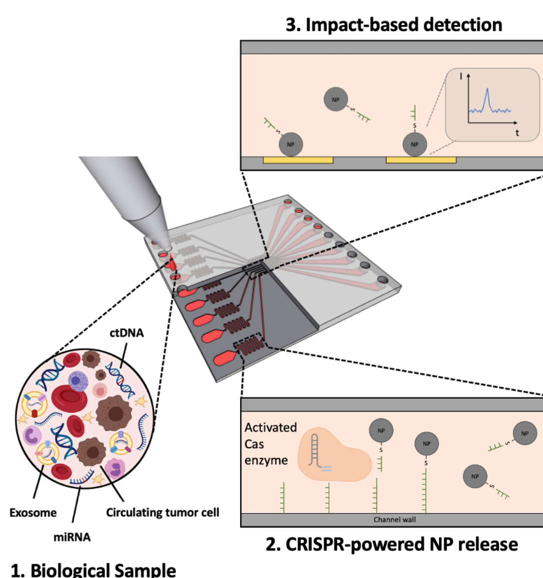

Figure 1: Digital CRISPR-powered sensor concept. *1) A biological sample is applied to the platform. 2) In the presence of the target, collateral cleavage activity is activated and immobilized AgNPs are released. 3) Detection of collision events on a biased microelectrode.*

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A Single-Step Magnetic Bead-Based Sandwich Immunoassay for the Rapid Detection of Ciguatoxins in Fish Samples

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Keywords: Ciguatoxins (CTXs), Ciguatera, Immunoassay, Magnetic Beads (MBs), Fish

Introduction

Ciguatoxins (CTXs) are known for being the causative agent of one of the most prevalent seafood-borne disease worldwide: ciguatera fish poisoning (CFP). CTX-producing microalgae used to be endemic from tropical and subtropical areas, but lately have been found in regions with more temperate waters, like Europe. This situation highlights the need for new bioanalytical tools to detect the presence of CTXs in fish and to protect consumers from CFP. In this work, a simplified colorimetric magnetic bead-based sandwich immunoassay is reported as a fast bioanalytical tool for CTXs screening in fish samples.

Results and Discussion

The development of a sandwich immunoassay typically requires multiple incubation steps for each one of the components of the assay. In this work, we propose the simultaneous incubation of all the components to simplify the protocol and shorten the analysis time. A schematic representation of the proposed sandwich configuration is shown in Figure 1.

Initially, the colorimetric signals obtained with the single-step configuration were lower than the ones obtained with the conventional approach **[1]**. Nevertheless, the optimization of reagents concentrations allowed to achieve comparable signals and similar analytical parameters between both approaches.

The effect of the fish matrix on the immunoassay performance was evaluated using naturally contaminated fishes. No significant differences were observed in CTXs quantifications obtained with the simplified assay in comparison with the conventional one. Finally, negative fish extracts were spiked with CTX1B at the FDA level of 0.01 µg CTX1B equivalents / kg, and recoveries around 100% were obtained.

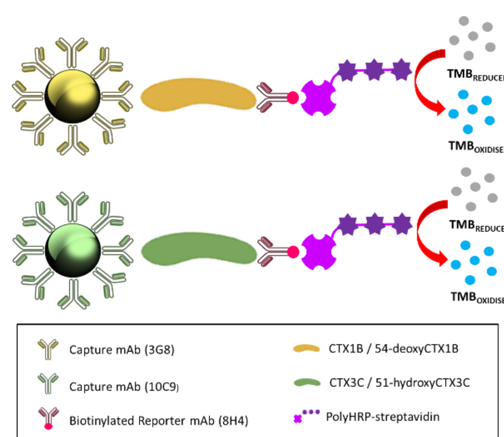

Figure 1: *Simplified magnetic bead-based sandwich immunoassay configuration for the detection of CTXs.*

Conclusions

The simplified single-step colorimetric magnetic bead based sandwich immunoassay was able to detect CTXs in fish samples at the FDA level and in only 40 min. This rapid and user-friendly bioanalytical tool is promising for its application in low-resource settings, as well as for research and monitoring activities.

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Neurotransmitter sensing using aptamer modified nanopipettes *in vitro* and *ex vivo*

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Keywords: Aptamer, nanopipette, nanopore, neurotransmitter, dopamine

Introduction

Small molecules can be rendered detectable down to picomolar levels through the fusion of nanopores with conformationally switching aptamers.[1] Aptamers are artificially designed single stranded DNA sequences, engineered to react specifically to a target of interest. We tether neurotransmitter specific aptamers (dopamine and serotonin) inside of quartz nanopipettes with openings of *ca.* 10 nm diameter. Upon neurotransmitter binding, the aptamers, covering the lumen of the nanopipette, undergo a conformational rearrangement, resulting in altered charge distribution within the pore. The aptamer specific modification in charge distribution translates to a measurable current shift. [2]

Results and Discussion

We have demonstrated that dopamine interaction with our nanopipette results in a decrease in current, both in *in-vitro* systems, where known dopamine concentrations are exposed to our sensor (Fig. 1) as well as in *ex vivo* brain slices, where we measure endogenously released dopamine (Fig. 2). We have developed a reset protocol, enabling us to calibrate our sensors prior to use.

To further validate the specificity of our sensor's signal, we have designed a reference nanopipette, functionalized with scrambled DNA sequences. These scrambled sequences are designed to have the same number and base pairs as the specific sensor, but in an altered order, hindering recognition and binding of the target analyte. These reference nanopipettes are exposed to the same ionic environment, allowing us to have a comparative measurement.

Conclusions

Measuring neurotransmitters in low concentrations and complex environments such as brain tissue, allows us to project applications of our sensors to better understand and potentially monitor neurological diseases.

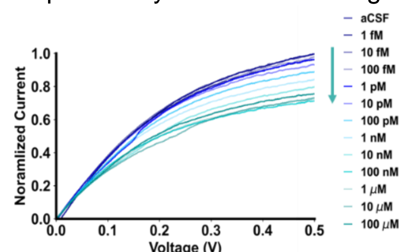

Figure 1: *Monitoring the current in response to exposure of known concentrations of dopamine in-vitro, while sweeping a potential. Dopamine binding results in a decrease in current.*

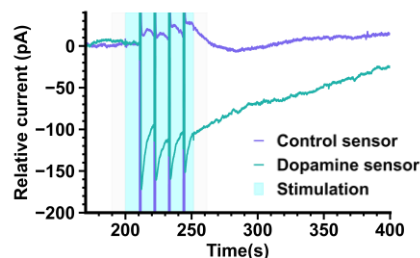

Figure 2: *Dopamine and control sensors are deployed into a brain slice. The tissue is subject to high frequency stimulation, resulting in a measurable endogenous dopamine release.*

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This work was funded by ETH Zürich. The authors would like to thank Anna Cavaccini for her technical support with ex-vivo brain measurements.

Magnetic nanoparticle based sensor for detection of *Brucella* DNA at point of care

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Keywords: Magnetic nanoparticles, DNA detection, Frequency mixing magnetic detection, Brucellosis

Introduction

Zoonotic diseases are responsible for most emerging human infections and have the potential to cause widespread outbreaks. Brucellosis is one of the most common bacterial zoonotic diseases worldwide; it is endemic in several developing countries in the Middle East and Asia. Currently, conventional diagnostic assays used for the detection of Brucella are mainly based on serological tests, blood culture, and molecular methods. These methods have limitations in terms of time and are not suitable for field testing. Therefore, there is a need to develop a rapid, selective, and accurate assay for early on-site detection of *Brucella* which helps hindering the spread of the disease.

Results and Discussion

The developed assay is based on the magnetic sensing of the sequence-specific hybridization event between the immobilized capture probe and its complementary biotinylated target DNA on porous polyethylene filter (PE) see Figure 1. The PE filter surface was modified with an amine group to promote covalent bonding with capture probe. The hybridization was done via flushing ssDNA by gravity flow through the filter. Hybridized biotinylated target DNA was detected by streptavidinated magnetic nanoparticles using frequency mixing magnetic detection (FMMD) technology [1]. The measurement was done inside a small portable magnetic reader.

In our study, the detection principle was successfully implemented. The signal amplitude was considerably higher in the presence of Brucella target DNA. The assay was able to detect the ssDNA target in less than 2 min hybridization and 3 min of magnetic nanoparticles incubation time.

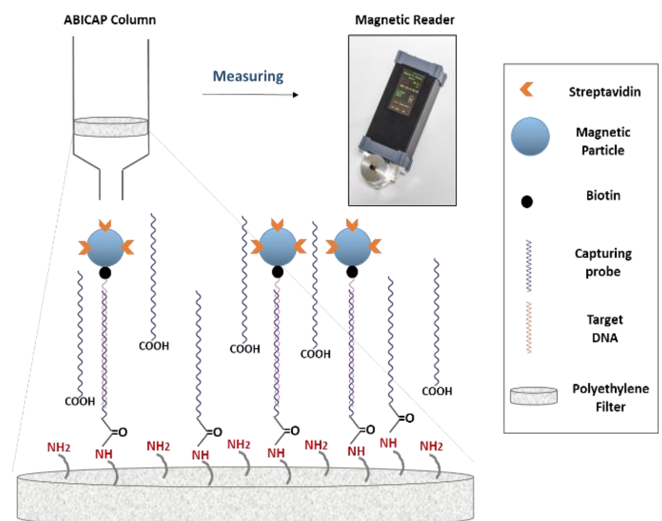

Figure 1: *The principle of detection of the designed magnetic based DNA sensor*

Conclusions

Compared with conventional methods, our developed assay is highly sensitive and specific in detecting Brucella DNA. Using a portable magnetic reader for mobile testing near the main source of infection may help control the spread of the disease.

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This work is supported by the German Federal Ministry of Education and Research (BMBF) within the framework of the Palestinian-German Science Bridge (PGSB).

Development of a homogeneous, one-step liposome-based assay for the detection of anti-SARS-CoV-2 antibodies

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Keywords: Liposomes, SARS-CoV-2 diagnostics, High-throughput screening

Introduction

Lipid bilayer vesicles, known as liposomes, are widely used as carrier and controlled release systems not only for medical but also for bioanalytical applications. [1] By using liposomes encapsulating the fluorescent dye sulforhodamine B (SRB) and modified with the receptor-binding domain (RBD) of SARS-CoV-2, a novel homogeneous assay for the detection of anti-SARS-CoV-2 antibodies was developed. Targeted liposome lysis was achieved by exploiting the complement system which is part of the innate immune system and a highly complex interaction of several serum proteins leading to formation of membrane attack complexes and thus lysis of pathogens or liposomes. [2] The complement system can be activated by various trigger molecules, in this case by the Fc-fragment of anti-SARS-CoV-2 antibodies bound to RBD on the liposomal surface.

Results and Discussion

Bioconjugation of RBD to liposomes using EDC/NHS chemistry was investigated and optimized. Subsequently, the interaction of RBD-modified liposomes with immobilized angiotensin-converting enzyme 2 (ACE2), the human receptor targeted by the SARS-CoV-2 virus, was investigated in heterogeneous assays to verify successful protein-coupling.

RBD-bearing liposomes were applied in complement-based assays after anti-SARS-CoV-2 antibodies were allowed to bind to the liposomes. This yielded in antibody-triggered complement lysis of RBD-liposomes correlating to the antibody titers of the patient sera. Inhibition curves were obtained by implementing free ACE2 as a competitor for anti SARS-CoV 2 antibodies to distinguish between neutralizing and binding antibodies.

Conclusions

This liposome-based platform facilitates a complement system-dependent readout, which can be applied in microplate-based high-throughput screenings (HTS) either for viral or immune status detection. The main advantage of this assay over other antibody detection strategies is the homogeneous assay format, which does not require multiple washing steps like the commonly used heterogeneous formats.

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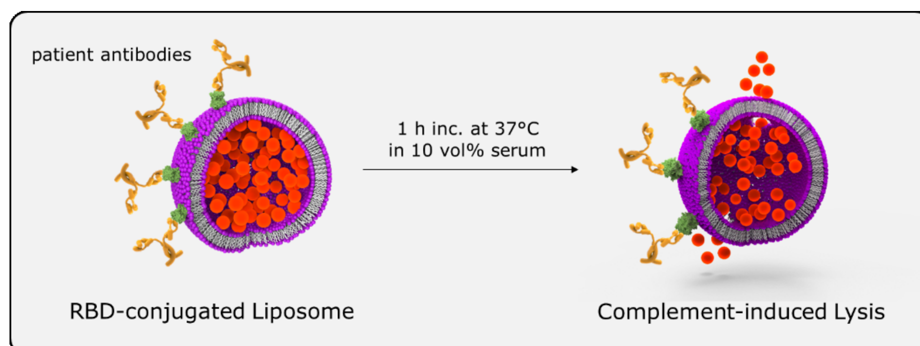

Figure 1: *Schematic of the developed homogeneous serum-based assay towards the detection of anti-SARS-CoV-2 antibodies using RBD-conjugated liposomes in a complement system-dependent readout.*

Development of a multiparameter sensing platform based on impedance and thermal sensing for monitoring of advanced biological applications

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Keywords: impedance spectroscopy, thermal sensing, data fusion

Introduction

Multi-device synchronous measurements are becoming more and more popular in research. Different sensors have their own limitations and uncertainties. By combining different sensing system and the fusion of their data, we can increase the accuracy and performance of the system [1].

In biomedical research, impedance spectroscopy has proven its power for the detection of various cellular properties (cell viability, cell-barrier formation, etc.) [2]. Besides, the use of thermal sensing has proven to be effective for the label-free detection of metabolic activity and cell count during cell growth [3].

This work combines the use of an impedance (IDE) and thermal (TPS) sensing element into one measuring platform. The combined measuring principle has already been established in our previous work [1]. However, the setup has to be tailored towards the detection of biological samples. By the combination of both sensing techniques, and fusion of data, a deeper insight into specific biological principles can be achieved.

Results and Discussion

The platform is based on the format of the gold standard in biomedical research, a 96-well plate. A sensor strip forms the bottom of an 8-well plate (⌀ = 7 mm), locating the sensing elements underneath the sample of interest. The impedance structure (⌀ = 7 mm) is located at the top of the strip, making contact to the biological sample (Figure 1). The thermal sensing structure (⌀ = 5 mm) is located on the bottom, electrically isolated from the sample (Figure 1). This way, by alternating the measurement of the impedance and thermal sensor, the electrical and thermal properties of the sample can be assessed.

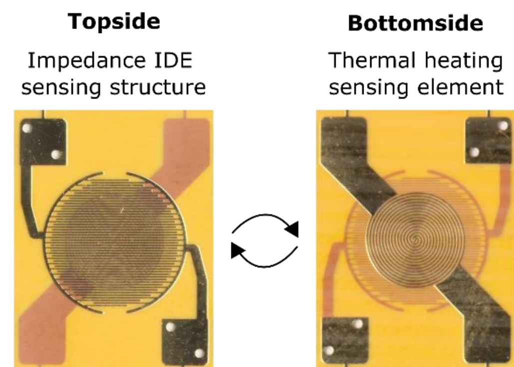

Figure 1: The impedance sensing element (IDE: width and spacing of 60 µm) and the thermal sensing element (spiral heater: width and spacing of 60 µm and 80 µm).

This enables us to monitor the electrical footprint of the sample, while assessing the change in thermal properties during a biological process.

Conclusions

This research aims to develop a multi-parameter sensing platform to monitor various biological processes and gain a deeper understanding of the underlying mechanisms. Different vertical pillars can be explored to demonstrate its application potential.

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This work was supported and funded by Fonds Wetenschappelijk Onderzoek (FWO) – Vlaanderen (1SD7923N).

Enhanced performance, stability and life-time of potentiometric ion-selective elelctrodes mediated by biochar transducer

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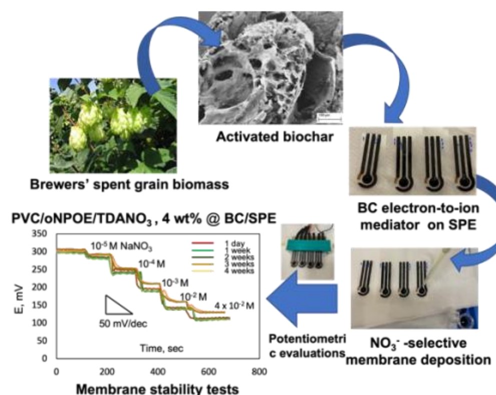
Keywords: ion-selective sensors, nanostructured biochar mediators, nitrate sensing

Introduction

Biochar (BC) is a highly porous carbonaceous material obtained from biomass pyrolysis [1]. BC bears different functional chemical groups on its surface, has high redox activity and may interact with different organic and inorganic compounds. Previosly BC materials have been actively used in waste manegment, elvironmental remediation and bio-energy production, and as mediators in chemical sensors development [2]. While activey exploted voltammetric sensors development [3], the BC application in potentiometric ISEs was investigated in the mach smaller degree. In this work, BC obtained from brewers' spent grain rich of common hop (Humulus lupulus) species was tested as ion-to-electron signal mediator for all-solid-state nitrate-selective electrodes development. The performance of BC mediated nitrate-selective sensors on terms of stability, reproducibility and life-time was compared to the properties of the electrodes with the same NO3- - sensing material, modified with conductive polypyrrole (PPy) layer as ion-to-electron transducer, commonly employed nowadays in all-solid-state of potentiometric electrodes [4].

Results and Discussion

The 1 mg/mL BC dispersions in ethanol was treated in an ultrasonic bath for 60 min. The BC/SPEs were assembled via drop casting of 5 uL of BC dispersions on bare graphitic SPE; the solvent was evaporated for 30 min in oven at 37 °C; BC depositions were repeated 3 times on the same SPE. PPy film was deposited on SPE electrodes from 0.15M solution of pyrrol in acetonite and 0.1M $TBAClO_4$ background electrolyte by CV in -0.3 - 0.9 V range at 100 mV/s scanrate for 10 cysles. The PVC polymeric membrane plastisized with oNPOE plasticizer in 1:2 ratio and doped with 4 wt% of $TDANO_3$anion excghanger was drop-casted on BC and PPy modified SPEs and tested in $NaNO_3$ solutions in the consentration range from 10^{-5} to $4x10^{-2}$ M consentrationa range on 0.01M HEPES pH 7.5 background, Scheme 1.



Scheme 1: *The schematic presentation of BC-modified* NO_3^-*-selective sensors preparation and testing.*

The application of BC mediator has significantly improved the stability and life-tiime of tested nitrate-selective sensors through improved electron transport efficiency between solvent polymeric and SP WE. The stabe close to Nernstian response slope of BC/NO_3-sensors, -48.2 ± 0.9 mV/dec, was registered over the one month of sensor testing. PPy/NO_3-sensors have shown less background potential stability, lower reprodusibility and sub-Nernstian slope of -40.7 ± 7.1 mV/dec while tested in parallel at same conditions. The obtained preliminary results indicate the biochar potential to serve as an effective ion-to-electron transfer mediator in the development of stable and reliable ISEs. The tests on BC obtained from different plant biomass application as mediators for all-solid-state ISEs development are in progress in our laboratories and will be discussed in details in or presentation.

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A highly sensitive antibiotic biosensor based on a field-effect capacitor modified with a bilayer of polyelectrolyte/plant-virus particles

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Keywords: *tobacco mosaic virus,* field-effect biosensor*,* enzyme nanocarrier, penicillin biosensor

Introduction

Tobacco mosaic viruses (TMVs) are nanotubular plant viruses with a length of 300 nm and a diameter of 18 nm. Engineered TMV variants with more than 2000 thiol groups on their protein coat are attractive nanocarriers for developing field-effect biosensors, enabling binding of a variety of biomolecules on their outer surface. Thus, TMVs displaying different analyte-specific enzymes were used to develop various types of capacitive electrolyte-insulator-semiconductor (EISCAP) biosensors [1].

Results and Discussion

A novel approach has been investigated to immobilize a high density of TMV particles on the Ta_2O_5 gate surface of an Al/p-Si/SiO_2/Ta_2O_5 EISCAP: the surface was modified by an additional positively-charged polyelectrolyte poly(allylamine hydrochloride) (PAH) layer prior to the immobilization of the negatively charged TMV particles (see Fig. 1). The biosensors went through physical characterizations, like atomic force, scanning-electron and fluorescence microscopy. Finally, TMV-assisted penicillin biosensors were prepared by immobilizing the enzyme penicillinase on TMV nanocarriers. These novel biosensors were examined by means of capacitance–voltage, and constant capacitance measurement techniques for a penicillin concentration range of 0.1 to 5 mM with a mean penicillin sensitivity of 113 mV/dec.

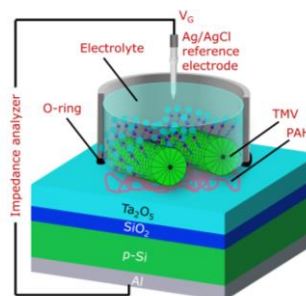

***Figure 1:** Schematic of the experimental setup used for the characterization of the PAH/TMV-modified EISCAPs.*

Conclusions

A positively charged polyelectrolyte layer was applied on an EISCAP surface to allow immobilization of a high density of TMV nanoparticles and then, functionalize them with enzymes, such as penicillinase, for penicillin detection. The new immobilization strategy can be upgraded to detect further target molecules with beneficial prospects in biotechnology, biomedicine and environmental analysis.

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Acknowledgements

This work was funded by the Deutsche Forschungsgemeinschaft (DFG: German Research Foundation)-446507449.

Tailoring the Peptide Interface for Protease Detection via Multi-parametric Surface Plasmon Resonance Spectroscopy

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Keywords: peptide interface, protease biosensor, MP-SPR

Introduction

Proteases are emerging biomarkers for a variety of medical issues, including cancer, cardiovascular, neuro-degenerative or inflammatory diseases. Accurate protease detection would improve our understanding of protease function and may lead to new treatment options for patients.

Proteases cleave peptides or proteins in the body. This natural process can be mimicked to develop novel protease detection assays based on synthetic peptides. While significant progress has been achieved in the field of peptide-based biosensors, several major challenges remain such as spurious signals due to non-specific binding and insufficient sensitivity.

Here, we aim to design a novel peptide-based sensing interface for the highly selective detection of a relevant protease (matrix metalloproteinase, MMP-9). The assay was monitored in situ via multi-parametric surface resonance (MP-SPR) spectroscopy. MP-SPR offers highly sensitive and label-free detection of molecular interactions.

Results and Discussion

The gold surface of SPR chips was modified by self-assembled monolayers of linker molecules followed by covalent attachment of artificial peptides. These surfaces were exposed to different concentrations of MMP-9 in the buffer. The entire process was monitored by MP-SPR (Figure 1 a). In this assay, we observed a signal reduction upon exposure to MMP-9. This reduction indicates that peptides are cleaved by the protease. Moreover, the signal reduction depends on MMP-9 concentration, a prerequisite for successful biosensor development (Figure 1 b). Control chips coated with peptides without cleavage sites did not show a signal decrease, suggesting the selective nature of the observed interaction. Using this assay, we could detect MMP-9 levels as low as 5 pM which is much lower than that reported in other papers [1, 2].

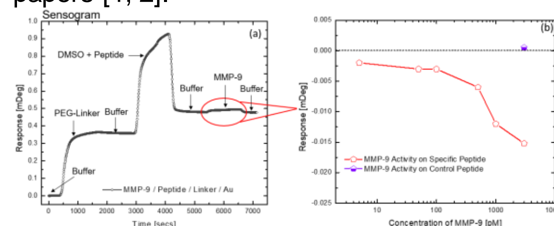

Figure 1: *(a) SPR sensogram displaying the immobilization steps and exposure to MMP-9, (b), and c) signal suppression vs. different concentrations of MMP-9 in PBS buffer (pH 7.4) at 30°C.*

Conclusions

These results demonstrate that by tailoring the surface with a specific combination of linkers and peptides, a robust interface for detecting MMP-9 protease with high specificity and sensitivity can be created. The established heterogeneous assay is versatile and can be transferred to other transducer types including electrical and electrochemical detection schemes.

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Acknowledgments

The authors gratefully acknowledge funding by the Ministry of Science and Health of Rhineland-Palatinate (project MultiSensE).

Towards modulating near-field plasmonic coupling for enhanced optical spectroscopy

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Keywords: responsive hydrogels, gold nanoparticles, plasmon-enhanced spectroscopy.

Introduction

Metallic nanostructures allow for tight confinement of electromagnetic field through the resonant excitation of surface plasmons. The associated enhancement of field intensity offers efficient means for the amplification of weak optical spectroscopy signals (such as fluorescence, Raman scattering, or infrared absorption). Typically, the metallic nanostructures are made static. A possible route to expand the spectrum of applications and performance of plasmon-enhanced spectroscopy tools is pursued, based on responsive hydrogel materials that act as artificial muscles and provide on-demand, reversible reconfiguration of plasmonic hotspots[1].

Results and Discussion

Responsive hydrogels are capable of on-demand swelling and collapsing around ~5/10x the dry thickness. When supporting an array of AuNPs and placed in close proximity with a metallic layer (Au, in this work), they allow for plasmonic hotspots to be excited at the AuNPs / Au surface gap, leading to strong enhancement of field intensity, as depicted in Fig. 1a).

Finite Element Method simulations are performed to understand the far-field and near-field properties of such device. Experimental work will be performed to confirm simulation results.

Results show that the collective LSPR (cLSP) excitation dip, given by diffraction coupling of LSP modes on neighboring AuNPs of the array, is splitted when a gold layer is placed at tens/hundreds of nm from the array, as depicted in Fig. 1b). This is due to coupling of the cLSP mode to SPPs at the flat metal surface.

Simulations show that the distance between AuNP array and Au layer and the polar angle of irradiation θ are critical parameters for the tuning of the excitation.

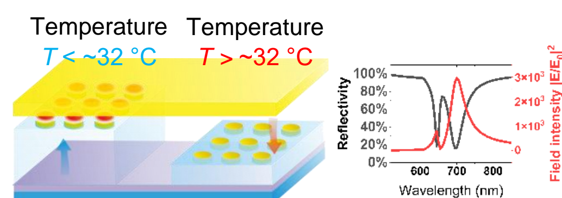

Figure 1: *a) Flow-cell concept, featuring reconfigurable onset of plasmonic hotspots. b) Splitting of LSP resonance.*

The LSPR excitation is sensitive to AuNP / Au layer distances, due to interference with the back-reflected light at the Au layer. This leads λ_{LSP} to blue-shift for longer distances.

When the polar angle θ is varied, anti-crossing of the λ_{LSP} and $\lambda_{cLSP/SPP}$ occurs, indicating a strong coupling between LSP and SPP modes[3].

Conclusion

The presented concept lays the theoretical foundations to the fabrication of such device. The strong field enhancement at the AuNP-surface gap can be exploited for probing of molecules by plasmon-enhanced optical spectroscopy.

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Acknowledgements

This work was supported by the Czech Science Fund, project APLOMA (22-30456J) and from Austrian Science Fund (FWF) through the Lise Meitner Programme (M 2925) and the project DIPLAB (I 5119).

Degradation of synthetic hydrogels by an *in vitro* automated reactive accelerated aging assay

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Keywords: Synthetic hydrogels, *in vitro* reactive accelerated aging, material degradation

Introduction

Hydrogels are very promising candidates for use in implant technology and biomedical sensors due to their easily achievable biocompatibility and wide variety of base polymers they can be made of [1]. However, employing them for *in vivo* applications requires extensive testing to obtain clearance for medical use. In order to reduce times, costs and the need for corresponding animal experiments, novel *in vitro* methods for material degradation studies need to be developed [2]. One promising approach for that purpose is reactive accelerated aging (RAA). By exposing materials to harsher-than-life conditions, such as increased temperatures and concentrations of reactive oxygen species (ROS), material degradation can be simulated at an accelerated rate. This RAA approach has so far been used for comparing aging of neural implants to results from animal studies and a good agreement was demonstrated [3].

Here we report on a study for applying this concept to the class of hydrogels. Therefore, we have set up an *in vitro* automated reactive accelerated aging (aRAA) based on physico-chemical processes mimicking aspects of *in vivo* immune reactions, to artificially age synthetic hydrogels.

Results

An aRAA setup similar to the one described in [3] was modified to be used for aging synthetic hydrogels (Fig. 1). We optimized controls to ensure the reaction conditions in the assay remain stable throughout experiments over prolonged amounts of time.

In initial experiments, we have aged different synthetic hydrogels as well as common encapsulation materials for biosensors [5].

We observed varying degrees of changes to the materials by macroscopic as well as microscopic evaluation. Differences in discoloration, material integrity and volume change were observed.

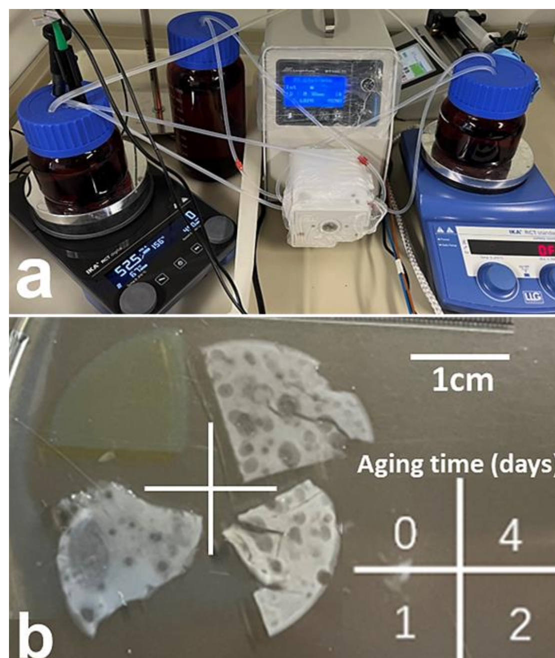

Figure 1: *(a) aRAA set up (b) PNIPAAm hydrogel doped with silver nanoparticles (50nm diameter) before and after different aging times*

Conclusions

The aRAA set up is suitable to simulate material aging *in vitro*. Different materials exhibit distinctive characteristics of degradation upon artificial aging. In the future, we aim to show the comparability of aRAA results to previously published datasets from animal and human studies and cell-based assays. Showing a correlation between *in vitro* and *in vivo* processes for material aging will allow optimizing the aRAA setup for broader use and significantly facilitating future studies by providing a time and cost-efficient way to study material degradation processes.

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AC electrokinetic immobilization of single biomolecules on nano-electrode arrays

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Keywords: single molecule, dielectrophoresis, nanoelectrode, immobilization, AC electrokinetics

Introduction

A key element in the construction of a biosensor is the immobilization of the bioreceptor. In common biosensors the bioreceptor molecules are immobilized as randomly oriented objects on one transducer, leading to a comparatively low ensemble signal due to the combined measurement of all analytes. Immobilizing individual, addressable biomolecules would allow to exactly locate the signal's origin achieving a new level of biosensing. This goal can be achieved by a label-free method of immobilization: Dielectrophoresis (DEP). DEP is a phenomenon in which a dipole is induced in a polarizable particle in an inhomogeneous (AC) electric field. By the right choice of voltage and frequency, this particle can be moved without damage and can be immobilized. Enzyme activity and antibody binding function are preserved after DEP application [1, 2] and biomolecules can be immobilized oriented parallel to the electric field lines [3].

Results and Discussion

Using regular arrays of many thousands of vertical silicon- or tungsten-based nano-electrodes with tip diameters ranging from 500 nm down to about 1nm it is possible to gain statistical information since thousands of experiments are carried out in parallel. Using nanospheres of different sizes as a model system, immobilization of just a single object on each electrode is ensured by choosing appropriate electrode dimensions and shapes in relation to the objects' size. Immobilization of exactly one particle at each electrode tip has been demonstrated for electrode tip diameters with half the particle size [4].

These results have been transfered to the successful DEP-immobilization of autofluorescent R-PE proteins as individual biomolecules on nano-electrode arrays. The proof of immobilization and singling is done via fluorescence microscopy in combination with a histogram method, revealing the blinking of molecules, immobilized as few or singles on the electrodes of the array. In this way the ensemble averaging of the analyte signal is avoided, still a statistically large sample size is investigated.

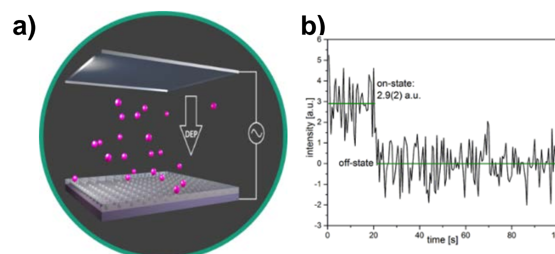

Figure 1: *a) Experimental setup; b)Time trajectory showing a single molecule event*

Conclusions

Electrically controlled immobilization and singling of nanospheres and proteins on nano-electrodes has been shown. The method is efficient at low voltages, fast, as the immobilization takes place after minutes, and highly parallel, so thousands of experiments can be carried out simultaneously. This leads to the possibility of single molecule investigations with still good statistics and points to new approaches in the field of biosensing.

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Acknowledgements

We gratefully acknowledge funding by the Brandenburg Ministry of Science, Research and Cultural Affairs, and the European Regional Development Fund.

Development of a DNA origami nanosensing platform for a near-patient analysis of ADAMTS-13 proteolytic activity

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Keywords: near-patient diagnostics, DNA origami biosensor, ADAMTS-13 activity

Introduction

To achieve high sensitivity and specificity with an efficient quantifiable signal is an objective for biosensors, in particular for diagnostic applications. DNA nanostructures have emerged as attractive platform, capable of addressing these challenges. A relevant class of targets that require better strategies for their detection via biosensors are proteases. One target being clinically relevant is the protease **A D**isintegrin **A**nd **M**etalloprotease with **T**hrombo**S**pondin repeats 13 (ADAMTS-13), which catalyzes the proteolytic cleavage of the von Willebrand factor (vWF) [1]. Insufficient ADAMTS-13 activity (<10%) due to inhibitory autoantibodies to ADAMTS-13 or an inherited dysfunctional enzyme causes the life-threatening disease thrombotic thrombocytopenic purpura (TTP). This rare disease is fatal in 72-94% when appropriate treatment is delayed [2,3]. Thus, a fast and efficient diagnosis to differentiate between TTP and other thrombotic micro-angiopathies can be essential for patient survival. Hence, the aim of this project is to develop of a fast and sensitive DNA origami-based biosensor for near-patient monitoring of ADAMTS-13 activity in plasma samples.

Results and Discussion

A FRET-based sensing strategy merging DNA and protein technologies for realization of protease biosensors was developed (Figure 1). Preliminary experiments showed compatibility of the aimed DNA clamp nanostructures with plasma and ADAMTS-13 assay conditions. Moreover, proof-of-concept TEV protease biosensors that close the FRET-labeled DNA clamp nanostructure by a 23-amino acid bridging peptide containing a TEV cleavage sequence proved to be suitable for detection of TEV protease activity. For the generation of ADAMTS-13 protease biosensors, the vWF73 peptide (Asp^{1596}-Arg^{1668}), published as the shortest effective ADAMTS-13 substrate [4], was successfully expressed in *E. coli* and purified to homogeneity by affinity and size exclusion chromatography. This peptide will be coupled to ssDNA using Cu-free click chemistry to bridge the two DNA clamp arms for detection of ADAMTS-13 activity in plasma (Figure 1).

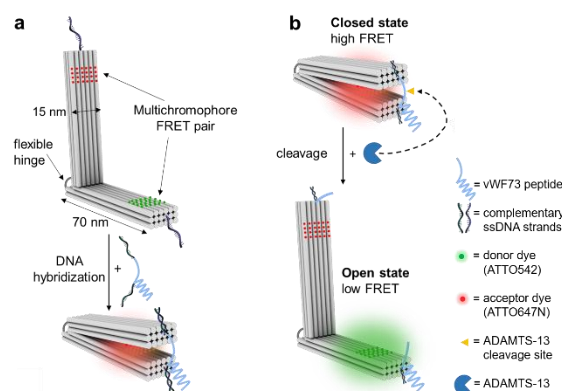

***Figure 1:** Proposed protease biosensor for the detection of ADAMTS-13 activity. **a)** The aimed nanosensor scaffold comprises a flexible DNA hinge carrying FRET pairs on its two opposing arms. The protease clamp is kept in a closed state by bridging the two arms with the vWF73 peptide. **b)** Cleavage of the peptide bridge by active ADAMTS-13 results in the opening of the clamp and the loss of the high FRET between donor and acceptor fluorophores.*

Conclusions/Outlook

Besides reproducible preparation of functional ADAMTS-13 protease clamps, FRET contrast and signal amplification strategies also need to be optimized. Furthermore, the stability and specificity of the protease clamp under assay conditions need to be demonstrated.

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Acknowledgements

This work is supported by the Deutsche Forschungsgemeinschaft (DFG).

Using conjugated primers and nucleotides as DNA labelling techniques for a lateral flow assay for detection of nucleic acid from *Legionella pneumophila*

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Keywords: Point-of-care-Testing, PCR, Lateral flow assay, *Legionella*

Introduction

Drinking water contaminated with *Legionella spp.* poses a great risk for public health. 8 - 10 % of patients diagnosed with legionnaires‘ disease (severe pneumonia caused by *Legionella* bacteria) subsequently die from it. **[1]** Therefore, regular controls of critical drinking water systems are one of the most important steps in the prevention of infections. The currently accepted protocol (ISO 11731:2017) to detect *Legionella* spp. in water samples is by plate culture. However, this method is insufficient due to long cultivation times (7-12 d) and the necessity of specialised laboratories. **[2]**

For this reason, we developed a rapid molecular detection method consisting of PCR and an antibody based lateral flow assay. To detect nucleic acid from *L. pneumophila*, via lateral flow test PCR products had to be double labelled with predefined marker molecules through primers or nucleotides.

Results and Discussion

For detection of *L. pneumophila*, we targeted different fragments in the *mip* and *rpoB* gene. As marker molecules FITC (or Fluorescein) and Biotin were used. These were introduced in the DNA fragments by using primers or nucleotides. Both labelling techniques did not interfere with DNA amplification during PCR. Amplicons labelled by conjugated primers or nucleotides respectively could be detected on lateral flow test strips (see figure 1). Although conjugated nucleotides offer the opportunity to label a DNA fragment multiple times, the use of FITC and Biotin conjugated primers yielded the most sensitive results on lateral flow tests (10 – 20 pg DNA). The entire assay (combination of PCR and lateral flow test) had a limit of detection of approximately 31 cells, which can be achieved in water samples by filtration.

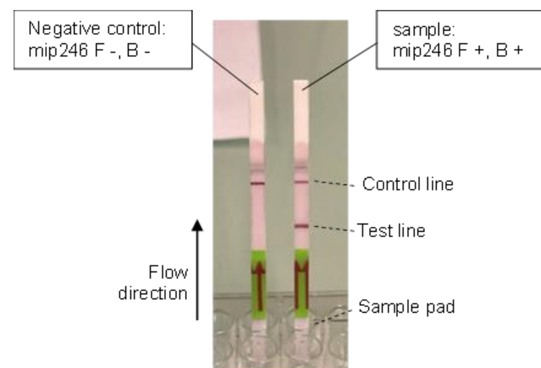

Figure 1: *Detection of PCR products on a lateral flow test strip. Left: negative control; unlabelled PCR products cause negative test result, indicated by single control line. Right: double labelled amplicons generate two lines and therefore a positive test result.* F = FITC, B = Biotin.

Conclusion

In this study we showed that it was possible to detect nucleic acid from *L. pneumophila* via lateral flow assay. It became apparent that the labelling of DNA fragments by conjugated primers was the most effective method for this assay. By processing of water samples through filtration a similar sensitivity to plate culture detection can be achieved in less time.

Our assay presents a clear improvement compared to the current method due to the drastically reduced return time for results. Furthermore, it has the potential to be fully automated and applied on-site.

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Rapid prototyping by laser engraving for a novel microfluidic platform for point-of-care sensing

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Keywords: rapid prototyping, microfluidic, point of care

Introduction

Microfluidics is a versatile and growing field with applications among others in chemistry, biology, and medical diagnostics. The unique properties that fluids exhibit in microfluidic channels allow for many standard lab operations to be scaled down and integrated into a small microfluidic chip. However, the channels must be adapted for their intended use, as the channel geometry defines the properties of the microfluidic system.

Here, rapid prototyping is an essential step in the development process. It allows for the quick transfer of a design into a suitable substrate. This can be achieved by methods, like CNC micromachining, mask-less lithography, or laser engraving. We studied the use of a simple laser engraving printer for the generation of microfluidic channel system for point-of-care biosensors.

Results and Discussion

Using a commercially available 10400 nm CO_2 laser with a power of 30 W, patterns generated as vector graphics could be engraved in a range of polymers. A preferred material was polymethyl methacrylate (PMMA) sheets, but also cyclic olefins (COC) and PET could easily be adapted for the process. Microstructures with a depth of 250 µm and a width of 150 µm were generated using vector mode. Patterns with larger area could be generated with the raster mode resulting in structures with a minimal width of 150 µm and depths ranging from 30 µm to 250 µm. After bonding, these designs could be assembled to fully functional microfluidic chips. The resulting microfluidic platform could be fitted with a variety of functional sections such as sampling zones, mixing, incubation, and detection areas. Typical passive valve structures could easily be generated through this process.

Furthermore, immobilization strategies for future bioassays in the microfluidic channels were explored. Paper-based elements, nitrocellulose, hydrogels, and magnetic beads were successfully integrated without negative impact on flow performance. The microfluidic system can also be equipped with laser-induced graphene electrodes for potential electrochemical detection of analytes on-chip.

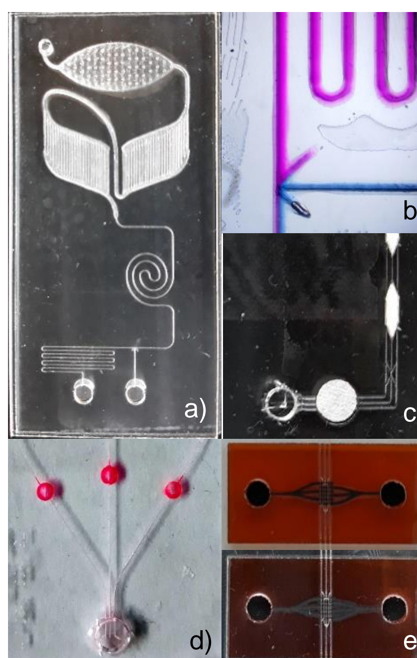

Figure 1: *a) Microfluidic chip with various functional sections; b) activated stop valve; c) paper elements in microfluidic channels; d) hydrogel in microfluidic channels; e) laser-induced graphene electrodes in microfluidic channel.*

Conclusions

This technique for rapid prototyping allows for quick development and production of microfluidic systems. Many basic and advanced functional elements could easily be fabricated. As immobilization strategies on chip were realized, the presented platform will be used to implement bioassays on a microfluidic chip in a point-of-care format. The platform also offers great possibilities for multiplex detection approaches.

Membrane-based immunoassays with photon-upconversion nanoparticles as highly sensitive detection labels

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Keywords: immunoassay, photon-upconversion, nanoparticle, membrane, dot-blot

Introduction

Conventional labels in immunoassays are represented by enzymes and fluorescent dyes. They often possess disadvantages such as low stability and sensitivity in case of enzymes and photobleaching and high optical background due to autofluorescence of sample matrix in case of fluorophores. To overcome these drawbacks, new labels based on nanomaterials are being investigated as suitable alternative. Photon-upconversion nanoparticles (UCNPs) are representing emerging class of nanomaterials that due to their unique properties can be used for detection of low abundant analytes. UCNPs are lanthanide-doped nanocrystals exhibiting anti-Stokes emission, enabling their detection without optical background due to the reduction of autofluorescence of biological samples. When conjugated with biorecognition molecules, UCNPs became convenient label for high sensitivity immunoassays [1].

Results and Discussion

Our work focused on dot-blot nitrocellulose membrane-based immunoassays with UCNPs as detection labels. First, we have synthesized Er^{3+} and Yb^{3+} doped UCNPs, modified their surface with a PEG-based linker to achieve water solubility and reduce the non-specific interactions, and finally conjugated them with biorecognition molecules, such as streptavidin or antibodies. Human serum albumin (HSA), whose increased concentration in urine indicates kidney damage [2], was chosen as model clinical analyte for optimization of the immunoassay. We have performed optimizing experiments to choose suitable membrane type and assay conditions, including blocking of non-specific binding, assay buffer, washing conditions etc. This allowed us to perform HSA immunoassay with limit of detection (LOD) 0.11 ng/mL, which is by three orders of magnitude more sensitive than membrane immunoassay with similar detection label presented in literature [3]. The obtained LOD is about one order of magnitude higher than in microtiter plate assay, which is broadly used and well optimized. Thanks to convenient sensitivity of our dot-blot assay, next steps will proceed to the analysis of HSA in real samples to verify assay robustness.

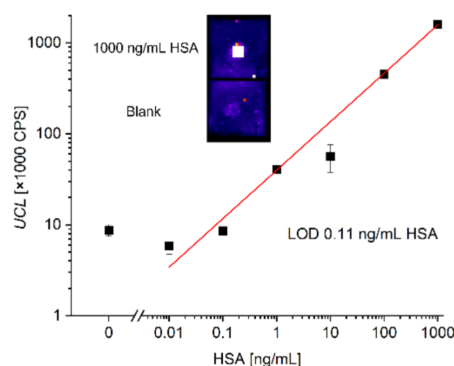

Figure 1: *Calibration for the detection of HSA with conjugate of UCNPs with streptavidin as detection label.*

Conclusions

Our data indicated that dot-blot immunoassays with UCNPs as detection labels can become an alternative for conventional microtiter plate immunoassays. Due to the unique properties of UCNPs, it is possible to upgrade these membrane-based immunoassays from semiquantitative to quantitative detection platform and use them for sensitive detection of biomarkers which is necessary for early diagnosis and prevention of various diseases.

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Acknowledgements

The work was supported by grant GA22-27580S from the Czech Science Foundation.

Water-phase exfoliated 2D transition metal dichalcogenides-based nanoarchitectures for electrochemical (bio)sensing

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Keywords: 2D-materials, sensors, nanomaterials, nanocomposites, immunosensors

Introduction

Two-dimensional (2D) transition metal dichalcogenides (TMDs) have gained increasing attention thanks to their versatile chemistry and unique catalytical properties. Nevertheless, their use in (bio)electro-sensing could be limited by their semiconductive nature; furthermore, tedious procedures for the exfoliation are usually required, employing organic solvents and/or polluting chemicals **[1]**.

Results and Discussion

Water-dispersed 2D TMDs were prepared by liquid-phase exfoliation (LPE) assisted by naturally derived compounds; the latters act as stabilizing agents (SA) allowing water-dispersibility, eventually conferring functional features according to their structure. With this approach, the ability of food-derived polyphenols to act as SA was tested, demonstrating their peculiar ability to confer redox functionalities to the resulting TMD nanosheets **[1]**. On the other hand, the bile salt sodium cholate was employed as functional SA to assemble 2D-TMDs with other nanomaterials through straightforward simple procedures, giving rise to nanocomposites with boosted electro-sensing features **[2]**. Eventually, this strategy was applied to obtain the surface decoration of WS_2 nanoflakes with AuNPs achieved using a simple water-phase interaction; the as-obtained nanoplatform was employed to build up an impedimetric immunosensor onto a screen-printed transducer for the determination of human serum albumin (HSA), immobilizing the specific antibody via cysteamine-glutaraldehyde chemistry. Exploiting the synergy between the two nanomaterials, the WS_2/AuNPs nanocomposite resulted in improved bio-recognitions capacity, enhancing the immuno-sensing performance, returning a satisfactory LOD (2 ng mL^{-1}) and extended linear range (0.005–100 μg mL^{-1}). The WS_2/AuNPs immunosensor was applied for the HSA determination in urine samples, obtaining satisfactory recoveries (92-112%) and reproducible results (RSD≤7.5%; n =3), demonstrating to be a useful tool for microalbuminuria screening.

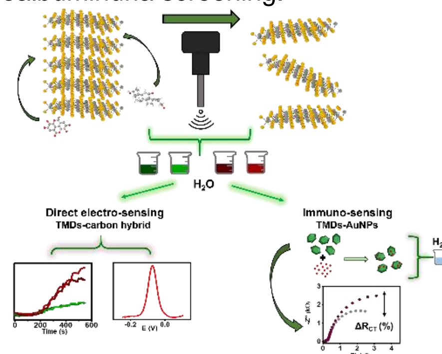

Figure 1*.* Graphical *sketch of 2D TMDs exfoliation and biosensing applications*

Conclusions

LPE in water-phase assisted by naturally derived molecules represents an effective alternative to build-up TMDs-based nanostructures with enhanced electro-(bio)sensing features; in light of this and given the high versatility, this approach still offers captivating opportunities for advanced and smart biosensing devices development.

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Acknowledgements

This research was funded by the European Union – Next Generation EU. Project Code: ECS00000041; Project CUP: C43C22000380007; Project Title: Innovation, digitalization, and sustainability for the diffused economy in Central Italy - VITALITY.

Taste organoid-based biosensor and its application in juice detection

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Keywords: biosensor, extracellular potential recording, taste organoid, taste recognition

Introduction

Taste is of great importance for human beings to perceive the world. Reconstituting human taste functions is extremely needed in the food and pharmaceutical industries. Previously, we developed a biosensor based on taste organoid and microelectrode array for highly mimicking the biological sense of taste *in vitro* [1]. The taste organoid-based biosensor showed great specificity in recognition of sourness, sweetness, bitterness, and saltiness, and distinguished varying degrees of the different basic tastes. In this study, as a proof of concept, we applied the taste organoid-based biosensor in real sample detection and analyze the lemon juice from viewpoints of sourness, sweetness, bitterness, and saltiness (**Figure 1**).

Results and Discussion

First, the taste organoid was formed by the taste progenitor/stem cells from the circumvallate papilla of C57 mice. Both immunofluorescence staining and real-time quantitive PCR showed that the taste organoid contained different kinds of taste receptors and had taste perception potential. In addition, the Ca^{2+} fluorescence imaging showed that the taste receptor cells in the taste organoid were sensitive to sour, sweet, bitter, and salt stimuli.

Next, the taste organoid was coupled with a microelectrode array chip to construct the taste organoid-based biosensor. Then, the taste biosensor was used to detect different concentrations of acetic acid, sucrose, phenylthiocarbamide (PTC), and NaCl to select channels with specificities that respond to only one type of the basic tastes and make the standard curves for sourness, sweetness, bitterness, and saltiness, respectively. The results suggested that the taste biosensor was able to recognize varying degrees of the basic tastes. For the evaluation of the lemon juice, the specific response signals of the taste biosensor to the lemon juice were analysed. The results showed that sourness was the most dominant taste in lemon, while the others contribute less to the flavour (**Figure 1**).

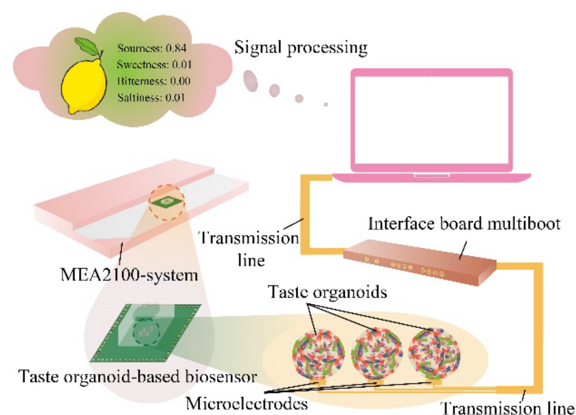

***Figure 1:** Application of the taste organoid-based biosensor in lemon juice detection. In the biosensor, different taste receptor cells in taste organoids were able to perceive different basic tastes, while microelectrodes coupled with the taste organoids were able to detect the extracellular potential changes generated by taste receptor cells. 25 mg/mL acetic acid, 1 g/mL sucrose, 10 mM PTC, and 6 mol/L NaCl were defined as degree 1 for sourness, sweetness, bitterness, and saltiness, respectively. Degree 0 means undetectable taste information.*

Conclusions

In this study, we used the taste organoid-based biosensor to “taste” lemon juice and evaluate it from viewpoints of sourness, sweetness, bitterness, and saltiness. The taste biosensor was able to analyse the samples in biological sense of taste and may facilitate the studies in the food and pharmaceutical industries.

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This work was supported by the National Key Research and Development Program of China (2020AAA0105900, 2021YFF1200803), National Natural Science Foundation of China (No. 32250008, 62271443), Natural Science Foundation of Zhejiang Province (Nos. LBY21H180001 and LY21C100001).

Silicon photonic supported by dielectrophoresis for detecting microbes.

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Keywords: cell-based diagnostics, dielectrophoresis, silicon photonic, microring resonator

Introduction

The effect of diffusion and mass transfer limitations in receptor-based biosensors become particularly significant when attempting to register larger bioparticles such as whole cells and bacteria, since few analytes are present in the sample solution and these binds less efficiently to the sensor surface. The target cells are generally delivered to the sensors via microfluidic solutions with microchannel heights of tens to hundreds of micrometres; consequently, most cells will pass the microchannel without adsorption on the sensor surface.[1] An applied AC field will interact with the cells and may be used to focus them onto a sensor surface via the dielectrophoretic force. In this work we have applied dielectrophoresis to focus microbes onto a silicon based microring resonator biosensor to increase its sensing performance

Results and Discussion

We designed a chip with silicon photonic integrated circuits (PIC) based on the IHP technology to realize a sensitive microring resonator that can be used for sensing purposes. FEM simulations allowed us to optimize electrode structures next to the waveguides that is used to focus the cells onto the core waveguide.[2] Based on the simulations the electrodes were realised with doped silicon structures on the device layer separated 1.5 µm from the core waveguide. The packaging of the chips whose dimension is only 2.5x2.5 mm and contains wire bonds, grating coupler and sensing areas was realised with a combination of lithography techniques as well a PDMS chip. As a model organism to detect, Legionella Parisiensis was chosen.

An applied voltage of 10 Vpp at 20 MHz frequency was identified as parameters that may focus living cells while not interacting with pathogenic irrelevant dead cells.

Furthermore, proof of principle measurements showed successful detection of the cells with the microring resonators causing the resonance peaks to shift to higher wavelengths.

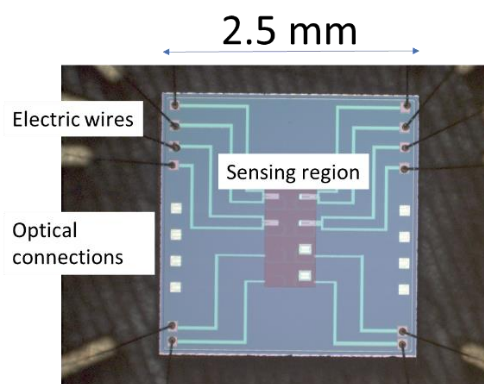

Figure 1: *The designed sensor Chip.*

Conclusions

We have explored the implementation of DEP in silicon photonic biosensors for detecting whole legionella cells. Suitable designs and electric parameters have been identified and proof of principle experiments show that microring resonator might be suitable for detecting legionella in drinking water.

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Acknowledgements

This work was funded by the German Federal Ministry of Education and Research (BMBF) within the program “Wissenschaftliches Vorprojekt Photonik” (FKZ 13N15712).)

Cells between hot and cold: Towards a new cell-based biosensor

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Keywords: Cell stimulation, spontaneous cell detachment, cell characterization, cell identification, cellular oscillations

Introduction

Sensitive techniques for cell characterization and identification are crucial for various diagnostic, therapeutic and pharmacological applications. Available techniques depend on either engineered receptors or labelling approaches, which are subject to cost- and time-inefficiencies. Therefore, alternative techniques are needed **[1]**. In this work, we report on a new cell-based sensor strategy that allows to facilely discriminate cancer cells and micro-organisms down to strain level **[2]**. The technique is based on the observation that eukaryotic cells, including cancer-cell lines and yeast strains, display spontaneous and collective detachment from the interface between a heated solid surface and a stagnant liquid phase, under a thermal gradient stimulus **[3]**.

Results and Discussion

We show that at a constant chip temperature, *T*, cells are subject to a temperature gradient, which induces spontaneous and synchronized detachment at a sharply-defined and highly reproducible time, t_d. The detachment time, t_d is identified from a sudden increase in the temperature of the liquid above the chip-liquid interface **[4]**. The collective cell detachment time, t_d, depends strongly on the cell type and chip temperature: t_d decreases exponentially as *T* increases, with scaling parameters that allow distinguishing reliably between two cancer cell lines and three different yeast strains. Furthermore, we show that cell metabolic activity plays a major part in the spontaneous detachment process: t_d decreases with nutrients (sucrose) and increases with treatments that negatively impact cell metabolism (e.g., DMSO and blebbistatin). The role of metabolic activity was confirmed by independent measurements using the commercially available alamarBlue™ cell viability reagent **[2]**. Under specific conditions of temperature, sucrose, and drug dosing, we observe synchronized oscillations during cell detachment, providing additional cell-specific fingerprints.

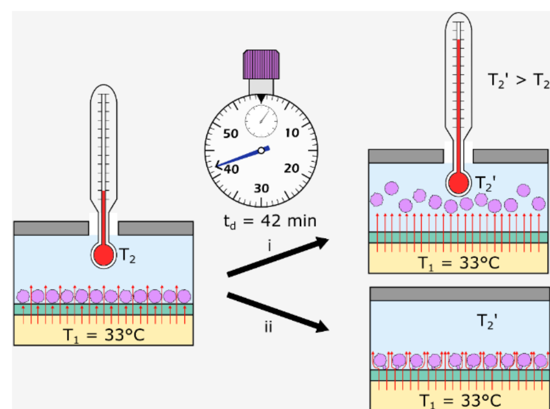

Figure 1: *Schematic of spontaneous cell detachment and detection principle.*

Conclusions

The results show that spontaneous cell detachment can be used to effectively characterize eukaryotic cells, including human cancer cells. The strong sensitivity of this effect to metabolic activity suggests applications in cell viability analysis and pharmacology, in which spontaneous detachment serves as a simple and fast tool for assessing drug response at cellular level.

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Acknowledgements

This work was financed by the KU Leuven project C14/15/066 and the Research Foundation Flanders FWO, project G.0791.16N.

Electrochemical immunosensing with a 3-dimensional microfluidic flow cell

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Keywords: Immunosensor, horseradish peroxidase/TMB, microfluidics, flow cell, amperometry

Introduction

Immunoassays, based on analyte recognition and capture by highly selective antibodies with high affinity, are intensively used in all fields of laboratory diagnostics and in screenings of food and environmental samples. Yet, for many purposes, online sensors are desirable, and, in principle, all immunoassay techniques can be integrated into lab-on-chip set-ups that can work as continuous monitoring devices. Yet, the challenge remains to develop platforms and elements that are fit for a quick transition of laboratory microplate assays to immunosensors.

Results and Discussion

Point of departure was a magnetic bead-based ELISA for the pharmaceutical and pollutant of the aquatic environment, diclofenac [1]. The immmunoenzymatic sensing approach has been described before [2].

Here, we developed a complete flow system, based on a core microfluidic flow cell, to establish the automated detection of one of the most used immunoassay substrates, 3,3',5,5'-tetramethybenzidine (TMB) via chronoamperometry. Other elements of the microfluidic set-up were pressure-driven flow control, automated valves, and electrochemical detection on a screen-printed electrode associated with a dedicated mini-potentiostat. Architecture and fluidic optimisation of the system showed that a specifically designed 3D flow cell enables higher flow rates (500 µL/min) than a standard enlarged microfluidic channel (50 µL/min), which has been used in an earlier study [3]. Using the 3D flow cell resulted in a significantly shorter time-to-result of 30 seconds and turned the system more robust against interferences from bubbles that commonly form in microfluidic chips. The electrochemical measurements showed improved signal-to-noise ratio (SNR) and therefore enhanced sensitivity for the model immunoassay for diclofenac (SNR = 59), compared to the analytical performance of the conventional laboratory microplate-based assay with optical detection (SNR = 19).

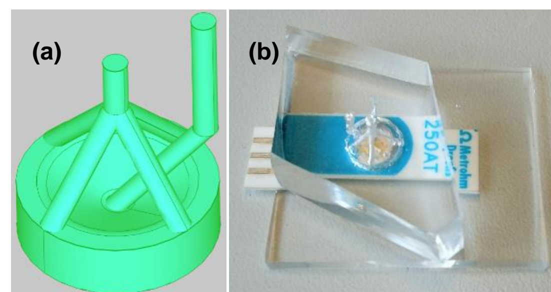

Figure 1: *a) 3D model of the inner structure of the microfluidic chip: Cell, inlet channel (centred) and outlet channels (cell edges) were printed separately to produce the complete ABS scaffold, merged from individual parts. b) The obtained 3D microfluidic chip with a flow cell chamber aligned on the screen-printed electrode and a channel structure for inlet and outlet connections.*

Conclusions

The use of a specifically designed 3D flow cell that enables perpendicular flow onto the electrode and hight flow rates facilitates the transformation of any conventional immunoassay into a sensitive immunosensor with automated and continuous detection.

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Acknowledgements

We thank the Indo-German Science and Technology Centre (IGSTC) and the Federal Ministry of Education and Research (BMBF) for funding (IDC-Water project, grant number: FKZ 01DQ18003A.

Single Chip Solution for electrical characterization

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Keywords: electric relaxation, passive electrical properties, impedance, ASIC

Introduction

Electrochemical impedance is useful for material characterization and becomes particularly interesting when the measurement is fast and the instrumentation is simple and inexpensive. Methods in the time domain are superior, especially with regard to speed, since measurements do not require sweeping though the frequency range. Instead, a broadband signal is applied and the response of the system is measured, from which the electrical properties can be derived. Of the possible broadband signals such as step functions, Dirac surge, multisine, maximum length sequence or chirp, the step function has the best potential for a quick measurement with simple hardware. In biological materials, the response to a step in potential or current is a sum of exponential functions or, for a simpler interpretation, a distribution of relaxation times. The electrode polarization that often occurs in measurements on biological systems leads to a $\sqrt{t}$ -dependency. Generally, the response to a step is a rapid change in current or voltage immediately after the step, with significant deceleration thereafter. With a frequency range of five orders of magnitude and the fulfilment of the sampling theorem, equidistant sampling would require at least 200.000 sampling points, which is problematic for continuous monitoring of an object. Gradual sampling with short intervals when the signal changes quickly and correspondingly longer times when changes are slowly reduces the necessary sampling points considerably, but leads to a violation of the sampling theorem. We avoid this by a stepwise integration between two sampling points, which yields an adaptive anti-aliasing filter. In current work, about 30 sampling points are needed for a complete reconstruction of the relaxation behaviour over a dynamic range of six orders of magnitude (500 ns - 50 ms). This corresponds to a spectrum between 10 Hz - 1 MHz when evaluating a single step. An ASIC was developed for the required hardware, which, in addition to a universal front end, also contains the entire timing control and the analog-to-digital conversion. To validate the concept, we made a discrete circuit with exactly the functionality of the ASIC. Its analogue part allows the use of 2, 3 and 4 electrode systems and the choice between potentiostatic and galvanostatic excitation.

Various work regimes are implemented, where the single step allows the fastest measurement. For biological or electrochemical applications, the multiple step method is provided, in which a charge balance with positive and negative potentials is achieved. For precise measurements a sampling principle is implemented, in which an offset-free square wave is applied and the integration time is increased with each positive step. A three-electrode arrangement with automatic compensation of the zero current potential is available especially for electrochemical applications.

Practical Considerations

In the case of measurements with individual steps, offset compensation is of great importance, as this will compromise the calculated result. Since a simple compensation along the entire measurement chain is not possible, the offsets of all stages are determined and mathematically corrected using test and calibration routines. The dynamic range of the fully calibrated device corresponds to that of high-quality laboratory devices, which is achieved with a 16-bit ADC for measurement, a 12-bit DAC for excitation and variable amplification in analog processing. The ASIC is a universal component, which, however, is intended for solutions that are specially adapted for a specific application.

Acknowledgements

This work is support by the BICCell project (Dechema / IGF, FKz: 21174 BR).

Kinetic analysis of ternary and binary binding modes of the bispecific antibody emicizumab

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Keywords: antibody, avidity, drug discovery, ternary complex, bispecific

Introduction

The binding properties of bispecific antibodies (bsAb) are crucial for their function, especially when two antigens are targeted on the same cell surface. Dynamic interactions between each of the antibody's arms and its cognate target cause the formation and decay of a biologically functional ternary complex **[1]**. How association and dissociation processes work cooperatively, and how they influence the avidity of the ternary complex, is still poorly understood.

Results and Discussion

Here, we present a biosensor assay for the simultaneous measurement of the binding kinetics of the therapeutic bsAb emicizumab (Hemlibra®) and its two targets, the blood coagulation factors IX and X (FIX, FX). We describe an automated workflow to characterize binary and ternary-binding modes, utilizing a Y-shaped DNA nanostructure to immobilize the antigens on a sensor and to emulate conditions on a cell or platelet surface by presenting the antigens with optimal accessibility for the bsAb flown over the sensor as analyte. We find that emicizumab binds FX much stronger than FIX (Kd = 0.05 µM vs. 5 µM, t1/2 = 20 s vs. 1 s) with profound consequences for the avidity of the ternary complex, which is dominated by FX's binding properties and a hand-off mechanism from FX to FIX. Moreover, formation and decay of the ternary complex depend on the bsAb concentration during the association phase.

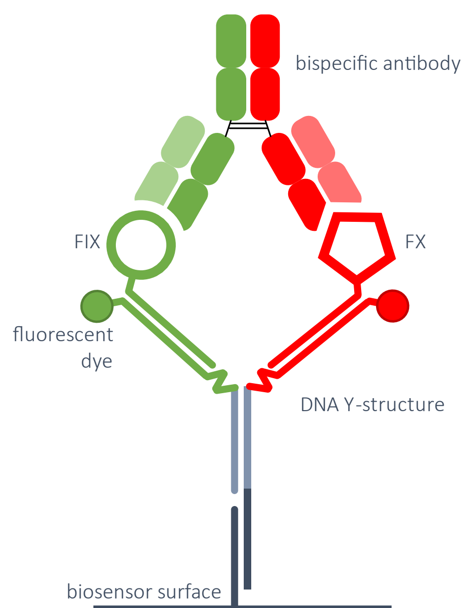

Figure 1: *The co-immobilization of both target proteins on the DNA Y-structure allows the characterization of binary and ternary binding properties while closely emulating biologically relevant conditions on a cell surface like stoichiometry, local concentrations and accessibility.*

Conclusions

Emicizumab's *in vivo* mode of action and the catalytic activation of FX can be rationalized from the analyzed binding kinetics. The assay and workflow are well suited for the screening of bispecific binders in drug discovery and provide valuable new kinetic information.

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Multiparametric characterization of Eukaryotic cells based on cell detachment through combined measurement of impedance, heat transfer, and mass-loading signals

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Keywords: HTM, QCM-D, EIS, Spontaneous cell detachment, Yeast strains

Introduction

A novel set-up combining the electrochemical impedance spectroscopy (EIS), quartz crystal microbalance with dissipation monitoring (QCM-D), and heat transfer method (HTM) demonstrates the real-time and multipara-metric detection of bio- and chemo-targets. This method reveals the electrical, structural, and thermal transport properties of analytes in a flow cell at the same condition **[1]**. In this study, combining three techniques provides novel insights regarding the mechanism of synchronized and spontaneous cell detachment with sharply defined time patterns, a phenomenon unknown in literature. This phenomenon is a natural cellular response to thermal stimuli that can be employed as a label- and receptor-free technique for cell characterization **[2]**.

Results and Discussion

cell suspensions in a flow cell with a quartz crystal substrate are exposed to a temperature gradient which induces synchronized and spontaneous cell detachment from the surface. This phenomenon depends on surface temperature, nutrients, and drugs. The application of combined QCM-D, EIS, and HTM provides complementary information about the mechanism of collective cell detachment with sharply defined and reproducible detachment time. The results represented similar detachment time and behaviour in QCM, EIS, and HTM measurements. HTM shows higher thermal conductivity after cell detachment, while EIS shows lower impedance amplitude due to easier charge transfer after the detachment. QCM-D measurement demonstrates a decrease in frequency signal based on the hydraulic mass effect with higher dissipation due to increasing damping after cell detachment. The effect of substrate temperature shows that cell detachment time (td50) becomes shorter by increasing the chip temperature **[3]**.

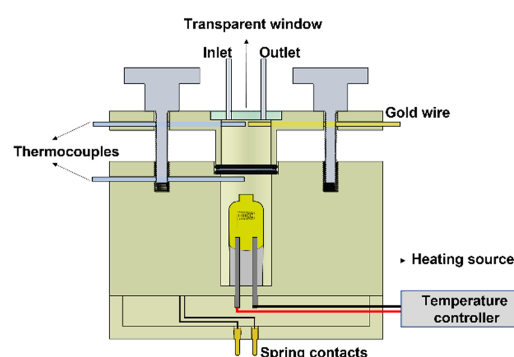

Figure 1: Cross-section schematic of the Triple Device flow cell. A 10 W halogen lamp was used as the heating source that is controlled by a temperature controller. Two 500 µm thick thermocouples monitor the chip temperature (T_1) and the liquid temperature (T_2).

Conclusions

The multiparametric combined set-up can compensate drawbacks of each technique taken alone, and the results show that the spontaneous cell-detachment technique can be used to effectively characterize and potentially identify eukaryotic cells, including human cancer cells.

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Acknowledgements

This work was financed by the Research Foundation Flanders FWO and Austrian Science Fund FWF.

Natural Biomimetic Spongin-Composites for Sensing Applications

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Keywords: natural polymer, biomimetic, biocomposite, sensor

Introduction

The development of sensing materials based on biomimetic receptors that mimic the biological binding site can overcome the disadvantages of biological materials (unstable, expensive to produce, denaturation) and address the ongoing trend to reduce the use of animals for the production of biological reagents [1]. In recent decades, the synthesis and application of biomimetic composites, especially in the field of sensing, has become a rapidly growing and promising field. In this context, renewable biopolymers, such as spongin have recently been used to develop new metal oxide-containing composites even using highly toxic metal-rich industrial waste [2]. So, under selected conditions, spongin can react with diverse metal ions including Fe, Hg, and Cu and obtain new outstanding features such as mimetic catalyst activity and also great functionality as a 3D porous sensing material Accordingly, we have developed various sensor composites based on the integration of atacamite ($Cu_2Cl(OH)_3$) and lepidocrocite (γ-FeOOH) to selectively detect various desired targets such as glucose, gallic acid, and dopamine.

Results and Discussion

The developed Sp.Fe and Sp.A. composites were observed and analyzed using an advanced optical imaging system, which revealed that green crystalline of atacamite and red-brown crystalline of lepidocrocite, remain firmly attached to the spongin fibers even after ultrasonic treatment. The performance of the prepared CPEs with Sp.A composite as the sensing platform for the detection of glucose and gallic acid was investigated using electrochemical methods. The practical feasibility of the proposed sensors was confirmed by their application in real samples with acceptable recoveries (for the detection of glucose in biological samples and beverages (Fig.2) and the determination of gallic acid in tea and wine samples). Also the CPEs with Sp.Fe composites as sensors towards glucose and dopamine showed excellent performance, even

Figure 1: *Optical microscope image of pure spongin (a), Sp.A (b) and Sp.Fe (c).*

in real samples. This superior analytical performance of the sensor is attributed to the unique structural design of the 3D microporous sponge framework, which efficiently enhances the activity of atacamite or lepidocrocite as an electrocatalyst.

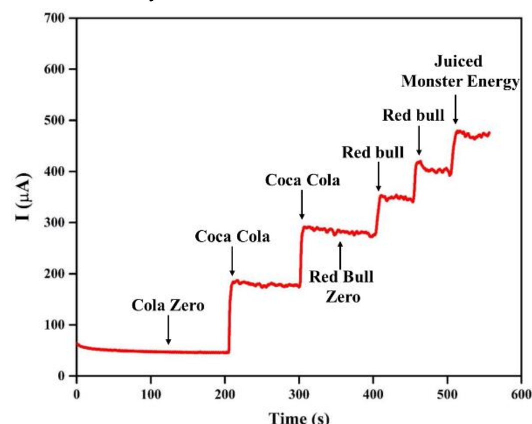

Figure 2: *Amperometric responses of the Sp.A modified CPE with successive additions of 20 µL different beverages in 10 mL 0.1 M NaOH solution at 0.6 V.*

Conclusions

The natural spongin composites as sensing materials for the fabrication of simple, cheap and reliable sensors for glucose, gallic acid and dopamine revealed high analytical performance, which could be attributed to the porous 3D sponge framework as an excellent template for the growth of catalyst crystals and the enhancement of their catalytic activity.

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Dipstick sensor based on molecularly imprinted polymer-coated screen-printed electrodes for the single-shot detection of glucose in urine samples - From fundamental study towards point-of-care application

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Keywords: molecularly imprinted polymers, non-invasive glucose monitoring, non-enzymatic glucose sensors, screen-printed electrodes, dipstick sensors

Introduction

Diabetes is considered a global burden both in terms of health-related and economic costs. Generally, most commercial devices use enzymes as recognition elements,[1] Their main limitations reside in their relatively short long-term stability. A promising alternative that could overcome these issues are molecularly imprinted polymers (MIPs).[2]

Results and Discussion

In this work, we have prepared bulk MIP particles[3] and screen-printed them on top of a carbon SPE to prepare a MIP-SPE platform in a cost-effective and reproducible manner. Moreover, the design of the fabricated platform made it possible to use the sensor with the Heat-Transfer Method (HTM) and Electrochemical Impedance Spectroscopy (EIS) **(Figure 1)**.

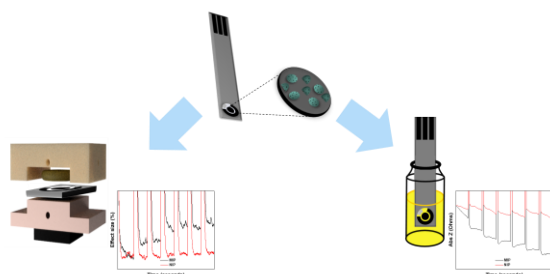

__Figure 1:__ Design and setup adopted for rebinding analysis using MIP-SPE as dipstick technology.

After evaluation of the sensitivity and selectivity of the MIP-SPE platform in PBS solutions, the applicability of the sensor for the diagnosis and monitoring of diabetes was investigated. To this end, the HTM and EIS response of the developed sensor in human urine samples was investigated **(Figure 2)**.

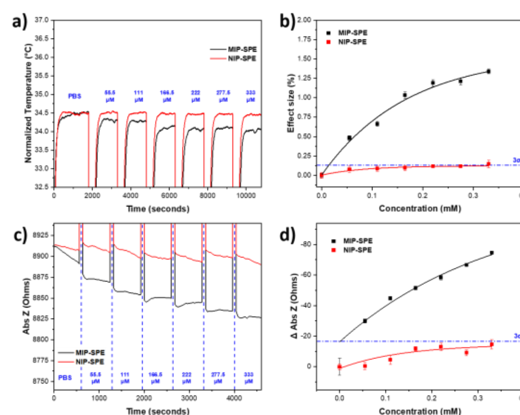

__Figure 2:__ HTM and EIS rebinding analysis in human urine samples.

Conclusions

The developed MIP-SPE sensor has demonstrated to allow both HTM and EIS analysis in PBS as well as urine samples. The combination of the MIPs with the scalable production process of the SPEs emphasizes the high commercial potential of the platform.

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Acknowledgements

This work was supported by the Interreg Euregion Meuse-Rhine, project "Food Screening EMR" (EMR159), funded by the European Regional Development Fund of the European Union.

Abstracts - poster presentations

Laser-patterned SU-8 based 3D microelectrode arrays enable extracellular recording in HL-1 cells

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[&] These authors contribute equally: Hu Peng, Inola Kopic

Keywords: Laser-patterning, SU-8, 3D microelectrode arrays, extracellular recording

Introduction

Microelectrode arrays (MEAs) are commonly used to study the electrophysiological behavior of cells. In recent years, there has been a growing interest to fabricate three-dimensional electrode arrays as novel platforms [1]. Here, we present a new process for the fast fabrication of SU-8-based 3D electrode arrays via laser-patterning technology. After fabrication, the final devices were electrochemically characterized. Furthermore, we recorded extracellular signals from HL-1 cells using the 3D electrode arrays as a proof of principle.

Results and Discussion

Our approach combines photolithography and laser-patterning technology to generate SU-8 based 3D electrode arrays for extracellular recording in HL-1 cells. A schematic of the fabrication process is shown in Fig. 1. In a first step, pillar structures were directly fabricated onto a substrate (Fig. 1a). An adhesion layer of titanium (5 nm) followed by the main conductive layer of platinum (100 nm) was subsequently deposited onto the sample using a PVD process to coat the entire surface of the sample including the printed 3D pillar structures (Fig. 1b). In a next step, laser patterning was used to define the feedlines and electrode structures. A layer of parylene C was deposited via a CVD process to insulate the sample by coating the entire chip, including the top of the pillars. To expose a defined ring electrode only at the tip of the pillar, we employed a laser ablation process (Fig. 1c). Fig. 1d and Fig. 1e show the image of six exemplary electrodes and a close-up image of an individual 3D electrodes' tip. Compared to e.g. inkjet-fabricated 3D ring electrode arrays, the devices exhibit a very regular structure at the cost of a limited aspect ratio.

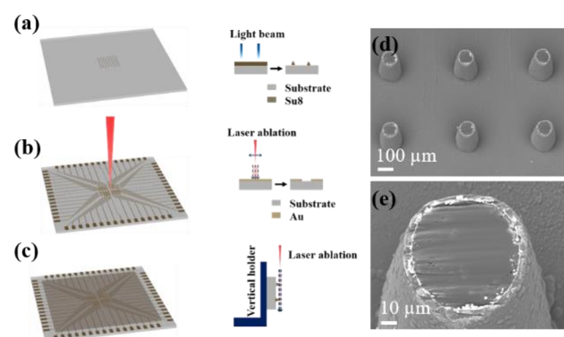

Figure 1: *(a-c) Schematic of the fabrication process for the SU-8 based 3D microelectrode arrays. (d) SEM images of tips after straight cut. (e) A close-up image of an individual tip.*

Conclusions

We presented a simple process for the fabrication of 3D MEAs with the assistance of laser-patterning technology. The 3D MEAs enable recordings away from the bottom of a planar substrate. We believe that this concept can be applied for bio/neuro-electronics within various 3D environments.

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Acknowledgements

We greatly appreciate the financial support from China Scholarship Council and funding from the German Research Foundation (DFG; grant number 446370753).

Exploration of membranes for fluorescence-based analysis of biological specimens.

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Keywords: membrane, fluorescence, staining, adhesion, bioanalytic

Introduction

Fluorescence-based analytical methods are frequently used for the qualitative and quantitative detection of microorganisms or biomolecules from patient or environmental samples. Such methods are very sensitive and specific, but require analysis surfaces with low autofluorescence, low abiotic properties and high binding capacity for reproducible and reliable results. Particularly in the case of small quantities of analytes, these must first be concentrated. For this type of investigation, filters and membranes are useful on which the analytes can be well immobilized, stained and optically analyzed.

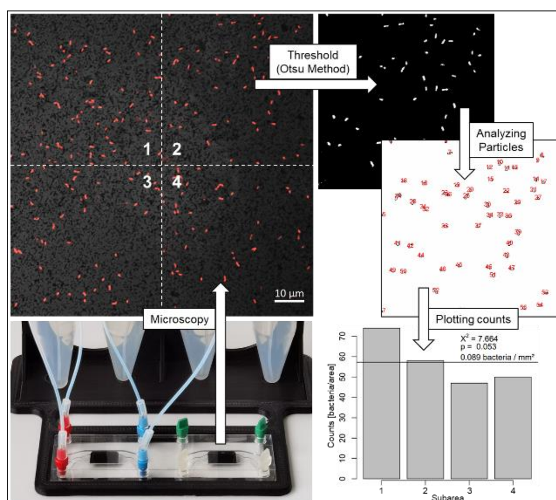

Figure 1: Analyzing bacteria on membranes. Acidithiobacillus ferrooxidans adhered to a PET membrane (PFA-fixed (4 %), propidium iodide-stained (red)). The autofluorescence of the membrane in a narrow wavelength range (gray) illustrates the pore density (pore size = 0.3 µm) and can be used as a reference signal. Imaging was done with a confocal laser scanning microscope (LSM800, Zeiss; 63× objective). After thresholding (Otsu), image subareas were analyzed and counting results were plotted as bacteria per area.

Results and Discussion

We compared different filter and membrane materials (nitrocellulose, polyethersulfone, polyvinylidene fluoride, polyethylene terephthalate, Polycarbonate). The auto-fluorescence of the materials was analyzed by fluorescence spectrometry and the surface was examined by different microscopic techniques (Epifluorescence, Laser Scanning, Atomic Force Microscopy). The adhesion and location of microorganisms on the surfaces were analyzed by fluorescence microscopy after nonspecific (nucleic acid dyes) and specific (fluorescent probes hybridization) staining, and additionally the growth on them was studied. For this purpose, the materials were dealt with 3D-printed holders and integrated in (micro-) fluidic devices. We were able to find a suitable planar membrane with low background fluorescence and good binding properties, which is highly applicable for fluorescence-based bioanalytical methods.

Conclusions

Membranes are used in a wide range of bioanalytical applications, but are not only useful for separating or concentrating substances. The direct analysis of samples on them is also advantageous and simplifies, for example, the diagnosis of pathogens that are difficult to cultivate. The membranes described can be used both on a larger scale, but can just as easily be incorporated into small instruments such as microfluidic chips, which can be automatically evaluated with fluorescence microscopy systems.

Acknowledgements

Federal Ministry of Education and Research:
Grant 13GW0424: "KEAnI – Komplettsystem zur schneller Erstellung eines Antibiogramms bei nosokomialen Infektionen"

Fabrication of an SU-8 based pyrolytic sensor array with integration of MOFs for Electrochemical detection

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Keywords: microelectrode arrays, electrochemical detection, MOFs

Introduction

Electrochemical sensors are used for real-time detection in various fields such as environmental or biomedical monitoring. Metal-organic frameworks (MOFs) show promise in enhancing sensitivity and selectivity of electrochemical sensors due to their high surface area and tailored functionalities **[1] [2]**. Here, we explore the potential of SU-8 pyrolytic electrode arrays, which offer advantages such as high stability and ease of fabrication **[3],** for integration with MOF structures to improve the performance for electrochemical sensing.

Results and Discussion

The process of creating pyrolyzed SU-8 based microelectrode arrays (MEAs) involves several steps, as shown in Figure 1.a. The process begins with photo-patterning SU-8 onto a planar substrate. After patterning, MOF-containing solutions are drop-casted onto the electrodes. Once the MOFs are deposited onto the SU-8 patterns, the structures are carbonized in a furnace under a nitrogen atmosphere. This step is crucial as it results in the formation of a carbon-based layer that contains the MOFs on top of the SU-8 patterns. After carbonization, a thin layer of SU-8 is patterned as a passivation layer. We fabricated MEAs comprising 64 electrodes with an opening of 25 µm diameter onto which the MOFs are drop-casted. To confirm the adhesion of the MOF on the pyrolyzed SU-8 structures, X-ray diffraction (XRD) analysis was conducted. The XRD plots of the normalized intensity versus 2-theta for pyrolyzed SU-8 and the combination of SU-8 with two different MOFs are shown in Figure 1.c. The appearance of two peaks at around 33° and 37° indicates the presence of Fe_2O_3 and, hence, the presence of the selected MOF on SU-8 after pyrolysis. This confirms that the MOFs do indeed adhere to the SU-8 patterns after pyrolysis.

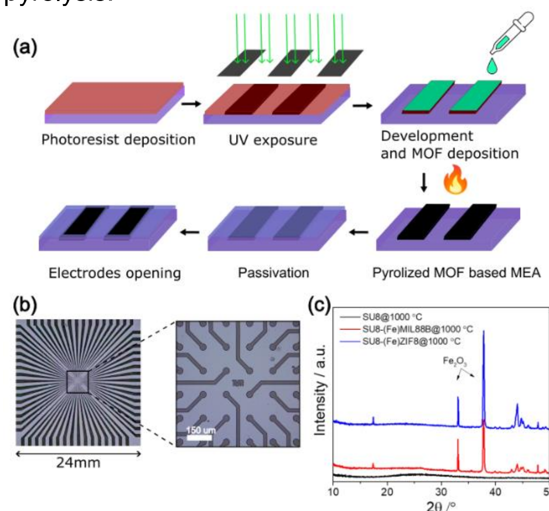

Figure 1: *(a) Photolithography process. (b) Fabricated SU8-based MEA after pyrolysis. (c) XRD plot of normalized intensity vs 2-theta for pyrolyzed SU8, SU8-(Fe)MIL88B and SU8-(Fe)ZIF8 at 1000°C.*

Conclusions & Outlook

Future investigations will focus on the electrochemical detection of various analytes. Furthermore, the deposition of multiple MOFs onto the MEA will enable the simultaneous detection of multiple analytes from a single sample.

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Acknowledgements

I would like to acknowledge the TUM Innovation Network: ARTEMIS.

Platelets on a plate: QCMs provide a cost-effective and rapid sensing alternative for thrombocyte viability monitoring

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Keywords: viability assay, blood product stability, miniaturization, quartz crystal microbalance, atomic force microscopy

Introduction

Modern medicine relies on fast and precise results to ensure optimal treatment for patients. For example, observing blood parameters and the quality of blood products in real-time is crucial in many settings, especially in ICUs and during surgery [1]. However, this complex task often requires costly devices and trained personnel [2]. In this work, we present a rapid and cost-effective sensor to monitor the viability status of human platelets (thrombocytes) in concentrates *via* measuring their aggregation behaviour. The sensing system relies on non-modified quartz crystal microbalances (QCMs), making determination of platelet viability possible in a matter of minutes. This allows for answering the question if a stored platelet concentrate is still safe to administer, as well as for determining the health status of the cells in the concentrate. The developed sensor displays the first steps towards affordable and rapid determination of platelet viability.

Results and Discussion

QCM sensor responses decline with growing age of the measured platelets (Figure 1).

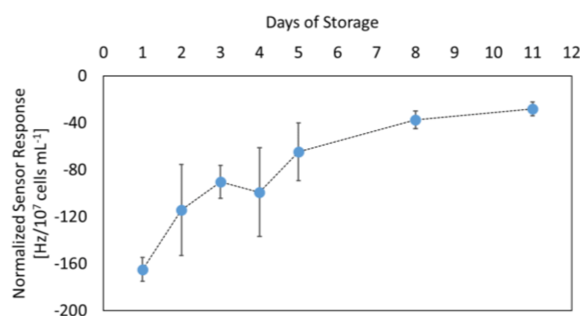

Figure 1: *Normalized QCM sensor responses of platelet samples at sub-physiological concentrations (i.e. 2-7*10⁴ cells/µL, measured with a CASY counter) in 1xPBS buffer (pH 7.4). Strong frequency shift in the first days of storage decline with growing age of platelets, indicating ongoing loss of viability in sample.*

QCM data shows that only approx. ¼ of the initial sensor response (i.e. approx. -170 Hz) can be recovered at the end of storage period. AFM imaging of the sensor surfaces depicts platelet viability in terms of aggregation and thereby binding strength to the QCM (Figure 2). Results were confirmed *via* a resazurin fluorescence assay, indicating significant cell mortality starting at the 3rd day (not shown).

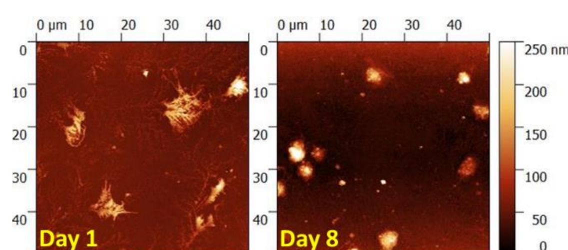

Figure 2: *AFM images of QCM surfaces after sensor measurement show strong aggregation and spreading of freshly stored platelets. Old cells lack that ability – which is linked to their binding strength and thereby viability.*

Conclusions

Thrombocytes change their aggregation properties during storage. Loss of the aggregation ability ultimately leads to decrease in binding strength/adhesiveness to the QCM surface, which in return causes a decrease in sensor response. Platelet viability is directly linked to the aggregation ability. Our system provides an easy and cost-effective alternative to standard platelet function testing and additionally leaves room for further miniaturization and modification.

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Acknowledgements

This project is funded by the University of Vienna and supported by the Austrian Red Cross, which we gratefully acknowledge.

Determination of Toxicity Equivalency Factors for Tetrodotoxin Analogues using a Single-Cell Biosensing Platform

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Keywords: Tetrodotoxin (TTX), Pufferfish, Cell-Based Biosensor, Electrophysiology, Patch Clamp

Introduction

Tetrodotoxin (TTX) is one of the most potent marine neurotoxins known, responsible for many poisoning accidents and some fatalities. At least 30 natural TTX analogues have been described, but their toxicities and interaction with the voltage-gated sodium channels (target of the TTXs) have not been fully elucidated. In this work, we propose the use of a single-cell biosensing platform based on patch clamp technology as a tool to evaluate the toxicity equivalency factors (TEF) of five TTX analogues and to explore their potential as a new bioanalytical tool for the rapid and automated detection of TTXs in pufferfish samples.

Results and Discussion

The automated patch clamp device from Nanion enables the simultaneous analysis of eight individual cells exposed to a sample or toxic compound. The cell patching and current recording is achieved by the use of a planar glass chip coupled to an electrode system. This automated system reduces the complexity of the assay, shortens the analysis time and increases the throughput of the analysis.

The murine neuroblastoma cell line Neuro2A (extensively used in toxicology) was used as a cellular model in this study.

The TTX analogues were purified from the liver of a toxic pufferfish (*Lagocephalus sceleratus*) caught in Greek waters. The inhibition of the voltage-dependent sodium channel currents of clamped Neuro2A cells exposed to different concentrations of TTX analogues is shown in Figure 1. The IC_{50} values of each curve were used to calculate the TEFs of the analogues as the ratio in relation with the IC_{50} of TTX.

Different tissues from three pufferfish specimens caught in Greek waters were also analysed with the automated patch clamp. Then, the toxicity of the fishes was neutralized with an anti-TTX monoclonal antibody in order to prove that the current blockade observed with the biosensing platform was caused by the TTXs present in the fish.

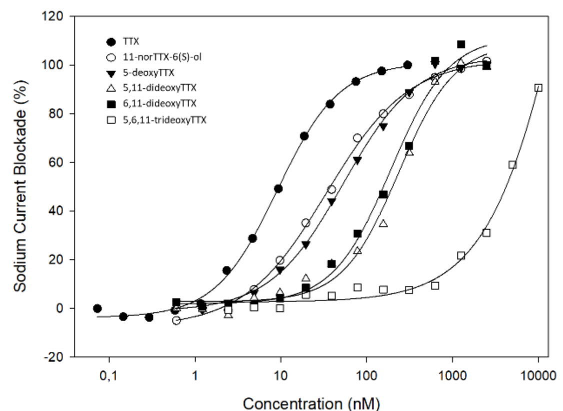

Figure 1: *Dose-response curves describing the toxicity of TTX and TTX analogues on patched Neuro2A cells.*

Conclusions

The single-cell biosensing platform used in this work showed to be a powerful bioanalytical tool for toxicology analysis, enabling not only the individual evaluation of five TTX analogues for TEF determination but also the toxic evaluation of naturally contaminated fishes, being attractive for its implementation in research and monitoring programs.

Acknowledgements

This research has received funding from the Spanish Ministerio de Ciencia e Innovación (MICIN) and the Agencia Estatal de Investigación through the CELLECTRA project (PID2020-112976RB-C21 and PID2020-112976RB-C22). The authors also acknowledge support from CERCA Programme/Generalitat de Catalunya.

Development of a screening method for small molecules binding to nucleic acid to characterize changes in secondary structure using switchSENSE® technology

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Keywords: nucleic acids, binding kinetics, Förster resonance energy transfer, small molecules

Introduction

Approximately 85 % of the genes are transcribed into RNA but only 3 % are translated into proteins (1). This is not reflected in the amount of available drugs, as most of them target proteins which relate to 0.05 % of nucleic acids. Thus, an effort must be made to fill this gap and focus on the transcriptome level of diseases. Non-coding RNAs fulfill important regulatory functions as small interfering (siRNA), transfer-RNA (tRNA) and other unknown ones (2, 3). Due to their importance, they are explored as novel drug targets, e.g. to develop alternative antibiotics.

Results and Discussion

We screened a set of small molecules to assess their effect on nucleic acid secondary structures while simultaneously measuring binding kinetics of the interaction utilizing a novel FRET-based biosensor assay. The assay employs DNA nanolevers immobilized on a biochip surface. The nucleic acid molecule with a FRET pair of fluorescent dyes are functionalized to the nanolevers. Structural changes within the nucleic acid affect the distance between the donor and acceptor dye. Consequently, the FRET-induced change in acceptor fluorescence signal intensity informs about conformational changes within the nucleic acid molecule. At the same time, precise kinetic rates can be derived from the assay, allowing a comprehensive characterization of the interaction. A detailed analysis of the binding mode of a small molecule binder implied that binding and nucleic acid folding are a one-step process. To validate not only small molecule hits but also examine the sequence dependency of the interactions, we screened different nucleic acid ligands to detect the essential binding sites.

Figure 1: *Binding and conformational change kinetics of nucleic acid with small molecule. The association of the analyte leads to a conformational change and therefore quenching in green and an increase in the red fluorescence signal due to FRET.*

Conclusions

The presented assay is a versatile tool for the screening of small molecules interacting with DNA. It provides not only binding kinetics but additionally reveals binding-induced structural changes and thus can be valuable in nucleic acid based drug discovery.

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Electrically actuated DNA origami nanolevers for protein size and conformational change analysis

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Keywords: switchSENSE, electrically-actuated, DNA origami, proteins, conformational change

Introduction

Protein function is directly linked to their three-dimensional structure. These diverse structures include the positioning of multiple side chains to form catalytic pockets in enzymes, or the complementary determining regions in antibodies. Additionally, many proteins perform their function through intricate conformational changes induced by ligand binding or through interaction with signalling molecules (e.g., nucleoside triphosphates, hormones, etc.) [1]. As a result, many small molecule and peptide-based drugs act by (de)stabilizing specific protein conformational states.

Drug discovery efforts will greatly benefit from an automated biosensor platform that allows the detection of conformational changes upon ligand binding, in tandem with measuring kinetic and equilibrium dissociation constants. Therefore, we introduce here a new molecular biosensor design that revolves around the use of DNA origami nanolevers that are seamlessly integrated with our current switchSENSE technology to enable relative protein size analysis and conformational change detection of large protein (complexes) under physiological conditions.

Results and Discussion

Our biosensor platform is based on the electrical actuation of DNA-based nanolevers tethered to a gold electrode. Alternating potentials applied to the sensor surface control the oscillating up and down motion of the nanolever. The nanolever's angular velocity is dictated by hydrodynamic friction with the solvent [2]. Thus, relative shape (changes) of a protein on the distal end of the nanolever can be directly inferred from the change in angular velocity. Due to their inherent flexibility and low charge density, however, conventional dsDNA nanolevers have limited protein size and ionic strength compatibility. To increase this compatibility we introduce here a long, rigid and slender DNA origami nanolever design, allowing us to measure the relative size and conformational change of larger protein complexes under physiological buffer conditions [3]. We demonstrate this through the measurement of a set of proteins with varying sizes. By using this novel biosensor approach, we can accurately characterize and discriminate protein size differences as little as ~8 kDa in real time.

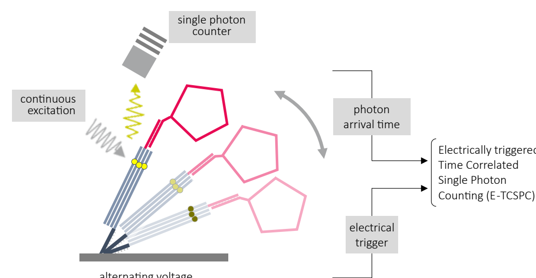

Figure 1: *Electrical actuation of DNA origami nanolevers carrying the protein of interest. The switching motion is tracked by distance dependent fluorescence quenching by the electrode surface. Protein size and conformational changes are derived from changes in angular velocity of the nanolever.*

Conclusions

Having confirmed the ability to discern small differences in protein size we will next set out to probe protein conformational changes induced by small molecule and peptide-based ligands.

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Acknowledgements

Funded by the European Union and supported by the UK Engineering and Physical Sciences Research Council (GA 101072980).

Simultaneous detection of glycated and non-glycated human serum albumin by a multielectrode array aptasensor for diabetes control

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Keywords: Electrochemical aptasensor, glycemic control, blood samples, point-of-care

Introduction

Assessment tests for glycemic control of patients with diabetes mellitus commonly use blood glucose and glycated hemoglobin A1C (HbA1C) levels for estimating acute and long-term glycemia, respectively. Glycated human serum albumin (GHSA) has been proposed as an alternative to HbA1C since it possesses a four times shorter lifespan and its blood concentration is not affected by hemic diseases. Here, an electrochemical aptasensor is proposed for the simultaneous detection of human serum albumin (HSA) and GHSA utilizing polymer-based multielectrode arrays.

Results and Discussion

Flexible multielectrode arrays (flex–MEAs) chips were employed facilitating a simultaneous dual-target detection of HSA and GHSA. Aptamer immobilization and target binding were studied by electrochemical impedance spectroscopy, chronocoulometry, and atomic force microscopy. Thermal pre-treatment of the receptor film was required to provide selective detection of the respective analyte and to avoid cross-selectivity between HSA and GHSA. The utilization of a polymer substrate for the sensor chip not only considerably reduced the material costs but also facilitated the easy separation and bending of the electrode arrays in opposite directions so that they could be incubated in separate vials containing, for instance, different aptamer solutions. As a result, both analytes can be measured in exactly the same sample, which implies the same sample composition and temperature, as well as less invasiveness for the patient since less sample needs to be donated and less care work since only a single measurement is needed. The electrochemical aptasensor developed in this work is characterized by high reliability for albumin and GHSA biomarker determination with wide detection ranges, combined with excellent recovery from diluted full-blood samples. Therefore, redundant signals from different electrodes of the same electrode set, all modified with the same receptor, were simultaneously recorded and analyzed, reducing the impact of device-specific variations. The use of polyethylene glycol backfill molecules effectively suppressed the nonspecific adsorption of blood matrix components and thus supported the highly selective detection of the analyte molecules of interest.

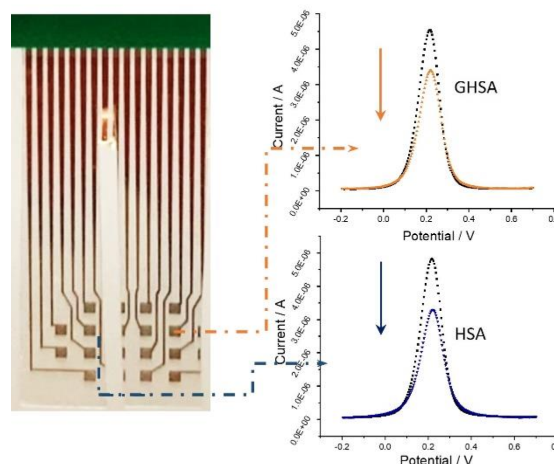

Figure 1: *DPV signal change of HSA and GHSA flex–MEA aptasensor with PEG backfills after incubation in a mixture of 100 nM HSA and 100 nM GHSA in blood, 100 times diluted.*

Conclusions

Here, we report on the first PoC ratiometric sensor for the simultaneous detection of HSA/GHSA in blood samples. Our flex–MEAs sensor not only utilizes low-cost polymer chips and aptamers but also immobilizes these receptors on different electrodes without expensive equipment, thus facilitating the quantitative and selective determination of midterm glycemia at the point of care without complicated sample treatment or expert staff.

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Acknowledgements

Lei Zhou acknowledges the financial support from China Scholarship Council (CSC 201708320255).

$(K_x, Na_{1-x})NbO_3$-Based Thin Film Surface Acoustic Wave Sensors as label-free Biosensors

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Keywords: surface acoustic wave, KNN, microfluidic, biosensor, lab-on-a-chip

Introduction

Surface Acoustic Wave (SAW) devices are promising platforms to achieve reliable, sensitive, and label-free point-of-care biosensors [1]. They are small, cost-effective, and simple to fabricate. SAWs can also be operated as filters, signal processing units, sensors, and actuators [2] and thus have been used for sample mixing, manipulation, sorting, sensing, pattering and atomization [3].

Typically, a SAW device consists out of three parts: a piezoelectric substrate (1), an input electrode (2) and an output electrode (3), which are coupled electro-mechanically to the substrate. In order to be used as biosensor, two additional components are needed: a linker molecule (4), which couples the substrate to the receptor, and capturing molecule (receptor) (5) which specifically interact with the analyte. A promising alternative to commonly used $LiNbO_3$ or lead-based $Pb(Zr,Ti)O_3$ (PZT) piezoelectric substrates is given by $K_x,Na_{1-x}NbO_3$ (KNN) thin films. KNN is a piezo- and ferroelectric material with ABO_3 perovskite structure [1] [3].

Results

SAW sensors were fabricated by growing ultrathin films of 30 nm thin KNN on $TbScO_3$ (TSO) via Metal Organic Vapor Phase Epitaxy (MOVPE). Strain engineering was used to increase the piezoelectricity [1]. At first, alternating multilayers of polyanions and polycations were deposited as model analytes by a microfluidic cell. The corresponding SAW signals for one, two, three, and four layers were measured and the limit of detection (LOD) was derived to be (58.41 +/- 3.27) fg. Subsequently, the impact of the temperature on the signal response was investigated and a considerable response on the SAW sensor signal was observed.

Conclusions

The polyelectrolyte multilayer measurement demonstrated the high sensitivity of this transducer and gives an insight in future application perspectives. The investigated SAW sensor showed high environment sensitivity, which could interfere the analyte detection and degrade the sensor performance.

To address this problem, a reference delay line will be implemented in future designs. This reference delay line will not interact with the analyte and provide exclusively information on environment parameters. Interfering signals from varying experimental conditions will be eliminated by drift correction in the following.

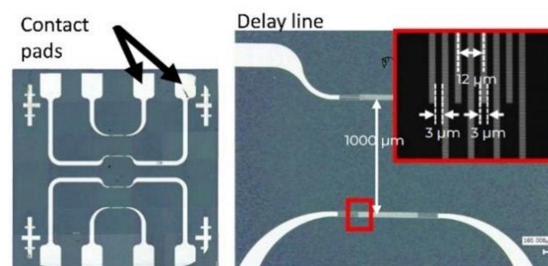

Figure 1: *Laser microscope images of a KNN SAW sensor with Ti/Pt IDTs. The small image was taken with a confocal microscope.*

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Acknowledgements

A. Offenhäusser, M. Blanznet, R. Kutzner, S. Trellenkamp, F. Lentz, and Helmholtz Nanoelectronic Facility are acknowledged for valuable support.

Intracellular RNA aptamer sensor with adaptable recognition site

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Keywords: RNA sensor, intracellular, FACS, aptamers

Introduction

Until now, the detection of biomacromolecules, small molecules, and ions inside cells is a challenge. Adding external sensors or sensing elements is associated to difficulties like hampered internalization or change of the intracellular environment. [1] To avoid the use of external sensors, substrate-binding proteins were chosen, which are not available for every analyte. Therefore, we developed a new intracellular sensor architecture based on engineered tRNAs that contains two aptamers (Fig. 1A). As a reporter system, GFP and its aptamer, which can quench GFP fluorescence, are used. The reporter aptamer is connected to the recognition aptamer, corresponding to the target molecule or ion. Upon binding of the analyte to its aptamer, the GFP aptamer conformation changes and GFP is released (Fig. 1A). Both the tRNA scaffold and the GFP reporter system can simultaneously be expressed and used in bacteria.

Results and Discussion

We developed an RNA sensor, which detects the antibiotic Neomycin B (NeoB) and its azide. NeoB azide is less toxic for bacteria and therefore a better model molecule. Both molecules bind to the NeoB aptamer, whereas NeoB has a higher binding affinity (100 nm) as the azide (28 μM). We showed that expression of GFP and the RNA sensor simultaneously results in quenching of GFP up to 60%, which can be reversed through addition of NeoB or NeoB azide (Fig. 1B). Due to the lower binding affinity of NeoB azide in comparison to NeoB, a significant higher target molecule concentration is needed for detection. The GFP fluorescence is detected via FACS (Fig.1B).

In addition to the usage in bacteria, the sensor design allows its use in vitro. When applied *in vitro,* the ratio of GFP against the sensor can be adjust precisely. A 1:10 GFP to sensor ratio induced approx. 40% quenching of GFP fluorescence in 25 min. Adding NeoB to the solution results in an immediate release of the GFP and an enhancement in fluorescence signal.

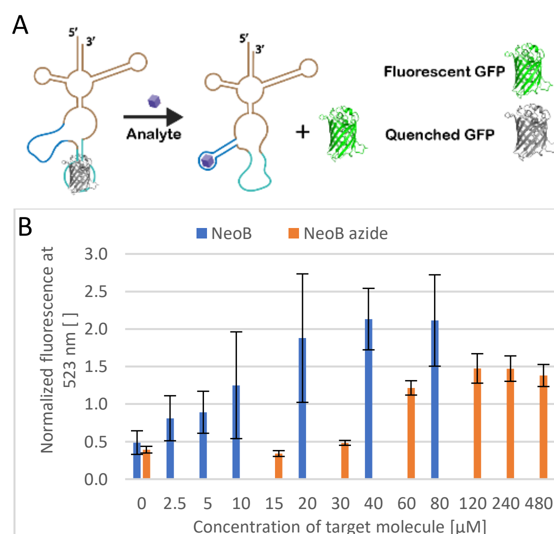

Figure 1: *(A) Mechanism of the RNA sensor with GFP as a reporter. (B) Normalised GFP fluorescence on a GFP positive control, measured via FACS, regarding different NeoB and NeoB azide concentrations in bacteria.*

Conclusions

The combination of two aptamers in one sensor opens up many new possibilities in the field of biosensors. The recognition aptamer can easily be exchanged for the detection of other target molecules, such as Co^{+} ions, TPP, or large proteins, such as surface proteins of viruses. The tRNA scaffold design allows the use of the sensor in bacteria or *in vitro*.

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Acknowledgements

This research was supported by RWTH Aachen University.

Understanding the potentiometric response of permselective hydrophilic nanopore-based sensors

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Keywords: Nernst-Planck-Poisson model, nanopore-based sensing, permselectivity, potentiometry

Introduction

The focus of ion-selective electrode research has been on the development of highly selective membranes for certain ions. "Ideally" non-selective membranes, however, may also find important applications, e.g., determination of total ion concentration or as detectors in ion chromatography. Conventional hydrophobic polymer-based membranes are not suitable for this purpose due to their inherent, hydrophobicity-induced selectivity (Hofmeister series). This can be eliminated by using hydrophilic ion-exchange membranes, such as the Donnan-exclusion-based electrodialysis membranes [1]. Gold nanoporous membranes modified with self-assembled monolayers of charged thiol derivatives could serve the same purpose, but dramatically reduce the synthetic effort to prepare pores with different sizes, charge signs, and charge densities. Previously, we implemented gold nanoporous membranes to prove the concept of permselective potentiometric response [2] and to fabricate solid-state ion channels with exquisite selectivity [3]. Here we report the potentiometric response of ion-exchanger-modified gold nanoporous membranes along with a full theoretical treatment based on the Nernst-Planck/Poisson (NPP) model.

Results and Discussion

We determined the potentiometric response of cation- and anion-exchanger-modified gold nanoporous membranes for a variety of ions and found that the membrane potential depends solely on the charge of the ions. This suggests that the interaction between the membrane and the ions is purely electrostatic, which enables the use of the NPP model. The theory reliably predicted not only the steady-state but also the transient potential responses. We systematically investigated the influence of the membrane properties (e.g., thickness, pore size, pore density) and the mass transport regimes on the potentiometric response.

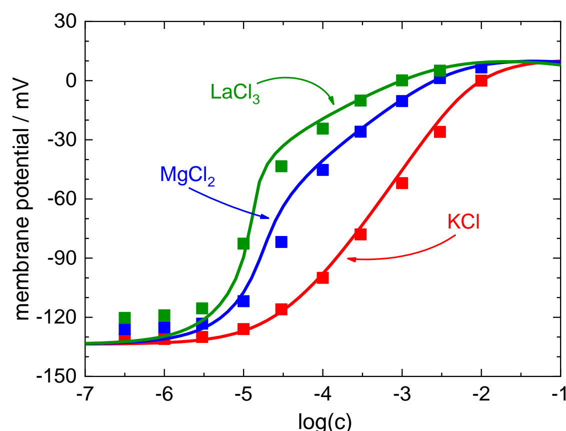

Figure 1: *Experimental (squares) and simulated (lines) potentiometric calibration curves of 2-mercaptoethanesulfonate-modified membranes with ∅ 4-nm pores. Simulations were performed with optimal values of diffusion layer thickness (75 µm) and nanopore surface charge density (-1.8 mC/m²).*

Conclusions

Hydrophilic nanoporous membranes can be used as non-selective potentiometric sensors. The implemented NPP model provides a strong foundation for tailoring nanoporous membranes for chemical sensing and separation.

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Acknowledgments

This research was funded by the National Research, Development, and Innovation Fund of Hungary (TKP2021-EGA-02). Mr. Solymosi was supported by the Ph.D. Excellence Scholarship of Gedeon Richter's Talentum Foundation (Gyömrői út 19-21, H-1103 Budapest, Hungary).

Oriented electrochemical molecularly imprinted-based biosensor for sensitive detection of alanine aminotransferase

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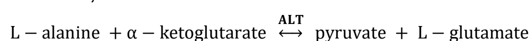
Keywords: Molecularly imprinted polymers; Alanine aminotransferase (ALT); Electropolymerization

Introduction

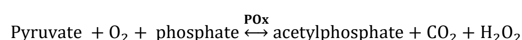
Alanine aminotransferase (ALT) is an enzyme found in various vital organs of the human body, and plays a crucial role in amino acids metabolism, energy production, and protein synthesis. As regular monitoring of ALT level is essential for evaluating liver function and assessing cardiovascular health [1], we developed an electrochemical tailor-made biosensor based on imprinted pyruvate oxidase enzyme (POx) using 4-aminophenol (functional monomer) on platinum microelectrode modified (PME)) with platinum nanoparticles (working electrode) and 4-aminoantypirine (4-AAP)/ sodium pyruvate as an electrochemical indicator.

Results and Discussion

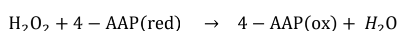
The imprinted electrode, was used for the determination of ALT utilizing 4-aminoantypirine as an indicator of enzymatic reaction, as follows:



$$\mathrm{L-alanine} + \alpha - \mathrm{ketoglutarate} \overset{\mathrm{ALT}}{\longleftrightarrow} \mathrm{pyruvate} + \mathrm{L-glutamate}$$



$$\mathrm{Pyruvate} + O_2 + \mathrm{phosphate} \overset{\mathrm{POx}}{\longleftrightarrow} \mathrm{acetylphosphate} + CO_2 + H_2O_2$$



$$H_2O_2 + 4 - \mathrm{AAP(red)} \rightarrow 4 - \mathrm{AAP(ox)} + H_2O$$

The change of the 4-AAP redox peak is correlated with activity of ALT in the sample under optimized conditions.

The fabrication steps started with successful electrodeposition of Pt nanoparticles to enlarge the electroactive surface area, which enhanced the sensitivity of the sensor.

The imprinting step using electropolymerization offers the formation of reproducible polymeric imprinted film under the optimized conditions including: the number of cycles, 4-AP concentration, extraction, and incubation time.

Sensitivity and detection limit (LOD) of the MIP modified PME biosensor for ALT detection are critical parameters. The calibration curve showed a linear relationship within the tested concentration range (see figure 1). The peak current increased with higher ALT concentrations due to increased generation of pyruvate. The limit of detection (LOD) was calculated to be 2.97 U/L with a high R2 value of 0.998.

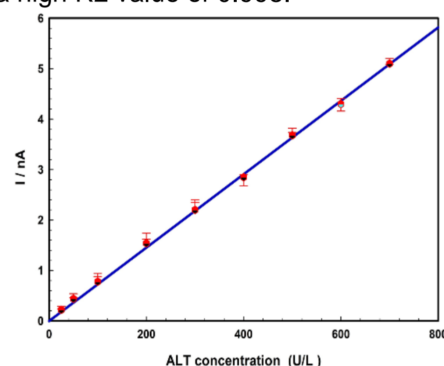


***Figure 1**: Calibration curve of POx MIP modified PME at different ALT concentrations (Error bars represent the standard deviation of three runs using the same electrode).*

The developed biosensor was also tested in respect to selectivity and didn't exhibit cross reactivity towards other tested enzymes including nicotinamide adenine dinucleotide (Beta-NAD), catalase (CAT), glutathione peroxidase (GPx) and L- glutathione reduced (GSH) enzymes. Finally, ALT was determined in human plasma samples with recovery values ranging from 99.80- 103.82% and RSD values of 0.27-2.01%. The results were comparable to those of the reference diagnostic kits without requiring protein extraction.

Conclusions

We presented herein a promising approach, which may be extended for application to involve other enzymes related to the POx cycle taking into consideration that sodium pyruvate can be replaced by the specific substrate in the active probe to assure the selectivity of the sensor towards the selected enzyme of interest.

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Capacitive enzyme-based interdigitated electrodes for detecting H_2O_2 vapour/aerosol for sterilization in medical industry

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Keywords: sterilization, hydrogen peroxide vapour/aerosol, enzymatic membrane, capacitive signal

Introduction

Hydrogen peroxide (H_2O_2) is a vastly used as sterilization agent in aseptic industry [1]. Noticeably, scientists have investigated online monitoring of aseptic food packaging, where concentrations of gaseous H_2O_2 up to 8% v/v are applied at elevated temperatures (up to 240 °C) [2]. Yet, in medical industry, lower concentrations of H_2O_2 vapour/aerosol are applied and monitored usually by bulky and expensive detectors. Here, we present a novel enzyme-based biosensor for the detection of H_2O_2 vapour/aerosol at low concentrations (<1000 ppm). Two identical interdigitated electrodes (IDE) are introduced; one IDE (active) is coated by the enzymatic membrane of horseradish peroxidase (HRP) to react toward H_2O_2, whereas a second IDE (passive) remains uncoated. The two IDEs form a differential set-up for the capacitive detection of the H_2O_2 vapour/aerosol.

Results and Discussion

Fig. 1 schematically presents the differential set-up of the IDEs, including passive IDE (right) and active IDE (with HRP membrane, left). Optical and scanning electron microscopy (SEM) as well as profilometry were employed for surface morphology characterization of the IDEs. When the IDE set-up is exposed to H_2O_2 vapour/aerosol, the HRP membrane (on the surface of the active IDE) reacts to H_2O_2. This enzymatic reaction affects the impedance and consequently, the capacitance of the IDE structure, following formula (1):

$$C = \frac{-\sin(\varphi)}{2\pi f Z} \quad (1)$$

Here, Z represents the impedance, φ is the phase angle between the impedance and the capacitance (C), and f the frequency.

The capacitive enzyme-based set-up can be used to assess the detection of the H_2O_2 vapour/aerosol in concentrations up to 1000 ppm by the novel IDE arrangement.

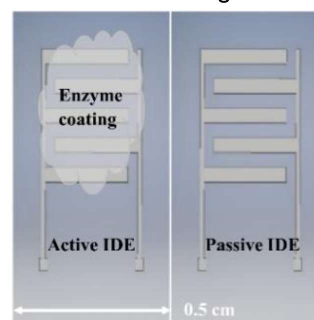

Figure 1: *Schematic of the IDE differential set-up; the active IDE (left, coated with the HRP enzymatic membrane) and the passive IDE (right, uncoated).*

Conclusions

A differential set-up of enzyme-based IDE biosensors has been developed for H_2O_2 vapour/aerosol detection. Due its flat shape, miniaturized size and cost-efficient production, this biosensor set-up can be used for future 2D- or 3D mapping of medical isolators.

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Transient magnesium-based resistive temperature detectors on flexible and biodegradable substrates

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Keywords: fibroin, polylactide acid, resistive temperature detector, magnesium, biodegradable

Introduction

Implantable sensors are not only relevant for glucose measurements but are also very attractive for monitoring patients undergoing certain surgeries, such as skin flap transplants. Here, the monitoring of parameters, like blood oxygen saturation, pH, color, or temperature is crucial [1]. Those sensors ideally consist of biocompatible and fully biodegradable materials that can be absorbed by the body after a certain time. We present a first approach for the realization of a biodegradable resistive temperature sensor (RTD). This sensor consists of a biodegradable substrate (polylactide acid (PLA) or fibroin) with a magnesium-type meander structure as resistive electrode on top, which is optionally passivated by additional magnesium (MgO) oxide layers.

Results and Discussion

On a biodegradable substrate (PLA or fibroin), different transient layer systems were applied: (i) Mg, (ii) MgO/Mg, and (iii) MgO/Mg/MgO. The magnesium layer was deposited as a meander-shaped electrode, whereas the magnesium oxide represents an adhesive (bottom) and protective (bottom & top) layer to increase the lifetime of the sensor. Therefore, the magnesium oxide layer was deposited onto the entire surface of the substrate as well as the meander structure to cover the edges, as shown in Fig. 1, top left. A video microscopic image of the RTD with a 3-layer structure (MgO/Mg/MgO) on fibroin is depicted in Fig. 1, bottom right. The sensors were characterized both under environmental conditions (30 °C to 43 °C) and under tissue-like conditions (37 °C), mimicked by a hydrogel. The calibration curve of the 3-layer RTD (MgO/Mg/MgO) on fibroin under environmental conditions is displayed in Fig. 1, too.

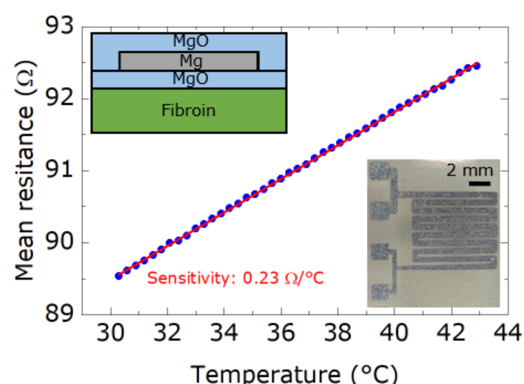

Figure 1: *Calibration curve of a magnesium-based RTD measured under environmental conditions from 30 to 43 °C in 0.3 °C steps. The RTD consists of a biodegradable fibroin substrate modified with two layers of magnesium oxide with a magnesium-based meander structure in between (top left corner). The picture in the bottom right corner shows the RTD before measurement.*

Conclusions

The developed, fully biodegradable, Mg-based RTDs on fibroin and PLA can be used under environmental and tissue-like conditions for temperature measurements. Depending on the substrate material, upcoming studies will focus on a further improvement of the stability behavior.

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Silk-fibroin as immobilization matrix for enzymes on light-addressable potentiometric biosensors

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Keywords: LAPS, urea, fibroin, immobilization methods, biosensor

Introduction

Light-addressable potentiometric biosensors (LAPS) determine the concentration of an analyte by measuring the surface potential. A fundamental challenge in biosensing is the reliable immobilization of enzymes on the chip surface without compromising its properties. Here, fibroin, obtained from the silkworm *Bombyx mori*, can be utilized as immobilization matrix [1]. The unique structure of fibroin offers the possibility of both physical entrapping and chemical binding of enzymes. Fibroin not only preserves the activity of the enzyme (several months), but also improves its stability to pH and temperature variations and offers high mechanical robustness [2].

Results and Discussion

Urease was immobilized on a LAPS chip (n-$Si/SiO_2/Si_3N_4$) using the silk protein fibroin as immobilization matrix. Here, different methods for the enzyme immobilization were studied: physical entrapment of urease in a fibroin membrane as well as chemical linking of urease to a fibroin membrane. The resulting LAPS-based biosensor was illuminated with an IR (infrared) laser beam modulated at 2.5 kHz. The resulting photocurrent for sweeping bias voltages in the range between –1.5 V and 0.5 V (with a step size of 8 mV) was recorded each minute for a period of 10 minutes after the addition of urea (0.2 mM to 6.4 mM) into 0.1 M NaCl solution.

Conclusions

The immobilization of urease on a LAPS chip using fibroin as immobilization matrix was successfully –demonstrated. Fig. 1 shows the shift of the recorded I–V curves (photocurrent–voltage) as a function of the increasing urea concentration.

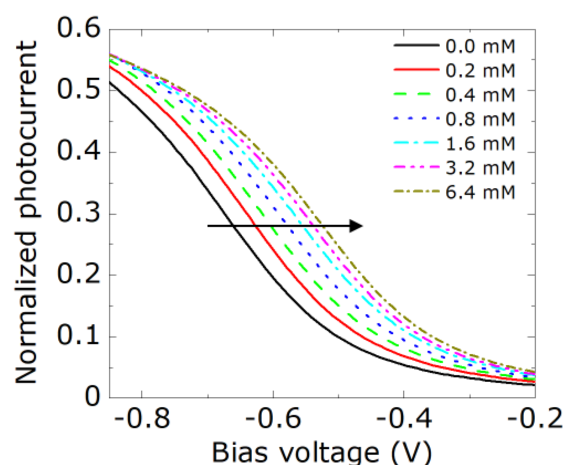

Figure 1: *Photocurrent-bias voltage curves of a LAPS chip modified with 30 U urease, immobilized in a drop-coated fibroin matrix consisting of 2-eq. 8wt% PureSilk, 1-eq. 50vol% ethanol and 1-eq. 3vol% glycerol solution for different urea concentrations.*

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Label-free field-effect detection of C-reactive protein using antibody-conjugated magnetic nanoparticles with a capacitive field-effect sensor

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Keywords: magnetic nanoparticles, field-effect sensing, C-reactive protein

Introduction

Immuno-sensitive field-effect devices for label-free protein detection via their intrinsic molecular charge have attracted considerable interest. However, field-effect detecting disease biomarkers in real biological samples (e.g., whole blood) remains a big challenge due to following major limitations: The counter-ion screening of the protein charge and the possible non-specific adsorption of other biomolecules present in the complex samples. One promising strategy to overcome these limitations is the magnetic field-promoted separation technique, i.e., magnetically fishing out biomarkers to be detected from the complex mixtures.

In this work, we present preliminary results on the label-free detection of C-reactive protein (CRP)-coupled antibody (anti-CRP)-conjugated magnetic nanoparticles (MNP, 30-100 nm in diameter) using field-effect electrolyte-insulator-semiconductor capacitors (EISCAP) **[1]**. CRP is a general inflammation and infection biomarker. Elevated levels of CRP may indicate increased risk of diabetes, hypertension, stroke and cardiovascular diseases **[2]**.

Results and Discussion

The EISCAP sensors consisting of an Al-p-Si-SiO_2 structure (Figure 1) were characterized in a low ionic-strength (5 mM) buffer solution before and after immobilization of CRP-anti-CRP-MNPs or anti-CRP-MNPs (for comparison) using capacitive–voltage and constant–capacitance methods. Dependence of the sensor signal on the CRP concentration has been studied. Details of experiments and obtained results will be presented and discussed.

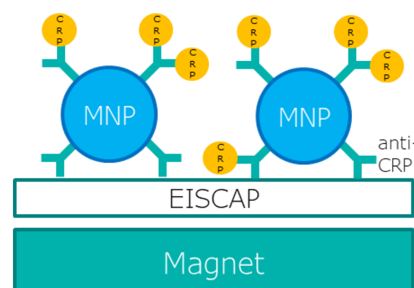

Figure 1: *Schematic structure of the sensor setup with immobilized CRP-anti-CRP-MNPs.*

Conclusions

Field-effect EISCAP sensors have successfully been applied for the label-free electrostatic detection of anti-CRP-MNPs and CRP-anti-CRP-MNPs.

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Acknowledgements

This work was funded by the Deutsche Forschungsgemeinschaft (DFG: German Research Foundation)– 445454801.

Magnetic Nanoprobe Based Competitive Immunodetection for Fast and Quantitative Detection of Antibiotic Residues in Milk

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Keywords: frequency mixing technology; immunofiltration; magnetic beads

Introduction

Ensuring high quality standards for food is of global importance. In order to ensure this, suitable rapid test methods are needed. Especially reliable and quantitative detection of low molecular weight compounds like e.g. antibiotic residues is challenging and often not possible with existing rapid test methods. Here we present a novel rapid test format based on competitive magnetic immunodetection (CMID) employing magnetic nanoprobes in combination with a competitive immunofiltration assay and frequency magnetic mixing technology (FMMT) [1] based detection.

Results and Discussion

CMID was demonstrated for quantitative detection of penicillin G and other antibiotics in whole fat milk (WFM). We employed special immunofiltration columns (IFC) with a porous matrix coated with antibiotics conjugate (Figure 1A). WFM was spiked with different concentrations of antibiotics and incubated with biotinylated monoclonal antibodies with binding specificity for the respective antibiotics. Subsequently pre-incubated samples were applied to the IFC. While passing the matrix in gravity flow, antibodies were able to compete for binding either to the antibiotics within the milk sample or to the coated antibiotics conjugate. Then magnetic nanoparticles were applied to the IFC to specifically label enriched antibodies. After a final washing step with PBS IFC were placed in the detection head of the mobile magnetic reader device (Figure 1B) and readout signals were determined based on FMMT. Magnetic signals correlate with the amount of enriched antibodies and were thus inversely proportional to the antibiotics concentration within the milk samples. Based on this assay principle, calibration curves were recorded for penicillin G (Figure 1C) and other antibiotics enabling detection limits below 1 ng mL^{-1} which is below the regulatory limits. Additional spiked WFM samples were analysed using cMID, revealing highly accurate recovery rates and assay precision.

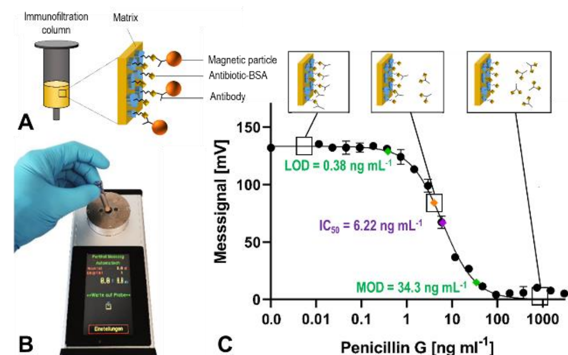

Figure 1: *Schematic representation of CMID assay principle (A), mobile magnetic reader devide (B) and representative calibration curve for penicillin G in WFM (C) showing Limit of detection (LOD), Maximum of detection (MOD) and* IC_{50} *value. European maximum residue limit for penicillin G is marked in orange.*

Conclusions

We were able to demonstrate that competitive magnetic immunodetection (cMID) is a fast, sensitive and reliable method to quantitatively detect antibiotic residues in milk. Furthermore, the assay can be easily adapted for the detection of other low molecular weight contaminants as e.g. mycotoxins [2].

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Revealing prevalent cancers by interrogating glycoproteins with sustainable immunoelectrochemical tools

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Keywords: electrochemical immunoplatforms, proteomic biomarkers, pancreatic and colon cancers

Introduction

The worldwide incidence and death toll of colorectal and pancreatic cancers (CRC and PDAC) have increased considerably since 1990. For this reason, both early detection and its regular follow-up are considered key factors in improving patient prognosis. In this sense, the determination of the total content of certain proteins and their aberrantly glycosylated fraction in oncologic processes could help to achieve the proposed goals.

Results and Discussion

In this work, two simple but highly competitive electrochemical immunoplatforms for the determination of total and glycosylated post-translational modified haptoglobin (Hp) **[1]**, and CA19-9 **[2]** (candidate biomarkers associated with colorectal and pancreatic cancer, respectively) are presented.

As seen in Figure 1, these biotools are uplifted in the use of magnetic immunocaptors and another antibody or a lectin as detector elements lastly labeled with HRP, which enables subsequent amperometric detection.

The presented bioplatforms exhibit attractive characteristics in terms of simplicity, affordability, and point-of-care application compared to the conventional available methodologies, highlighting low detection limits (0.07 and 0.46 ng mL^{-1} for total and glycosylated Hp, respectively, and 1.5 U mL^{-1} for CA19-9), and short assay times (< 2 h).

The workability of these quantitative bioplatforms for the analysis of secretomes from cultured CRC cells with distinct potential to metastasize (Hp) or serum samples from healthy and PDAC-diagnosed subjects (CA19-9) was assessed to definitely confirm full exploitation of all the above exposed enticing attributes.

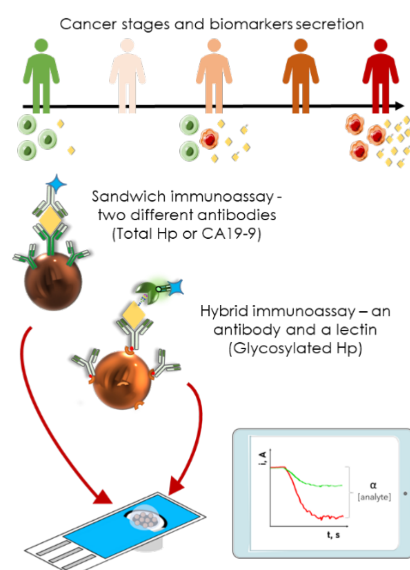

Figure 1: *MB-assisted immunoplatforms for the amperometric determination of total and glycosylated Hp or CA19-9.*

Conclusions

Our findings clearly revealed the unquestionable ability of these modern electrochemical immunoplatforms to discriminate between healthy and cancer-diagnosed subjects, as well as to assess disease progression, positioning these simple but effective methodologies as advanced electroanalytical tools with proven real biomedical applications, and the hope of aiding in the accurate diagnosis of prevalent and high mortality cancers.

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Evaluating the Resin-Solvent Compatibility of 3D Printed Biosensors

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Keywords: microfluidics, stereolithography, resin-solvent compatibility

Introduction

Custom microfluidic devices for biosensing purposes are currently in high demand. However, traditional fabrication methods, which are expensive and time-consuming, have prompted researchers to pay attention to the exploration of 3D printing. Stereolithography (SLA) is a technique for photopolymerization of liquid resin-containing monomers and photoinitiators upon UV light illumination. SLA enables the fabrication of internal microchannels with entangled designs. As a result, there has been a boom in research focused on SLA-based microfluidic devices [1]. However, it should be noted that SLA parts tend to leach unreacted monomers when they come into contact with liquid solutions [2]. This leaching may alter the functionality of the biosensor, for example, when SLA parts are used as moulds for PDMS [3].

Results and Discussion

To address this concern, our study investigates the leaching rates of various commercial resins exposed to different solvents that are commonly used in biosensors. We conduct leaching experiments both statically (immersion of an SLA part in a solvent) and dynamically (flow of solvent through an SLA-based microchannel). In addition, we investigated how post-processing techniques, such as simple washing, UV exposure, and heat treatment, can influence the leaching. By gaining insights into leaching tendencies and exploring potential remedies through post-processing, we aim to enhance the reliability and performance of custom microfluidic biosensors, thus contributing to their broader applications in biosensing and related fields.

Conclusions

The results show that post-processing of printed parts plays an important role on the leaching behavior of SLA parts. Adequately post-processed SLA parts show less tendency to leach uncured monomers into the solvents. Another criteria on the leaching of resin components is flow rate of the solvent that flow through the channels of biosensor. The faster the flow rate, the less amount of leached resin measured in the solvent. There was a big difference between flow and nonflow experiments: regardless of what post-processing is applied to microfluidic channels, there are still more amounts of uncured resin in the internal microchannels of the biosensor.

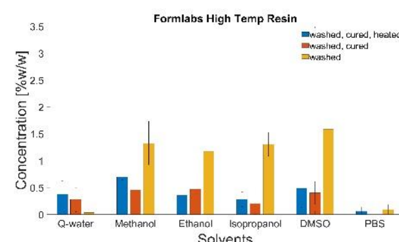

Figure 1: Concentration of leached resin in each solvent (calculated by infrared spectroscopy).

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Acknowledgements

This work was supported by the Czech Science Foundation (contracts #21-19779S and #22-20012S)

Improved electrochemical biosensor for C-peptide detection in urine samples

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Keywords: Electrochemical Biosensor; C-peptide; Urine Biomarkers; Diabetes Mellitus

Introduction

Diabetes mellitus type 2 (T2DM) and other disturbances of insulin metabolism are serious and complex diseases, affecting nearly 600 million worldwide by 2021. As hyperinsulinemia and insulin resistance is one of the most important factors in the progression of T2DM, surrogate biomarkers such as C-peptide have shown its relevance in risk assessment and disease monitoring. C-peptide is co-secreted with insulin in equimolar amounts and makes it a good indicator of beta cell function and/or hyperinsulinemia. A point-of-care testing device for C-peptide measurements would find widespread application to assist in the identification of people at risk for impaired glucose tolerance, e.g. T2DM or gestational diabetes in pregnant women. We thus aimed to develop a self-assembled monolayer (SAM) based electrochemical biosensor for the detection of human C-peptide in urine.

Results and Discussion

The biosensor relies on a competition format utilising a SAM to covalently linked C-peptide to a thiolated linker molecule immobilized on a gold electrode. An enzyme labelled anti-C-peptide antibody was used as a reporter antibody and, following competition and substrate addition, the reduced product was amperometrically detected. The developed biosensor is reliable, highly specific, and showed a good sensitivity with a limit of detection of 2 ng/mL. In addition, a good repeatability (intra-assay) and reproducibility (inter-assay) with <11% RSD (n=3) was observed.

Conclusions

This proof-of-concept has the potential to be integrated into a portable assay for point-of-care testing to support the assessment and monitoring of individuals at risk for type 2 or gestational diabetes.

Acknowledgements

We would like to thank the funding organization Center for Biomarker Research in Medicine (CBmed) who supported this research work.

Highly Crystalline Iron Oxide Nanoparticles for Nonlinear Magnetic Detection Methods Synthesized by Oxidative Aging

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Keywords: magnetite, biocompatible nanoparticles, superparamagnetic

Introduction

In the field of medical diagnostics and therapy, iron oxide nanoparticles (IONPs) offer auspicious possibilities. For utilization of IONPs as tracer material for magnetic particle imaging (MPI), for hyperthermia treatment or for nonlinear magnetic detection, particle sizes between 15-30 nm and a high crystallinity are required [1,2]. In this context, we present oxidative aging as synthesis method to achieve crystalline magnetite nanoparticles with a narrow size distribution and a biocompatible coating.

Results and Discussion

The synthesis by oxidative aging comprises precipitation of ferrous hydroxide and subsequent oxidation by KNO_3 to octahedral magnetite crystals (Fig. 1) [2]. The dependence of IONP size on the iron precursor concentration was investigated. With increasing iron concentration, increasing size was observed. The synthesized IONPs were coated with different biocompatible ligands such as dextran or dopamine to stabilize them in aqueous solution.

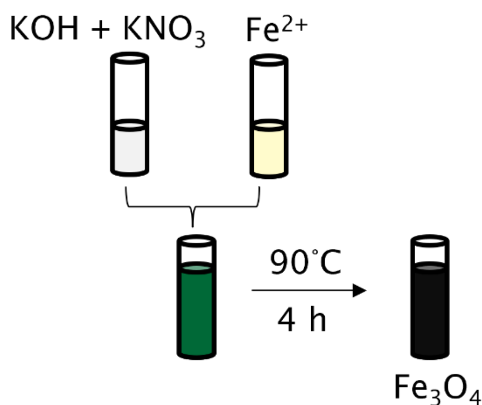

Figure 1: *Synthesis of magnetite IONPs. Ferrous hydroxides are precipitated and from this magnetite NPs develop via oxidative aging.*

The synthesized IONPs were characterized by dynamic light scattering, infrared spectroscopy, frequency mixing magnetic detection (FMMD), and scanning electron microscopy (SEM) (Fig. 2).

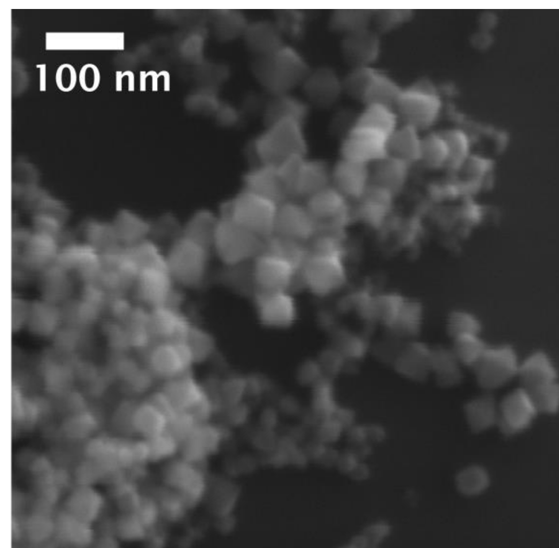

Figure 2: *SEM image of octahedrally shaped IONPs obtained by oxidative aging with an average size of 30 nm.*

Conclusions

Highly crystalline magnetite nanoparticles with sizes suitable for nonlinear magnetic detection methods like MPI or FMMD were synthesized by oxidative aging. The NPs are equipped with a biocompatible coating which is an excellent prerequisite for biomedical applications.

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Acknowledgements

We acknowledge funding by DFG grant number Ka 5450/1-1 and KR 3864/8-1.

Optimization of ion-selective membranes on PEDOT:PSS OECT microarrays for real-time and multiplexed sensing of sodium and potassium ions in the sweat concentration range

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Keywords: PEDOT: PSS OECTs, microfluidic, ion-selective membranes, multiplexed sensing

Introduction

In recent years, PEDOT: PSS-based organic electrochemical transistor (OECT) has been intensively studied and various applications have been explored, including neural interfaces, cell electrophysiological recording, wearable electronics as well as monitoring the compositions of sweat for health monitoring [1, 2]. The OECT sensor has been used to monitor the ions such as Na^+, and K^+ concentrations in sweat; however, multiplexed sensing of such ions with high sensitivity and selectivity is still challenging. In this work, sodium (Na^+) and potassium (K^+) ion-selective membranes (ISMs) are integrated with OECT microarrays by spin coating technique for multiplexed sensing of Na^+ and K^+ ions. A microfluidic system was integrated into the OECT for real-time measurement of ions in a controlled flow-through manner. Furthermore, the influence of the compositions and thickness of the ISM on device performance was statistically investigated.

Results and Discussion

In this work, PEDOT:PSS OECT microarrays with 16 channels were successfully fabricated. A controllable thickness of the ISM OECTs was archived by spin coating technique ranging from a few hundred nanometers up to a few micrometers. The schematics of ISM-PEDOT: PSS OECTs and the image of one encapsulated device are presented in figure 1 a. A microfluidic system was integrated with the OECT array for multiplexed sensing of Na^+ and K^+ ions. The sensors showed high sensitivity to the desired ions while high selectivity to another ion as shown in figure 1b. Changing the composition of the ISM and thickness strongly affects the sensitivity and selectivity of the sensors. A reduction in membrane thickness leads to an increase in sensitivity while a decrease in selectivity. An optimized composition and thickness of the ISM were archived and were used to monitor the concentration of ions in measured buffers.

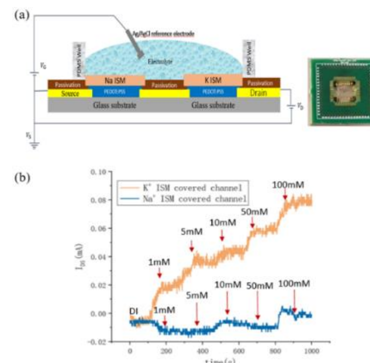

Figure 1: (a) The schematics of ISM-PEDOT:PSS OECTs and encapsulated device. (b) Real-time detection of ions in a buffer solution for K^+ ion on a chip with integrating different ISM membranes on different channels of the OECT arrays.

Conclusions

Microarrays of OECTs with the integrated ISM membranes for the multiplexed sensing of Na+ and K+ were successfully fabricated. We have found an optimal composition and thickness of the ISMs to have high sensitivity and selectivity to the Na^+ and K^+. In the future, an extension to detect other ions, like Ca^{2+} and NH_4^+ will be investigated. Furthermore, the platform will be transferred to a flexible substrate for applications in sweat monitoring.

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Acknowledgements

Microfabrication was carried out in the Central Laboratory for Micro- and Nanotechnology (ZMNT) at RWTH Aachen University. Technical support by ZMNT staff is gratefully acknowledged. CL gratefully appreciates the financial support from China Scholarship Council. Financial support for this project came from RWTH Aachen University.

High-performing printable electrode for H_2O_2 detecting enzyme-free sensors

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Keywords: H_2O_2 detection, nanocatalysts, laser-induced carbon nanofibers, point-of-need devices

Introduction

Hydrogen peroxide (H_2O_2) is an important biomarker found in biological samples at very low levels, which requires sensitive and selective detection. Enzymes are widely used in electrochemical sensors for H_2O_2 detection. Nevertheless, their limited stability and high cost make enzyme-based sensors less suitable for on-site detection. In contrast, metal nanocatalysts, especially embedded within carbon nanofibers, can offer such electrocatalytic activity with superior stability and affordable cost. We thus developed a high-performing enzyme-free sensor realized by laser-induced carbon nanofibers (LCNFs) containing *in situ*-generated metal nanocatalysts. Herein, polymeric nanofibers doped with metal salt were converted in such 3D carbon networks using CO_2 laser. In the present study, LCNF-Pt/Ni is investigated to promote the electrochemical detection of H_2O_2 under physiological pH [1].

Results and Discussion

We investigated the effect of various Ni:Pt ratios on the detection sensitivity. LCNFs containing 100% Pt yielded the best oxidative response (at +0.5V), providing a limit of detection (LOD) of 1.4 µM. Yet, such a high oxidative voltage causes the presence of interfering signals from other electroactive species. With 100% Ni, a LOD of 40 µM (at +0.5V) and similar interfering signals were observed. Interestingly, initial results with LCNFs containing 75%Ni + 25%Pt suggested that a constant voltage at -0.4 V can be applied to reduce H_2O_2 (Figure 1). However, with chronoamperometry at -0.4 V, H_2O_2 in µM-range was not detectable. We assume that the previous anodic scan from 0 to 0.8 V (Figure 1) acts like a pre-treatment of the nanocatalysts on the electrode surface. To confirm this assumption, linear sweep voltammetry w and w/o 10 mM H_2O_2 in PBS was carried out from 0 to -0.8 V w and w/o an anodic pre-treatment at +1.0 V for 20 s. As expected, the treatment strongly enhances the difference between signals with and without H_2O_2 at -0.4 V.

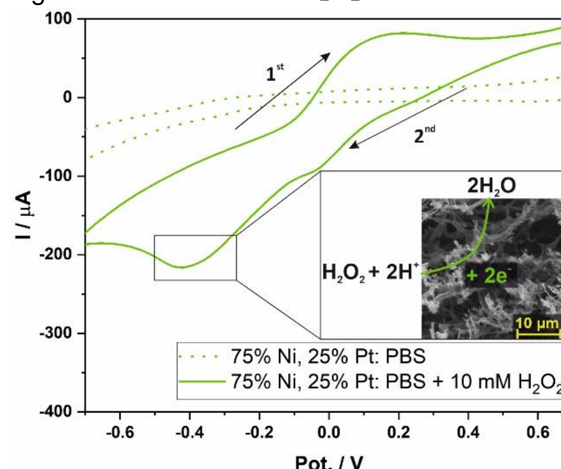

***Figure 1:** Cyclic voltammogram of the 75% Ni + 25% Pt LCNF in PBS (pH= 7.4) solution with and without 10 mM H_2O_2. Potential is given against Ag/AgCl electrode. Black arrows indicate the scan direction.*

Conclusions

Further optimization towards an optimum pre-treatment condition will consequently allow the detection of H_2O_2 with not only high sensitivity but also high selectivity. Furthermore, electrode flexibility, mass-production capability, and low material cost make our strategy suitable for development of H_2O_2 based wearable sensors.

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Acknowledgements

The authors would like to thank the research grant from Deutsche Forschungsgemeinschaft (project no. 457100614).

Enzyme functionalized miniaturized capacitive field-effect sensors with ultra-thin layers

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Keywords: penicillinase, field-effect sensing, penicillin, ALD

Introduction

Miniaturized electrolyte-insulator-semiconductor capacitors (EISCAPs) with ultra-thin gate insulators, consisting of Al–p-Si–Si_3N_4 (1 nm and 2 nm)–Ta_2O_5 (5 nm) and Al–p-Si–SiO_2 (3.6 nm)–Ta_2O_5 (5 nm) have been studied with regard to their pH-sensitive sensor properties in former studies [1]. Therefore, the SiO_2- and Si_3N_4-gate insulators were deposited by RTO (rapid thermal annealing) and RTN (rapid thermal nitridation), respectively, while the Ta_2O_5 layer was fabricated by ALD (atomic layer deposition). Since these EISCAP systems demonstrated a good pH response, the feasibility of surface functionalization with enzymes - here Penicillinase – was investigated.

Results and Discussion

A schematic measurement setup with the different layer structure sensors and penicillinase modification are given in Figure 1.

The two different layer structures showed a dependence of the sensor signal in the penicillin concentrations from 0.5 mM - 20 mM. Clear signal steps can be achieved for both sensor types.

The obtained penicillin sensitivity is in good agreement with conventional immobilization procedures.

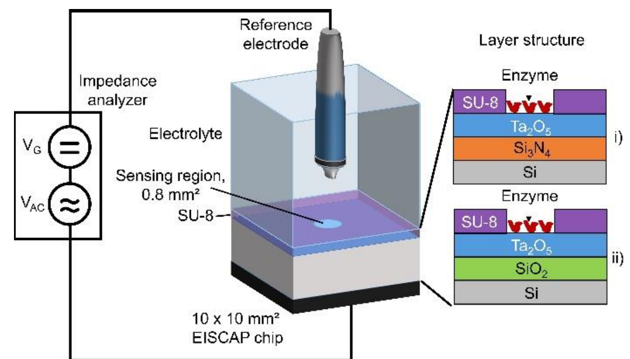

Figure 1: *Schematic measurement setup with impedance analyzer and EISCAP sensor chip. Both the sensor chip and the Ag/AgCl reference electrode (RE) are in contact with the analyte solution (left) and schematic sensor setup r with immobilized penicillinase (right).*

Conclusion

The lateral miniaturization of enzyme modified EISCAP sensor chips might enable further automation by applying the enzyme layers via nanospotting. Such are then applicable in fluidic systems giving the opportunity for complete lab-on-chip applications.

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Multi-functional ssDNA for DNA origami biosensor application

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Keywords: ssDNA, aptamer, SERS, atomic clusters

Introduction

For the development of novel biosensors with improved performance, the incorporation and combination of various nanotechnologies, such as functional nanoparticles (NPs) and DNA origami, is critical [1,2].

The DeDNAed project is developing a novel bioanalytical sensor-platform with advanced sensitivity and versatility, using SERS as an ultrafast optical analysis method. To this end, DNA origami will be used as a "nano-breadboard" to precisely control the nanoscale positioning of biorecognition elements (bioREs) with respect to the plasmonic hotspots of NPs, which are both positioned on the DNA origami using ssDNA [3].

To combine these technologies while ensuring high spatial precision we use short oligonucleotide sequences, a well-established method for attaching NP and active bioREs to DNA origami [4]. Furthermore, our concept incorporates metallic atomic cluster (AC) integrated within the bioRE, providing enhanced fluorescence properties compared to other NP-based systems, while their synthesis is based on novel etching methods that avoid denaturation of the bioRE [5].

Results and Discussion

The initial results on the development of an aptamer-based bioRE are presented here, with a strong emphasis on the synthesis of the AC and optimization of their fluorescent properties.

The bioRE is composed of three segments: one for DNA origami attachment, one for target specific binding, and one for AC coordination.

The three segments have been independently and in combination analyzed and optimized, demonstrating their combined functionality.

Conclusions

To summarize, we successfully synthesized a DNA oligonucleotide sequence for the creation of a bioRE compatible with DNA origami bearing AC. This element consists of three functional segments: a complementary DNA oligonucleotide strand for attachment to the DNA origami, an aptamer for specific target binding, and a sequence for coordinated synthesis of fluorescent AgACs.

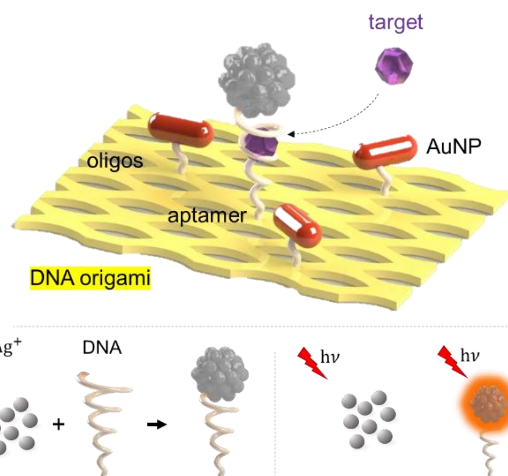

Figure 1: *Schematic of DNA origami scaffolds for the arrangement of plasmonic ssDNA attached NP arrays for SERS signal amplification, with an AC-decorated DNA aptamer as bioRE.* Bottom: *Multifunctional ssDNA oligonucleotide including a sequence for the coordination of fluorescent AgACs.*

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This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 964248. We like to thank Andreas Herwig and Michael Mertig of the Kurt-Schwabe-Institut Meinsberg for the DNA origami synthesis.

Volumetric Power Extracted from Symmetric and Asymmetric Glucose/O_2 Fully Printed Enzyme-based Biofuel Cell

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Keywords: enzymatic fuell cells; graphite printed electrode; glucose oxidase; symmetrical and asymmetrical electrodes

Enzymatic fuel cells (EFCs) have been proposed to catalyze oxidation of fuels at anodes and/or reduction of oxidants at cathodes and provide electrical power. The EFCs can be divided into two main groups: (a) mediated electron transfer (MET) devices, in which redox species are used to transport the electrons between enzyme and electrode surface; (b) direct electron transfer (DET) based biodevices, where the enzyme is able to communicate directly with the electrode [1].

Moreover, there are some issues in defining the power output especially considering anodes and cathodes with different sizes. For example, a H_2/O_2 EFC combines a 6 cm^2 bilirubin oxidase-modified cathode with a 1.2 cm^2 hydrogenase-modified anode to balance the catalytic performance as a function of the H2-air mixture used [2]. The raw power output must surely now be reported in all cases? It now seems valuable to define the volumetric power of bioelectrodes in mW cm^{-3} [3].

The aim of this work is to study the influence of electrode size on the performance of glucose/oxygen enzymatic fuel cell (EFC). The proposed EFC will encompass an efficient MET bioanode, employing glucose oxidase (GOx) modified graphite printed electrode (all materials will be enclosed in a newly formulated ink), and a DET biocathode, consisting of bilirubine oxidase (BOD) modified graphite printed electrode [4,5]. The EFCs will be assembled symmetrically and asimmetrically using three different electrode sizes: (a) 0.1 cm^2, (b) 1 cm^2 and (c) 10 cm^2. All Power output will be normalized by the EFC volumes instead of the electrode areas. Finally, the EFCs were tested in human serum and saliva to explore their sensitivity (decrease LOD) and their potential application a self-powered glucose biosensor integrated in wearable devices.

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Acknowledgements

The following funding agencies are acknowledged: Academy of Finland projects #316881, #316883 ‘‘Spatiotemporal control of Cell Functions’’, #332106 “ProSiT—Protein Detection at the Single-Molecule Limit with a Self-powered Organic Transistor for HIV early diagnosis”; Biosensori analitici usa-e getta a base di transistori organici auto-alimentati per la rivelazione di biomarcatori proteomici alla singola molecola per la diagnostica decentrata dell’HIV (6CDD3786); Research for Innovation REFIN—Regione Puglia POR PUGLIA FESR-FSE 2014/2020; PMGB ARS01_01195; NoOne-A binary sensor with single-molecule digit to discriminate biofluids enclosing zero or at least one biomarker, ERC Stg2021, GA:101040383; PRIN project prot.2017RHX2E4 “At the forefront of Analytical ChemisTry: disrUptive detection technoLogies to improve food safety – ACTUaL”; IDF SHARID (ARS01_01270); Åbo Akademi University CoE “Bioelectronic activation of cell functions”; University of Galway College of Science and Engineering Scholarship and CSGI are acknowledged for partial financial support.

D-(-)-Fructose Amperometric Biosensor based on Self-Templated Highly Porous Gold (h-PG): Morphological and Spectroscopic Characterization

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Keywords: enzyme amperometric biosensors; highly porous gold; electrodeposition; fructose.

In the last decades, nanomaterials have shown great advantages in terms of functional properties for a wide range of technological applications [1]. Metal nanoparticles provide a lot of advantages compared to macroelectrodes, such as enhancement of mass transport, catalysis, high effective surface area and control on the electrode conductive microenvironment. Highly porous gold (h-PG) can be synthesized by using achemical approach and electrodeposition. The main advantages of the electrodeposition method are thethickness, roughness and size control of the h-PG layer [2].In this work h-PG was directly electrodeposited onto a gold electrode (AuE) by using a two-steps method: 1) sweeping the potential in a 10 mM $HAuCl_4$ solution (supporting electrolyte 2.5 M NH_4Cl); 2)applying a potential -3 V vs. Ag|$AgCl_{sat}$ [3,4]. Initially we optimized the different parameters that can affect the electrodeposition process, such as scan numbers, concentration of precursor solution, etc.Afterwards, the nanostructured electrodes were characterized by scanning electron microscopy (SEM) and X-ray Photoelectron Spectroscopy (XPS). The so modified electrodes were further characterized with $Fe(CN)_6^{3-/4-}$ by cyclic voltammetry and electrochemical impedance spectroscopy, and compared to the naked gold electrode (AuE) in order to determine the electroactive area (A_{EA}), electron transfer rate constant (k^0), real surface area (Areal) and the roughness factor (ρ) [5]. Finally, the electrodes were modified with FDH and poly(vinyl alcohol), N-methyl-4(4'-formylstyryl)pyridinium methosulfate acetal (PVA-SbQ) to detect D-(-)-fructose in fruit juice samples as potential on-line biosensor for food industrial production processes [6].

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Acknowledgements

The following funding agencies are acknowledged: Academy of Finland projects #316881, #316883 ''Spatiotemporal control of Cell Functions'', #332106 "ProSiT—Protein Detection at the Single-Molecule Limit with a Self-powered Organic Transistor for HIV early diagnosis"; Biosensori analitici usa-e getta a base di transistori organici auto-alimentati per la rivelazione di biomarcatori proteomici alla singola molecola per la diagnostica decentrata dell'HIV (6CDD3786); Research for Innovation REFIN—Regione Puglia POR PUGLIA FESR-FSE 2014/2020; PMGB ARS01_01195; NoOne-A binary sensor with single-molecule digit to discriminate biofluids enclosing zero or at least one biomarker, ERC Stg2021, GA:101040383; PRIN project prot.2017RHX2E4 "At the forefront of Analytical ChemisTry: disrUptive detection technoLogies to improve food safety – ACTUaL"; IDF SHARID (ARS01_01270); Åbo Akademi University CoE "Bioelectronic activation of cell functions" and CSGI are acknowledged for partial financial support.

Point of care detection of microRNAs with electrochemical biosensors for early diagnosis of drug-induced liver injury

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Keywords: Drug-induced liver injury, microRNA detection, electrochemical impedance spectroscopy, biosensors, point-of-care diagnostics

Introduction

Drug-induced liver injury (DILI) is a major challenge in clinical medicine and drug development. DILI is responsible for nearly 50% of acute liver failure in Europe and the United States [1]. Paracetamol overdose is the commonest cause of DILI in the Western world [2]. In addition to paracetamol, NIH LiverTox database (livertox.nih.gov) has identified >1140 drugs which can cause serious liver injury. DILI can lead to early market removal of new drugs after launch, resulting huge financial losses for pharmaceutical companies. There is a need for rapid diagnostic tests for DILI. MicroRNA 122 (miR-122) is an early biomarker for DILI which is stable and translational across species, and reported to increase in the blood before standard-of-care markers such as alanine aminotransferase activity. We developed point-of-care compatible miR-122 detection using electrochemical biosensors for early diagnosis of DILI in human and veterinary medicine.

Results and Discussion

We used biosensors for electrochemical impedance spectroscopy (EIS) for direct, amplification free detection of miR-122 with screen-printed electrodes functionalised with sequence specific peptide nucleic acid (PNA) probes. We studied fundamental parameters influencing sequence-specific microRNA binding by altering the probe length, orientation of probe immobilisation, target overhangs, and degrees of base overhang on the electrode surface [3]. Our established model analysis and experiments showed that target overhangs on the electrode surface caused shorter probes to produce stronger EIS signals, and the EIS signal was proportionally higher with increasing lengths of the overhangs. To enhance assay performance and minimise sample volume requirements, we designed a closed-loop microfluidic system [4]. Our assays displayed clinical sensitivity and specificity over non-complementary and single nucleotide mismatch targets. With real samples, it displayed high selectivity for liver (miR-122 high) comparing to kidney (miR-122 low) derived samples extracted from murine tissue. During clinical sample analysis, our EIS assay distinguished human DILI patients from healthy individuals with a comparable performance to qPCR detection of miR-122 (Figure 1), and dog DILI patients with a significant difference from the healthy controls.

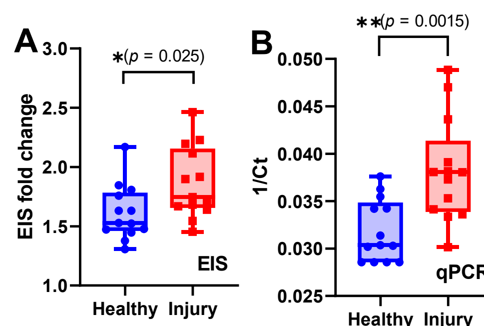

Figure 1: *(A) EIS signals of miR-122 detection in liver injury and healthy human serum samples (ROC-AUC: 0.77); (B) Benchmark qPCR Ct values (ROC-AUC: 0.83).*

Conclusions

In conclusion, direct, amplification free detection of miR-122 using EIS was achievable at clinically relevant concentrations and in clinical samples. Current work focusses on the sample preparation integration and realising a full sample-to-answer system which can be deployed to manage DILI in healthcare settings or at patients' home.

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Acknowledgement

We thank Rosetrees Trust for funding (CF1\100010)

Use of DNA thermodynamics for low-abundance mutation detection by DNA hybridization

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Keywords: nucleic acid thermodynamics, mutation detection, Langmuir adsorption theory

Introduction

Duplex formation via hybridization provides a detection technique where one of the strands is designed to match a mutant sequence and probe its presence. In a majority wild-type background consisting of single-nucleotide variations, the probe is however subject to cross-hybridization, lowering the detection sensitivity of low-abundant mutants. We use thermodynamics-based probe design and Langmuir theory to increase sensitivity and quantify the amount of mutant in a sample.

Results and Discussion

Figure 1 shows a hybridization-based sensor which uses three probe sequences: a wild-type matching probe, a mutant matching probe and a reference probe, the latter designed to have equal affinity to wild-type target as the mutant-matching probe. Single-stranded sample DNA is fluorescently labelled and allowed to bind to the probes. The detection signal is defined as

$$S = \log \frac{I_{Pmut}}{I_{Pref}}$$

A mixture of wild-type and mutant target will result in $I_{Pmut} > I_{Pref}$, signalling the presence of mutant DNA. To limit the amount of cross-hybridization, a large number of wild-type probes are introduced to deplete wild-type target. This enhances the effective mutant to wild-type ratio. The Langmuir adsorption theory provides the theoretical framework and was used in the linear regime to characterize the signal **[1]**. Using synthetic samples at a low mutant ratio, Van Hoof *et al.* showed that depletion of wild-type target achieves an order of magnitude improvement in the limit of detection **[2]**. However, clinical samples suggest a non-linear description is needed at higher mutant ratios, which we have been able to obtain. To characterise the dose-response $S(r)$ in terms of mutant to wild-type ratio r, a calibration experiment needs to be performed.

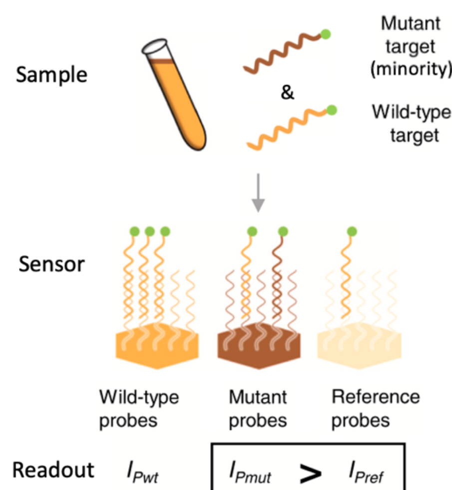

***Figure 1**: Experimental design and readout using reference probes for mutant detection.*

Subsequently, by inverting the theory, a signal measurement allows accurate determination of the amount of mutant in a sample.

Conclusions

We are currently exploring other methods to selectively deplete wild-type targets and extend the model for use with solution-based hybridization sensors **[3]**, which promise a cost-effective biosensing technique usable at room temperature.

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Acknowledgements

This work is supported by Special Research Fund with grant number BOF22OWB11.

Differentiation of background fluorescence by fractional contributions using fluorescence lifetime measurements

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Keywords: Fractional contributions, fluorescence lifetime, microparticle, microfluidic

Introduction

Flow cytometry is a powerful tool for the characterization and sorting of suspended single cells and is applied in areas such as cancer therapy [1] and detection of pathogens [2]. Background fluorescence from unbound fluorophores and autofluorescent components can complicate differentiation of such target cells. This study investigates the determination of fractional contributions using fluorescence lifetime to reduce the influence of background fluorescence and increase differentiability of targeted cells and particles.

Fluorescent microparticles (Fluorobrite® Microparticels, Polyscience Inc., US) with a diameter of 45 µm were measured in a microfluidic system with a diameter of 100 µm (10000211 Straight channel chip (4 parallel channels), microfluidic ChipShop GmbH, GER) with various fluorophores. The fluorophore shown here is Atto 465-NHS-ester (53404, Merck, Germany) at a concentration of 82.6 mM and deionized water was used as solvent.

Results and Discussion

Mixing the fluorophore Atto 465 into the solution containing the microparticles, an increase in the measured background fluorescence is observed. This background fluorescence is not constant over the scanned region, since the concentration of the fluorophore may differ (Figure 1 A). When a particle is excited by the laser, the measured intensity increases. However, if the background fluorescence is too high, it is difficult to distinguish the intensity of a particle from the background fluorescence. Under worst case conditions, either the particle is not recognized, or a particle is falsely identified.

By determining the fractional contributions, the fluorescence of the particles can be precisely classified, independent of the intensity of the background fluorescence (Figure 1 B). This is also possible when the intensity increases by less than 20 % due to a particle (lifetime difference $\Delta\tau = 2.4\ \mathrm{ns}$).

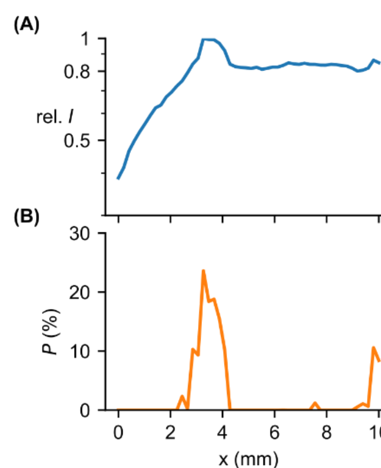

Figure 1: *Differentiation of microparticles ($\tau_p = 3.4\,ns$) in a solution containing a fluorophore Atto 465 ($\tau_f = 5.8\,ns$) with a fluctuating (A) fluorescence intensity. Determination of the (B) fractional contribution of the microparticles allows for intensity-independent differentiation.*

Conclusions

Our findings showed that using fluorescence lifetime as a parameter for differentiation of fluorophores is a viable alternative to intensity-based differentiation, particularly in scenarios where high levels of background fluorescence are present. One possibility is that this method could be used in the context such as fluorescence lifetime-based in vivo flow cytometry [3].

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Acknowledgements

This work was funded as part of the Fraunhofer LIGHTHOUSE PROJECT "RNAuto".

Electrochemical aptasensor with enhanced antifouling stability for highly sensitive neurotransmitter detection

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Keywords: aptamer, neurotransmitter, polyethylene glycol, antifouling

Introduction

Accurate determination of serotonin provides significant knowledge in neuroscience and enables application in clinical diagnostics of brain diseases. Herein, we present an electrochemical aptasensor based on sensing interface functionalized by mixed self-assembled monolayers of ssDNA aptamers and hydrophilic polyethylene glycol (PEG) molecules for sensitive and selective detection of serotonin (*Fig. 1*). They are immobilized on a gold electrode surface. The aptamers probes with methylene blue (MB) redox tag are capable of recognizing serotonin specifically accompanied by a conformational change. Using PEG as blocking molecules minimizes nonspecific adsorption of interfering species and thus endows an enhanced antifouling ability in complex environment. The variation conjoined with analyte binding can be monitored by square wave voltammetry (SWV).

Results and Discussion

The original serotonin aptamer strand with long chain lengths owns an invariant large stem-loop structure, which causes only small signal decrease during target binding and low sensitivity during electrochemical detection [1]. To gain the highest signal response, a truncated aptamer sequence with 32bp was designed for target recognition and a 3 times higher signal change is obtained compared to the use of original aptamer. Moreover, the composition of the mixed aptamer-PEG receptor layer was optimized by varying the incubation time in PEG solution [2]. Short incubation time could not form dense monolayers for effective prevention of matrix protein adsorption. On the contrary, an excessive incubation time forms a highly compact PEG monolayer, which led to a decrement of the signal response because aptamer requires a particular space on the transducer surface for 3D conformational adaptation for target binding. Correspondingly, a PEG incubation time of 4h was found to be optimal for highly efficient serotonin binding and fouling prevention.

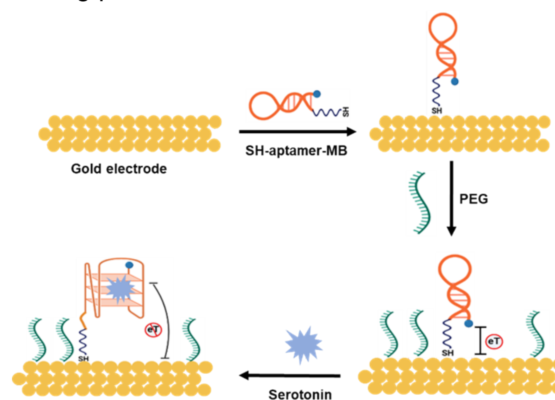

Figure 1: *Schematic illustration of the working principle of the electrochemical aptasensor for serotonin detection with PEG blocking molecules. Upon target binding, the aptamer is thought to undergo a conformational change into a G-quadruplex complex that forces MB tags away from the electrode, impairing electron transfer and leading to a detectable decrease in redox current.*

Conclusions

Combining the excellent antifouling properties of PEG and the high specificity of aptamers, the developed aptasensor exhibited a wide detection range and a pM LOD for serotonin. In addition, the proposed aptasensor showed a high selectivity and stability. It can also be used for serotonin assays in complex biological media such as human serum and aCSF.

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Acknowledgements

Ziheng Hu acknowledges the financial support from China Scholarship Council (CSC202006890001).

Functional taste organoid-based MEA biomimetic sensor for bitter substance detection

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Keywords: biosensor, taste organoid, MEA, taste sensing, bitter

Introduction

The taste system of living organisms has a very sensitive taste perception ability, which can help animals identify harmful substances and ensure life and health [1]. Taste biomimetic sensors inherit the advantages of biochemical sensory systems, and are slightly superior in sensitivity, response time, specificity and other indicators, and show broad application prospects in food safety, environmental monitoring and disease detection [2]. *In-vitro* 3D organoids can simulate the state of organs *in vivo* to the greatest extent and are one of the ideal models for current research [3]. This work developed a taste organoid-based biosensor to detect and identify bitter substances.

Results and Discussion

In Figure 1, size of taste cell spheres at 4d (Figure 1A), 10d (Figure 1B) and 16d (Figure 1C) were showed, which displayed expanding diameter and distinct globular multicellular structure with extension of time. These spheres can be harvested in as fast as 10 to 14 days, with a maximum diameter of around 150 μm.

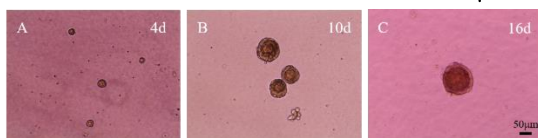

Figure 1: *Morphological changes of taste cell spheres at 4d (A), 10d (B) and 16d (C).*

Next, taste organoids were coupled with MEA chips to detect organoid responses to bitter substances. Initial signals were recorded for 5 min before and after adding the bitter substance. The recording results showed that there was low voltage signal less than 200μV with 191 spikes detected before bitter stimulation (Figure 2A) and greater than 500μV with 293 spikes detected after stimuli (Figure 2B). This indicated that the prepared taste bud organoids have a responsive capability to bitter stimulation, which suggests a good homogeneity of prepared organoids. Meanwhile, it also illuminated that the taste bud organoids could be utilized as sensitive elements for the development of biosensors towards taste sensation.

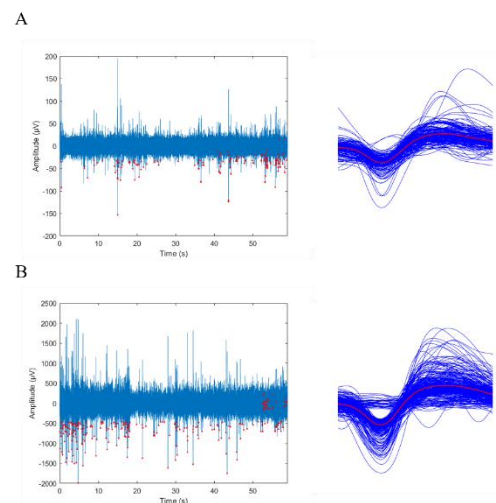

Figure 2: *Membrane potential changes were recorded from attached taste bud organoids via the MEA chip* (A) without *stimulation and (B)* with *100μM saccharin. Left: original signals; Right: clustering of recorded spikes from original signals.*

Conclusions

In this study, a functional taste bud organoid-based MEA biomimetic sensors was established for bitter sensation. This study provides an efficient approach for the development of bionic sensing system towards food and drug industry.

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Acknowledgements

This work was funded by the National Natural Science Foundation of China (Grant No. 32071370, and 32271427).

Synthesis of nipecotic acid molecular imprinted polymers (MIPs) for the screening of nipecotic acid bioisosteres

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Keywords: molecular imprinted polymers, nipecotic acid, bioisosteres, GAT1 radioligand

Introduction

Given the role of the GABAergic system in various CNS diseases, *in vivo* visualization of the GABAergic system can be a valuable diagnostic tool to increase our understanding of the pathogenesis of these disorders. In order to perform such non-invasive imaging the development of, among others, GABA transporter 1 (GAT1) addressing radioligands is highly desirable. However, despite several research attempts, no GAT1 radioligands are available to date. Instead, GAT1 radioligands developed so far suffer from low brain uptake, which can be attributed to their zwitterionic nipecotic acid moiety (Figure 1) [1,2]. To allow the development of more viable GAT1 radioligands in the future, the incorporation of nipecotic acid bioisosteres could be a promising solution. In order to aid this development, this research aims to develop nipecotic acid molecular imprinted polymers (MIPs) that might function to screen nipecotic acid bioisosteres. It is expected that bioisosteres that bind well to the MIP should also bind well into the GAT1 binding site and could thus be promising candidates for incorporation into GAT-1 radioligands.

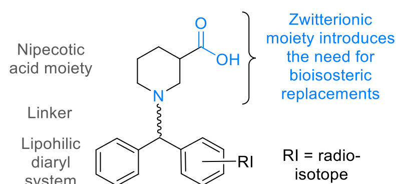

Figure 1: *General structure of GAT1 radioligands.*

Results and Discussion

Due to the low solubility of nipecotic acid in apolar solvents, electropolymerization was used for the synthesis of the MIPs. Inspired by the GABA MIPs developed by Zheng *et al.* [3], nipecotic acids MIPs were constructed by electropolymerizing *ortho*-phenylenediamine (*o*-PD) on Au electrodes (0 - 0.8 V, 10 cycles) from a PBS solution (pH = 5.8) containing 4.5 mM *o*-PD and 0.1 mM nipecotic acid. Preliminary impedance measurements show a good specific binding of the nipecotic acid template (Figure 2), indicating promising development of nipecotic acid MIPs.

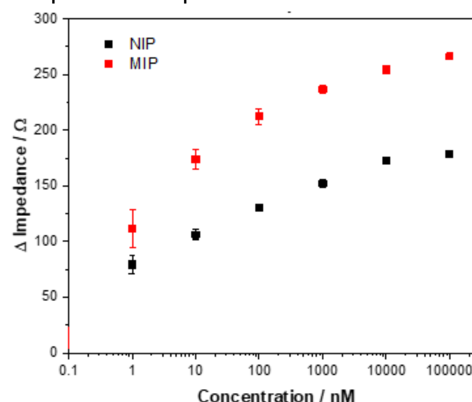

Figure 2: *Dose response curve of the nipecotic acid MIP and NIP.*

Conclusions

Preliminary impedance measurements indicate the promising development of nipecotic acid MIPs. After optimization, these MIPs might be used for the rapid and facile screening of nipecotic acid bioisosteres to facilitate the development of potent GAT1 radioligands.

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Acknowledgements

The project was funded by the Sensor Engineering department, Faculty of Science and Engineering, Maastricht University

Antibody Binding to Plasmonic Nanostructure: A Validation Study Using Experimental and Simulation

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Keywords: LSPR sensors, FDTD simulation, nanostructure, biosensor, biochemical functionalization

Introduction

LSPR sensors allow the label-free detection of molecules in real-time with high sensitivity, making them particularly suitable for the detection of surface binding events, e.g., of molecules or pathogens. However, the quantitative characterization of the biochemical functionalization of the metal nanostructure (e. g. with respect to layer thickness, layer refractive index and lateral layer density) is a demanding challenge. This contribution describes a simplified approach for a theoretical description in which a number of assumptions is made to obtain simulation results of the optical response of the nanostructure to binding events, which are in good agreement with experimental data.

Results and Discussion

The plasmonic response for molecular binding to a gold nanopillar array is simulated by thin dielectric layers in Ansys Lumerical. In a first step, the model is validated by comparing simulated results with experimental realizations using well-defined Al_2O_3 layers from atomic layer deposition on the nanostructured sensor substrates. Subsequently, this model is used to simulate the actual sensor application to detect diclofenac (DCF) [1].

In an indirect immunoassay DCF molecules are immobilized on the PEGylated gold nanostructure. The binding of a highly affine monoclonal anti-DCF antibody is then detected as a shift in the transmittance spectrum. Here, two different bifunctional PEGs with M_w=800 Da and M_w=3000 Da were compared. The molecular weight of these molecules determines the thickness of the PEG layer and, hence, the distance of the antibody binding event to the nanostructure, which substantially affects the resonance shift.

In the model, evenly distributed layers are assumed as the exact formation and lateral distribution of these films (PEG/DCF and antibody) on the nanostructure is unknown and very challenging to determine. For the thickness and refractive index of the PEG layer, the values of planar layers from [2] were transferred to the nanostructure. Since the number of antibody binding sites is also unknown due to the uncertainties described, antibody surface coverage is represented by a sweep of the refractive index of the antibody layer in the model and determined by matching experimental data with the simulation results.

Our experiments show a plasmon resonance shift of 4.2 nm when using the 3000 Da PEG, while a shift of 4.5 nm was measured for the 800 Da PEG. Matching these experimental shifts with the simulation results, a refractive index of 1.419 and 1.417, respectively, was determined as an average of the antibody and surrounding buffer on the nanostructured surface. Considering an average dielectric constant for proteins of ε_r=3.23 (n=1.797) according to [3], the volume fraction of the antibody in aqueous solution is 18.2% and 18.6% for the 800 Da and 3000 Da PEG, respectively.

Conclusions

The study demonstrates a valid simulation model which accurately predicts the shift of the plasmonic resonance wavelength upon molecular binding despite a number of assumptions and uncertainties. The model created can be used in the future to feed an automated optimization algorithm to maximize the plasmonic response of this type of gold nanostructures and to achieve maximum sensitivity to molecular binding events.

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AC field assisted deposition of influenza viruses on nanoelectrodes

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Keywords: immobilization, dielectrophoresis, AC electrokinetics, influenza, virus

Introduction

A rapid characterization of viruses and virus subtypes is of great biomedical interest. Here we present the use of AC electrokinetic forces, like dielectrophoresis and AC electroosmosis, as a simple and fast method to functionalize nanoelectrode arrays as a potential biosensor. The permanent immobilization of polystyrene nanoparticles, antibodies and other proteins on electrodes has already been demonstrated. The sensor itself consists of four individual arrays, each built up of 6256 tungsten nanoelectrodes with a diameter of 500 nm each. The immobilization, detection and characterization of influenza material is done without any prior chemical modification of the electrode surface.

Results and Discussion

The accumulation of virus material over time has been observed, showing that the largest amount has already been drawn to the electrodes within 60 seconds and reached a saturation after 180 seconds of applied AC electric field. Due to side effects such as fluid streaming, a concentration gradient is created decreasing from the outer to the inner electrodes. It has been demonstrated, that the virus material is permanently immobilized even after switching off the electric field. Furthermore, each functionalized electrode can be considered as a single event. Comparing these single events it seems like the virus material is distributed randomly across the nanoelectrodes. But after deconvolving the fluorescence image and merging the images of around 100 electrodes it reveals that the major part of virus material is collected at the electrode edge. This is in line with theory, as this is the region of the highest field gradient, and thus here the AC electrokinetic forces have the greatest impact on the sample.

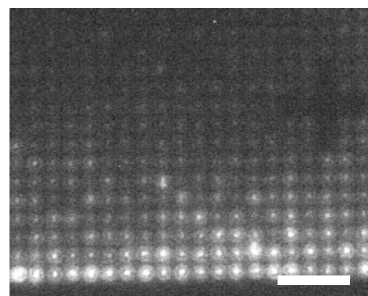

Figure 1: *Fluorescently labelled influenza virus material immobilizes on a nanoelectrode array; scale bar 10 µm*

Conclusions

The universal chip design does not limit the application to influenza viruses but also works for different viruses, bacteria, parasites or any other object that can be manipulated by AC electrokinetic forces. Each electrode can be used as part of an on-chip resonant circuit, whose frequency changes with surface coverage of the electrode and, hence, serves as a measure of the amount of viruses attached to the electrodes. So in future, the evaluation by fluorescence microscopy can be changed to an electrical evaluation. Thus, combined with microfluidics this chip has the potential for a small and rapid Point-of-care system.

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We gratefully acknowledge funding by the Brandenburg Ministry of Science, Research and Cultural Affairs, the European Regional Development Fund and the German Academic Scholarship Foundation.

Characterisation of molecular interaction of AflatoxinB1 DNA Aptamer with MST and switchSENSE technology

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Keywords: switchSENSE, MST, surface interaction, aflatoxin aptamer, atomic nanocluster

Introduction

Aflatoxin B1 (AFB1, 312.3 Da) is one of the predominant fungal carcinogenic toxins found in agricultural produce [1]. Detection of such small molecules is possible by HPLC (MS and FD) and ELISAs, which is cost-ineffective, especially in the developing countries [2]. For monitoring of such deadly toxins and tracking toxicity levels on-site rapid, affordable, and efficient biosensors are crucial. SERS has recently shown the capability of detecting femtomolar concentrations of aflatoxin which can be applied for portable, superiorly sensitive and versatile bioanalytical sensor-platforms [3]. One such biosensor platform is being developed in the DeDNAed project, for an ultrafast and ultrasensitive detection of small molecules. An AFB1-aptamer [4,5] is one of the target-biorecognition element (BioREs) chosen as demonstrator for the DeDNAed biosensor platform. In this study, molecular interaction characterisation was established using MST and switchSENSE® technology. The MST assays were conducted on the Monolith© NT.115, using a Cy5 label at the 5' end of the aptamer [1]. For switchSENSE experiments, fluorescence proximity sensing mode was used to measure the binding rates of the interaction on HeliX+©.

Results and Discussion

The HeliX+© biochip surface (see Figure 1C) has an upright dsDNA-nanolever, present on the Au-electrode with a constant negative voltage. The dsDNA nanolever carries a fluorophore (red or green) on one arm and the ligand on the other (the aptamer in our case). The fluorescence intensity (F) depends on the proximity of the fluorophore and changes (ΔF) depending on the ligand-analyte interaction. ΔF% is recorded in real-time as the result of the interaction. With switchSENSE technology, when interaction was performed with the green dye, proximity ΔF% was 2.5 and the KD observed was 115 µM. When performed in the red dye proximity ΔF% was 1.5 and K_D observed was in the range of 2-30 µM.

To enhance ΔF% and the sensitivity range of the aflatoxin-aptamer interaction, we propose introduc-ing an additional sequence for the coordination of fluorescent atomic clusters into the aptamer. The MST measurements already showed that these adaptations do not affect the aptamers affinity.

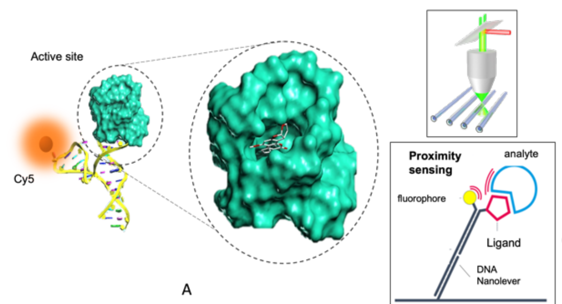

Figure 1: A) Secondary/tertiary structure of the aptamer with Cy5 label and the molecular docking results of AFB1 to the aptamer (adapted from [1]). B) Schematic representation of MST measurements. C) Fluorescence proximity mode principle of switchSENSE measurement (adapted from DBS© website).

Conclusions

The results from the switchSENSE measurements compare well with those obtained with MST, which are also in the lower µM range and have been reported before [1]. Nevertheless, the toxicity levels of Aflatoxin are in the range of 20 ppb – 300 ppb (FDA). Hence, to achieve such sensitivities in POCT settings, highly sensitive optically SERS detection systems, such as is the developing aim in the DeDNAed project, are essential for an effective monitoring of such toxins.

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This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 964248.

Development of advanced luminol modified gold nanoparticles for quantitative dual detection lateral flow assays

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Keywords: nanoparticle, chemiluminescence, lateral flow assay

Introduction

Rapid tests on the basis of lateral flow assays are well known through COVID antigen or home pregnancy tests. Ideally suited for the point-of-care due to their simplicity, they are yet not sensitive enough for most biomarkers. Therefore, our strategy employs modification of the most employed label, colloidal gold [1], with a chemiluminescent probe. These new labels offer not only a visual readout through the gold nanoparticles (AuNP), but also the advantages of chemiluminescent detection, such as background-free nature, large dynamic range and especially the desired very low detection limits in the attomolar range. [2]

Results and Discussion

The modification of AuNPs with luminol was implemented in four consecutive steps. Starting from 40 nm citrate-capped particles, a ligand exchange with 3-mercaptopropionic acid was conducted. Next, polymeric trimethyl chitosan (50% quaternization) was covalently coupled via EDC/sNHS to the carboxy groups on the AuNP surface. In the third step, the remaining free glucosamine moieties of trimethyl chitosan were functionalized with NHS-esters of PEG-biotin and PEG-alkyne. The biotin bound to poly streptavidin will mimic the antibody-antigen-antibody sandwich complex on a lateral flow immunoassay giving a best-case scenario while the alkyne function was used in the last step in a click reaction. For this, a novel azide-terminated luminol derivative was designed and coupled to the alkyne in a copper catalyzed azide alkyne cycloaddition. In the end, we predict that a quantitative chemiluminescent readout on lateral flow assay strips will lower the limit of detection by several orders of magnitude while retaining the benefits of qualitative optical evaluation through the colloidal gold.

Conclusion

By modification of AuNPs with luminol, the most common label for lateral flow assays becomes more versatile. Thus, the applicability of this point-of-care format may be expanded to new fields while potentially rivaling the sensitivity of lab-based ELISAs.

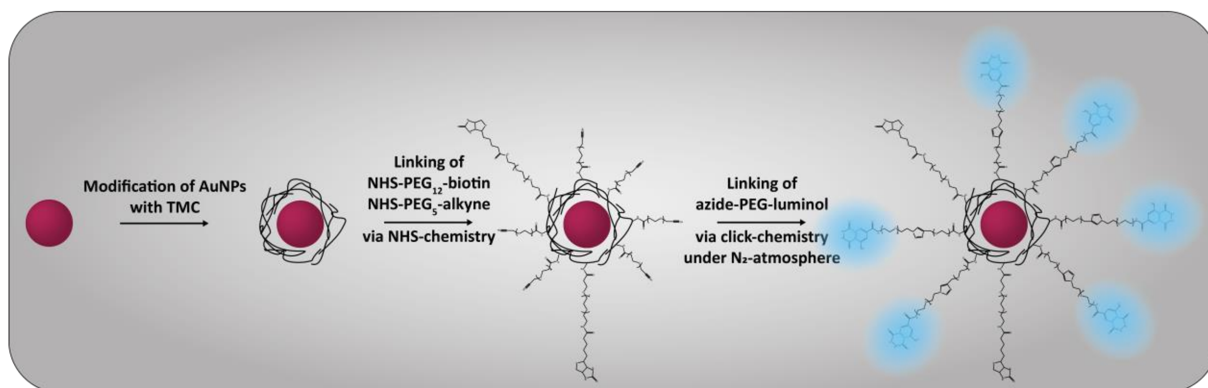

Figure 1 *Modification scheme of gold nanoparticles with a novel luminol derivative.*

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Acknowledgements

Part of this project was supported by the Federal Ministry for Economic Affairs and Climate Action (BMWK) based on a decision by the Deutsche Bundestag.

Food contaminants sensing by Molecular Imprinted Polymer-based biosensor

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Keywords: Molecular Imprinted Polymer, Electrochemistry, biosensor

Recently, molecular imprinted polymers (MIPs) played an important role in the development of reliable and stable sensors, considering the biomimicking nature of this platform with respect to several biorecognition elements like antibodies, DNA or enzymes, for instance enclosing nanomaterials as nanozymes [1,2] MIPs are synthesised through a polymerization method to create semi-specific positions for targets in the molecular dimensions by using a monomer (e.g., o-phenylenediamine, pyrrole etc.) and a target molecule [3].

In this work, we developed a MIP based electrode to sense 2,4-dichlorophenossiacetic acid (2,4-D) at ultralow/traces concentrations combining electrochemical techniques (Cyclic Voltammetry, Electrochemical Impedance Spectroscopy) and electronic measurements, carried out using an Organic ElectroChemical Transistor (OECT) biosensor. At first, we characterised the modified platform by using several electrochemical techniques to demonstrate the effective removal of the templating molecule and the possibility to perform the rebinding/washing step multiple times, hence proving the stability of the prepared electrode surface. Furthermore, we performed a spectroscopic characterisation of the modified surface by means of X-Ray Photoelectron Spectroscopy (XPS) to prove the efficient template removal. Finally, the platform was also used to perform low-trace sensing measurements using OECT biosensors.

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Wearable Array Printed on Smart Water-based Conductive Inks for Multiparametric Analysis

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Keywords: Ink, biosensor, sensor array

Disposable and low-cost electrochemical devices developing results a challenging but crucial purpose for biomedical applications, as demonstrated by the increasing demand for production processes that allow the manufacture of disposable and portable electrochemical devices, promoting the reduction of the volume of samples, in-situ detections, and lower cost. [1] A very promising approach is the implementation of conductive inks, typically used for the development of disposable electrochemical sensors. They trigger the possibility of building screen- or stencil-printed electrodes with similar efficiency with respect to solid electrodes. [2]

In particular, biocompatible inks can be formulated and stencil-printed on a flexible support that could be easily integrated within smart-devices, permitting a continuous and minimally invasive monitoring of different analytes. In this work, we develop a sensor array able to detect O_2, CO_2, lactate and pH at the same time in a real human fluid. For this aim, a six electrodes array was developed and stencil-printed. Four electrodes were used as independent sensing electrodes, resulting in a graphite electrode embedding a molecule/biomolecule specific for the target molecule (birilubine oxidase, carbonic anhydrase, lactate oxidase and polydopamine). The last two electrodes were used as reference (Ag) and counter (graphite).

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Continuous monitoring of glucose levels in sweat using wearable electrochemical biosensor

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Keywords: amperometric enzyme electrode, impedance, Prussian blue, Bluetooth LE

Introduction

Monitoring of glucose in sweat is considered as convenient alternative to blood and subcutaneous measuring approaches. However, the production of sweat is quite fluctuating depending on physical activity and temperature. We have constructed a 5-electrode flexible sensing system combining amperometric graphite / Prussian blue / glucose oxidase channel for the analyte, and impedance monitoring of the presence of sweat. The wearable measuring system was combined from "components of the shell" – LiPo battery, modular potentiostat EmstatPico running custom MethodScript protocol, and Xiao BLE microcontroller for data pre-processing and Bluetooth LE transfer to Android smartphone. In addition, the Xiao module measured temperature of the skin and accelerometer followed activity of the user.

Results and Discussion

The optimized version (immobilization procedure, working parameters, signal processing) of the biosensor was attached on the skin and four parameters were recorded – biosensor current, sweat impedance, temperature and accelerometer signal. As reference, the commercial Dexcom G6 biosensor was used for long-term experiments, and blood glucose was occasionally measured with the Fora DM30b glucometer for calibration purposes.

The example of a long-term recording from the biosensor and the Dexcom reference system in Figure 1 indicate few hours (10:00 - 16:30) of a rest state (office workload) where the production of sweat was negligible (confirmed by a very high impedance signal), thus the biosensor does not measure in this dry state.

When the subject started physical activities (walking, sport), the production of sweat resulted in restart of the biosensor and generation of response. It appeared critical to evaluate the impedance of the biosensor part contacting the skin; the values changed from GΩ (dry skin) to few kΩ (heave sweating).

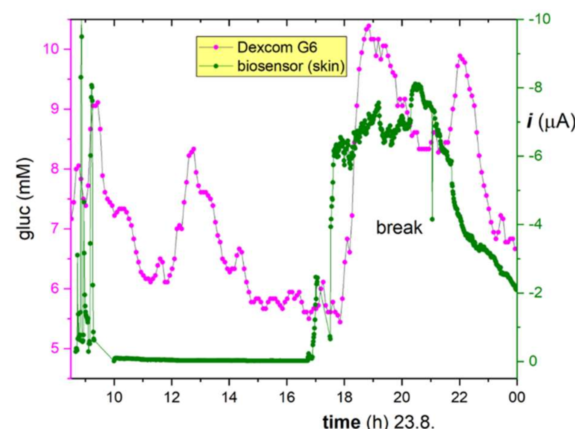

Figure 1: *Example of the long-term biosensor response (current, green trace) to sweat glucose levels. The parallel glucose concentrations (pink trace) were obtained from the commercial Dexcom G6 system.*

When measuring, the sweat biosensor response well correlated with the data from the Dexcom G6 inserted in the subcutaneous space; however, some time shift of peak values was observed.

Conclusions

The measurement of glucose (and other metabolites, too) in sweat seems feasible in situations when sufficient amount of sweat is generated (hot weather, physical activity). For reliable interpretation of the signals, one has to quantify the presence of sweat and ensure that the whole active part of the biosensor is completely wetted with sweat.

Acknowledgements

Support of this research from the Umana Vita (Malta) company is gratefully acknowledged.

Molecularly imprinted nanoparticles and time-resolved fluorescence for the ultra-low detection of human serum albumin

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Keywords: molecularly imprinted nanoparticles, fluorescent decay, time-resolved fluorescence spectroscopy, serum albumin

Introduction

Molecularly imprinted nanoparticles (nanoMIPs) are synthetic nano-receptors with tailor-made recognition prepared by a template-assisted synthesis [1]. Fluorescent molecularly imprinted polymers are raising interest for the design of optical sensor [2,3]. Combining nanoMIPs, as synthetic receptors characterized by high affinity and selectivity for the target analyte, with fluorescence, as a mean to report the presence of the analyte, permits to develop highly sensitive sensing systems. In the present work, fluorescent nanoMIPs (Fluo-nanoMIPs) were synthesized and used to detect the presence of human serum albumin (HSA) by means of the fluorescence lifetime decay.

Results and Discussion

Fluo-nanoMIPs were synthesized using a total monomer concentration of 0.2% w/v and HSA as template. Fluorescence was entailed by adding the monomer fluorescein O-methacrylate in the polymeric network. Physical characterization by dynamic light scattering (DLS) showed the Fluo-nanoMIPs had hydrodynamic size of about 100 nm, scanning electron microscopy (SEM) confirmed the size of the nanoparticles. The formation of binding cavities suitable to selectively recognize HSA was confirmed by monitoring the fluorescence intensity of Fluo-nanoMIPs incubated with increasing concentrations of HSA, whereas human transferrin did not show interaction. Fluo-nanoMIPs were tested in time-resolved fluorescence spectrometry. A bi-exponential fitting with fixed τ_1 was chosen to analyse the data. From these measurements, a decrease in the τ_2 was observed in the femtomolar range of HSA.

Conclusions

Our results showed that time-resolved fluorescence of Fluo-nanoMIPs specific for the recognition of the model protein HSA can be used as sensing material to develop an in solution optical sensing system with a promising ultra-low response (femtomolar range), that can be exploited to develop both diagnostic and environmental sensing systems.

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Acknowledgements

LP and DM thanks Strategic funding Area Covid-19 University of Trento being awarded of the OPTOVIR project.

AMB and AM thanks MUR for DM 351/2022 PNRR, Missione 4, componente 1.

Atomic nanoclusters-modified antibodies synthesis and characterization as biorecognition and transduction elements in DNA-origami based biosensors

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Keywords: atomic nanocluster, nanobiosensor, DNA-origami, point-of-care diagnosis, SERS

Introduction

In this work, a previously reported enzyme-free methodology for labelling antibodies using atomic nanoclusters (NCs) embedded in its structure has been set up [1] [2]. The resulting antibodies still maintain the affinity for target antigens. Here we propose a non-conventional immunoassay where the nanocluster-modified antibodies act as a probe and incorporates both, the biorecognition and transduction element. The transduction is performed employing changes in SERS signal when the analyte is bound to the antibodies carrying NCs. The DNA origami will serve to incorporate the elements of the biosensor with nanometric precision, also incorporating nanoparticles (NPs) to enhance SERS signal. This approach offers important advantages over conventional immunoassays, including improved sensitivity, quick turnaround, and suitability for point-of-care (PoC) diagnostics.

Results and Discussion

A secondary antibody (anti-rabbit IgG) has been selected to develop this research because it can be employed to detect any IgG from rabbit providing a broader range of application. Two different types of NCs have been synthesized within this work. On the one hand, bimetallic NCs composed by gold and platinum (Au/Pt NCs-IgG). This nanomaterial exhibit peroxidase-like activity. The affinity of these antibodies carrying NCs for target analyte has been tested with a paper-based immunoassay. The detection is performed taking advantage of the catalytic properties of the NCs with the oxidation of a non-soluble chromogenic substrate. The signal is visible by the naked eye and can be further analyse for quantitative results. In Figure 1.A. a scheme of a paper-based immunoassay for the detection of an IgG from rabbit based of Au/Pt NCs-IgG is showed. On the other hand, semiconductor NCs composed by cadmium sulfide (CdS NCs-IgG) has been synthesized. In this case the reduced size of the NCs bring them fluorescent properties. This optical property is used to detect the presence of target analyte on paper-based immunoassays and to test the affinity for target analyte after the synthesis. In this case it is necessary to illuminate with UV-light to reveal the signal. In Figure 1.B. a scheme of a paper-based immunoassay for the detection of an IgG from rabbit based on fluorescent properties of CdS NCs-IgG is showed.

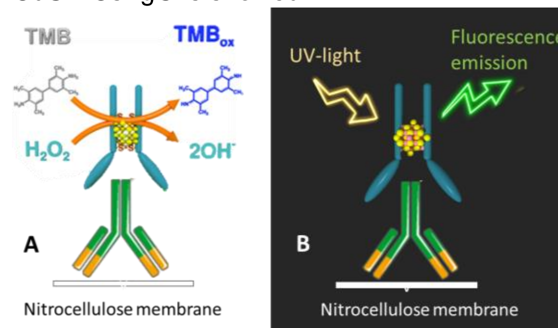

Figure 1: *Paper-based immunoassay for the detection of an IgG from rabbit based on Au/Pt NCs (A) and CdS NCs (B) embedded in an anti-rabbit IgG structure.*

Conclusions

The results of the paper-based immunoassays indicates that the antibody still have affinity for target analyte after the synthesis and can serve as a probe in DNA origami-based biosensors. The proposed strategy offers a new platform for the development of efficient POC nanobiosensores.

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Acknowledgements

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 964248.

Affordable manufacturing technologies for nanostructured films-based lab-made electrochemical biosensors

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Keywords: Lab-made devices; nanomaterials; electrochemical sensors.

Introduction

In the electro(bio)analytical scenario the use of commercial screen-printed electrodes is highly widespread, nevertheless, they present limitations regarding their default design, rigid substrate, and analytical performance. Recently, different low-cost strategies to fabricate (bio)devices have emerged as smart alternatives to the more expensive and cumbersome clean-room-based fabrication methods **[1]**. In particular, the manufacturing of tailored analytical devices integrating nanomaterials (NMs) based conductive films is still a hot topic; to overcome tedious, expensive, and not sustainable conventional fabrication techniques, several efforts are devoted to implementing effective and affordable technologies to produce nanostructured analytical devices. In this framework, low-cost substrates/materials and emerging manufacturing technologies, represent a captivating opportunity, and although much progress has been made, there are still unexplored rooms.

Results and Discussion

This presentation will be focused on using functional NMs, mainly produced by avoiding the use of solvents and pollutant chemicals, as building blocks for the implementation of completely lab-made analytical devices. An overview of nanomaterials' sustainable production, nano architectures assembling, and their integration into freestanding conductive films, flexible sensors and biosensors, and all-in-one devices will be given (**Figure 1**). Attention will be paid to the fabrication of devices integrating 0D, 1D, and 2D NMs using low-cost substrates as flexible polymers, thermoplastic sheets, cellulosic components, and benchtop microfabrication technologies as xurography, thermal lamination, and CO_2 laser scribing and cutting. The application of the developed devices to implement second- and third-generation flexible enzymatic biosensors will be presented, together with their use for the determination of agri-food and biological interest target compunds.

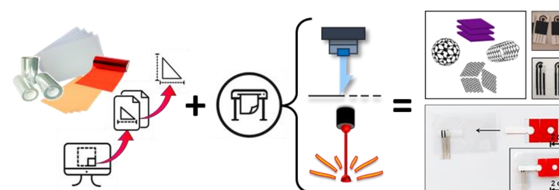

Figure 1: *Sketch of manufacturing strategies and pictures of the obtained biodevices*

Conclusions

The main goal of this presentation is to prove how NMs produced using emerging sustainable strategies can be easily integrated into tailorable cutting-edge bioanalytical devices manufactured via within everyone's reach technologies.

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Acknowledgements

This research was funded by the European Union – Next Generation EU. Project Code: ECS00000041; Project CUP: C43C22000380007; Project Title: Innovation, digitalization and sustainability for the diffused economy in Central Italy - VITALITY

PCR-based amplification of the pathogen *Legionella pneumophila* for visual SYBR Green I endpoint detection

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Keywords: Quantitative PCR, Visual endpoint detection, Point-of-care (POC) diagnostic system

Introduction

The environmental pathogen *Legionella* can cause severe respiratory diseases in humans. **[1]** Infection by *Legionella* species, especially by *Legionella pneumophila*, occurs through inhalation of aerosols, such in showers or air conditioners which are contaminated with *Legionella*. The rapid and specific detection of pathogens, as in the case of *Legionella pneumophila*, is critical for the success of antibiotic therapy in patients, especially those at risk. Current detection methods range from serological and antibody tests to urine antigen tests and the standard reference method, the bacterial cultures. **[2]**

Our aim is the development of a sensitive point-of-care system, whereby the detection time will be rapidly reduced. For this purpose, the PCR, gold standard DNA amplification method and a visual endpoint detection system based on the fluorescent dye SYBR Green I, were combined.

Results and Discussion

For this purpose, the specific *Legionella pneumophila mip* gene was selected. First, the genomic DNA of *Legionella pneumophila* was amplified by quantitative polymerase chain reaction (qPCR) followed by the detection step with the fluorescent dye SYBR-Green I using non-purified amplicons. Figure 1 (A) shows the first qPCR results, which showed the successful amplification with a negative control at a PCR length of 60 cycles. The results in part (B) show the visual detection under UV-light, where up to three cells can be clearly detected using the *mip* gene as an amplification target.

This is a first proof of concept for the rapid detection of *Legionella spp.* The next step will be to increase the amplification time to speed up the overall process. Therefore, the assay could also be transferred to isothermal amplifications such as the recombinase polymerase amplification (RPA).

In order to develop a genus-wide detection assay for the pathogen *Legionella spp.*, further genes have to be tested.

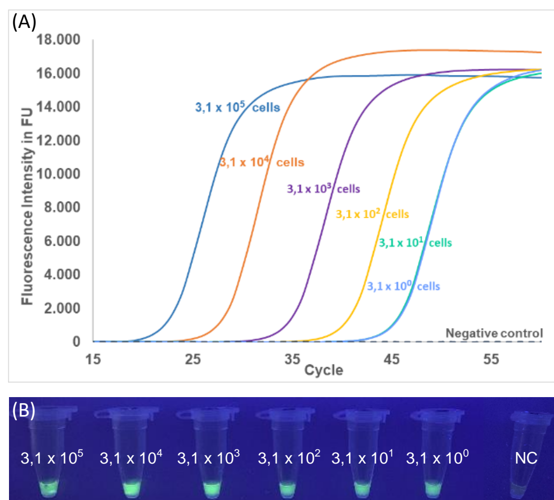

***Figure 1:** (A) Quantitative PCR amplification results of the mip gene: the fluorescence intensity is shown in relation to the cycles.*
(B) Visual endpoint detection of the mip gene. Therefore, 1 µL of 1000x SYBR-Green was added to the tubes, excitation under UV-light.

Conclusion

The presented system provides the basis for a solution of the sensitive amplification with a rapid visual endpoint detection of the pathogen *Legionella pneumophila*. Further work could allow the development of an assay suitable for on-site detection, for example, to perform rapid tests for drinking water.

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Time- and life-saving detection of sepsis pathogens via multiplex PCR and microarray of nine of the most prevalent pathogens

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Keywords: Microarrays, Hybridization, Multiplex-PCR, Pathogens diagnosis, Sepsis

Introduction

The clinical picture of sepsis can be traced back to a dysregulated immune response due to a bacterial infection leading more often to life threatening conditions. **[1]** A fast and precise diagnostic is crucial for the patients outcome, as with each hour passing the mortality rates increase. The diagnostic serves to determine a suitable antimicrobial therapy. Currently the detection is carried out via time-consuming (> 48 h) microbiological identification. **[2] [3]** The use of polymerase chain reaction (PCR) for the detection will not only enhance the sensitivity and specificity but also reduces the time between sampling and personalised therapy. For the identification of the specific sepsis pathogens, the microarray technology offers an approach that can enter competition with other massive parallel analysing technologies.

Results and Discussion

Due to the need for rapid action and intervention, we developed a method where the sepsis pathogens can be identified within a few hours including depletion by using a microarray-based multiplex PCR assay. The designed assay included the detection of nine of the most prevalent pathogens. In figure 1, an exemplary detection result is shown (*S. pseudopneumoniae* and *A. baumanii).* To identify the pathogens, we developed 60 probes that bind to a specific sequence on the 16S gene of the rRNA in prokaryotes. In addition, we used five different primer combinations in a multiplexed assay to detect the respective organisms. For both exemplary organisms, hybridization of the amplicons to their specific probes was accomplished. The probes delivered distinct signals, which were clearly distinguishable from the background signals.

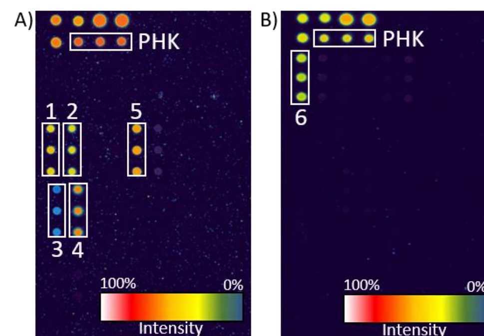

Figure 1: *Microarray hybridization results of multiplexed PCR products using five primer combinations. A) S. pseudopneumoniae and B) A. baumannii. The used multiplex primer resulting in a false colour display representing the relative fluorescence intensities of the bound amplicons. 1-4: Streptococcus Spp., 5: Streptococcus pneumoniae, 6: Acinetobacter baumannii, PHK: positive hybridization control.*

Conclusions

In this study, we have developed a method that allows sepsis pathogens to be amplified via multiplex PCR, which then were detected and identified via microarray hybridization using specific probes. This experimental design covers around 95% of the most prevalent pathogens. In conclusion, this method grants a result within a few hours and therefore possibly minimises the mortality rate of sepsis patients.

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Acknowledgements

We would like to thank our sponsors the ILB, the WFBB and the EU (EFRE).

Performance of an automated device for loop-mediated isothermal amplification with a colorimetric readout

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Keywords: LAMP, Isothermal amplification, Automation, Fluorescence

Introduction

The loop-mediated isothermal amplification (LAMP) is gaining popularity in analytics and diagnostics. Their special feature – being an isothermal amplification method that can multiply nucleic acids in less than one hour at a constant temperature with high specificity and sensitivity. **[1]** The preparation of LAMP components can be reduced to a few steps that can be handled by an automated pipetting device. In order to use the LAMP as a point-of-care system, that is less error-prone for interpretation of the results, it is missing an adequate user-friendly and quick readout. This we unified in an automated running device for LAMP with a colorimetric readout. To show the feasibility of the presented device the DNA of the bacterium of the genus *Salmonella spp.* with the target invA was used. The colorimetric endpoint detection was accomplished by the turnover of the SYBR Green I dye.

Results and Discussion

The genomic *Salmonella spp.* DNA was detected within 30 minutes of amplification. With one microliter of the nucleic acid dye SYBR Green I in a 1000-fold dilution and a false colour display the given image in figure 1 has been obtained. The picture was taken by a CMOS camera immediately under a LED after adding the dye to the amplified products. After the picture was taken the software automatically adjusted the image with different processing and filtering techniques. The result was the false colour display image. The colourless solution on the left showing the negative samples, without template (NTC). Meanwhile the radiant colourful solution on the right are positive controls, as seen in figure 1. It was possible to overcome the conventional way of detection via bacterial culture, in terms of time will be reduced from days to hours. **[2]**

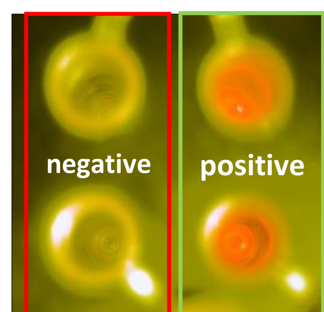

Figure 1: *Camera image illuminated with an LED and automatically processed by false colour display within 30 minutes of amplification. On the left side are samples containing only the LAMP components without template, which can be read as negative, because of the absence of colour. On the right side of the image are samples containing the Salmonella DNA in a double determination experiment, which can be seen as positive.*

Conclusions

It was possible to determine all samples correctly by the output image, without any further processing steps. The LAMP was successfully used for a rapid amplification within 30 minutes. After the amplification, the direct readout of the assay was achieved by a simple colorimetric readout.

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Acknowledgements

We would like to thank our sponsors the ILB, the WFBB and the EU (EFRE).

Rapid on-site detection of common pathogens in under 15 minutes using low cost point-of-care devices for LAMP

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Keywords: Rapid endpoint detection system, Portable point-of-care device, Low cost, LAMP

Introduction

Nowadays, the requirement on modern healthcare management is rapidly rising in terms of costs, time and personnel. **[1]** As seen in recent years, the emergence of new pandemics showed that it is advantageous to create adaptable systems for the efficient detection of pathogens. We constructed a 3D printed, portable, pathogen-detecting device that is able to measure fluorescence signals by its intensity, emitted through nucleic acid amplification.

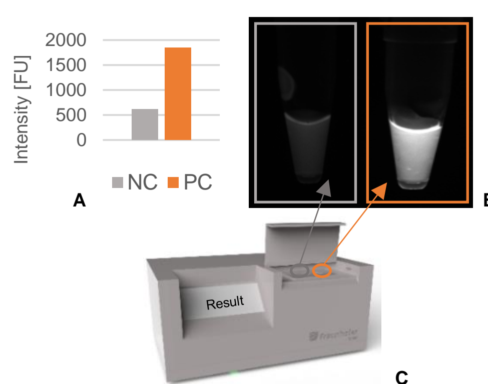

Figure 1: *A) End-point-detection after 12 min. nucleic acid LAMP amplification using SYBR Green. The resulting fluorescence signal for a negative (NC) and for a positive control (PC) was measured. B) Visualization of the different SYBR Green I signals using UV-light. C) 3D printed, low cost LAMP-Detection module*

Results and Discussion

The nucleic acid amplification was performed by using the loop-mediated amplification technique (LAMP). **[2]** This methodical approach brings the advantage of an isothermal setup which reduces not only the experimenting time, but also the amount of necessary power supply to common batteries. The detection of the generated SYBR Green I signal will be achieved by combining a LED with a photodiode sensor. Due to the endpoint detection, a fast confirmation or disproval of contamination is made possible. The user-friendly design of the test procedure allows for the on-site application. To prove the practicability of the portable device, SARS-CoV-2 cDNA of the N gene was amplified in less than 15 minutes, including an automated endpoint quantification (figure 1 A) of the intensities with a direct display “positive” or “negative” (figure 1 C) result. The N gene was successfully detected after 12 minutes of amplification time. In figure 1 B the different fluorescence intensities between both samples can be seen.

Conclusions

The design of a user-friendly on-site test was established. The requirements for a point-of-care detection system of cost reduction and time efficiency were successfully fulfilled. Further refinement regarding sensitivity and applicability of the chosen conditions is necessary. The only variable for future challenges should be the primer combination.

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Acknowledgements

We would like to thank our sponsors the ILB, the WFBB and the EU (EFRE).

Acoustic Sensor Detection of the Early-Stage Ovarian Cancer Biomarker Lysophosphatidic Acid with a Dual-Protein System

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Keywords: acoustic detection, ovarian cancer, lysophosphatidic acid, gelsolin, actin

Introduction

Ovarian cancer (OC) is the most fatal gynaecological cancer as it is usually detected late, when the survival rate decreases from >90% (stage I) to <30% (stage IV).[1,2] The only available blood test detects cancer antigen 125, an unreliable biomarker that is up-regulated in about 50% of early stages. Lysophosphatidic acid (LPA) is elevated in 90% of stage I OC, making it a promising biomarker for early-stage detection. Furthermore, as LPA levels correlate with disease progression, a potential LPA blood test can detect and stage OC.[2,3]

Results and Discussion

The electromagnetic piezoelectric acoustic sensor (EMPAS) and thickness shear mode with dissipation monitoring (TSM-D) system are mass-sensitive sensors that were used to detect LPA. EMPAS and TSM-D discs were functionalized with antifouling linkers terminated with Ni-NTA to bind with the histidine tag of recombinant gelsolin. Gelsolin(1-3)-actin is a protein complex that dissociates upon LPA binding. After exposing the functionalized surface to the complex, the resonance signal decreases due to the complex binding to the surface. The sensor was then incubated with different LPA concentrations, causing the signal to increase due to the loss of actin (Figure 1A). The resonance shift correlates with [LPA], enabling detection and quantification of LPA (Figure 1B).

Conclusions

An early-stage OC test is an important step towards realizing mass screening, which will lead to a significant decrease in OC mortality rate. Detecting the OC biomarker, LPA, highlights the possibility for identifying early-stage OC. We applied the gelsolin(1-3)-actin complex to measure LPA from 0.5 to 50 μM.

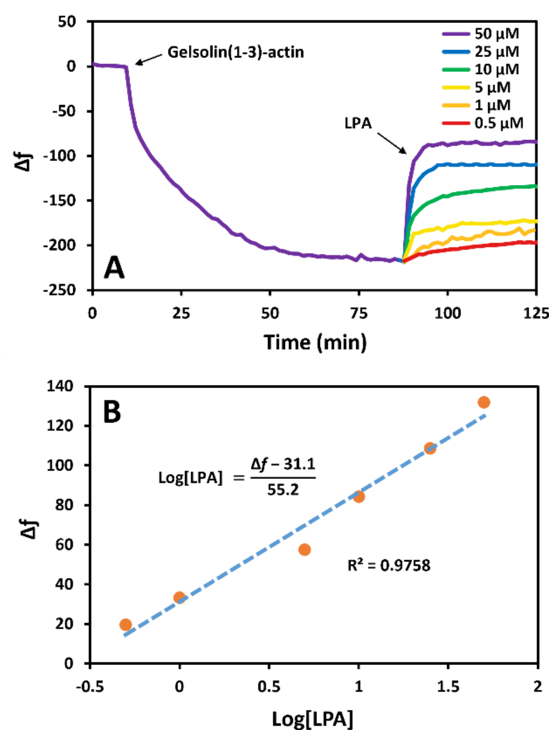

Figure 1: *(A) Frequency shifts of TSM-D following LPA incubation at various concentrations. (B) Linear correlation of Log[LPA] with frequency variations.*

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Acknowledgements

The project was funded under the European Union's Horizon 2020 research and innovation program through the Marie Skłodowska-Curie grant and the Canadian Institutes of Health Research.

Observation of DNA strand interaction with SERS

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Keywords: SERS, nanoparticle, DNA, bio-detection

Introduction

Surface-enhanced Raman spectroscopy (SERS) has demonstrated its ability as powerful tool that can provide us information about the structure and the conformation of molecules such as DNA.

In this work, we used an Hamamatsu commercial SERS substrate **[1]**, to study the interaction between a DNA sequence consisting of 20 Bases of poly-Thymin (PolyT) with its complementary poly-Adenin (PolyA).

Results and Discussion

The PolyA strand is grafted at the surface of the gold nanostructured surface using a thiol group at the 5' extremity of the DNA strand. the SERS substrate is incubated in 450 µl of PolyA (10-4 M) in TE buffer for 15 hours. The surface is then washed to remove the PolyA excess. We assume that we form a monolayer of PolyA. Some solutions of PolyT with different concentrations (10-7, 10-6, 10-5 and 10-4 M) are successively deposited on the SERS substrate. We performed Raman mapping on the surface and we recorded 400 spectra using a 633 nm excitation wavelength.

we can observe the 735 cm-1 band assigned to the ring breathing mode of the PolyA and some variations of its intensity depending on the position on the map. By changing the concentration, we observe a decrease of the standard deviation of the average SERS intensity of this band as well as a decrease of the standard deviation of the intensity of this band.

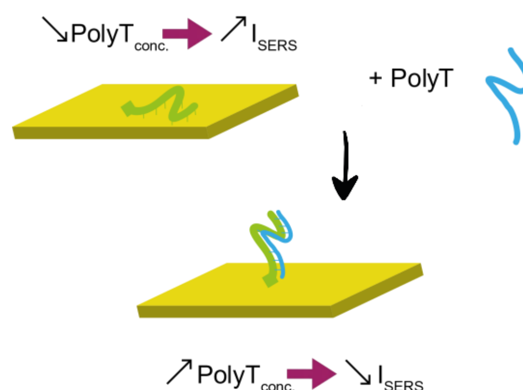

Figure 1: *scheme demonstrating the impact of increasing the concentration of polyT on the flexibility and orientation of polyA.*

Conclusions

We interpret this intensity change by some modification of the orientation and flexibility of the PolyA DNA strands interacting with the PolyT **[2]**. The increase of the concentration of Poly-T induced a loss of flexibility of the PolyT/PolyA molecular complex see figure 1. This study provides a new approach for the reliable quantification and structural analysis of biological molecules.

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Acknowledgements

This work is supported by funding from the European Union's Horizon 2020 research and innovation FET Open programme under grant agreement No 964248 (DeDNAed).

Development of immunochemical assays for the determination of quorum sensing peptides of *S. aureus* for diagnosis purposes

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Keywords: ELISA. quorum sensing, antibody, AIP, *S. aureus*

Introduction

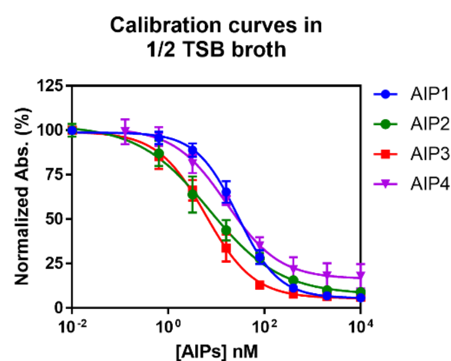
Quorum-sensing (QS) is a cell-to-cell communication process based on the release and sensing of low molecular weight chemical signals, called autoinducers (AIs). In S. aureus, these molecules correspond to cyclic thiolactone autoinducing peptides (AIPs I-IV), whose production is regulated by the accessory gene regulator (agr) system during an infection process3. AIPs control its own biosynthesis and modulate the genetic expression of virulence factors and survival mechanisms.

Results and Discussion

Antibodies for the detection of each AIP (I-IV) have been produced, characterized, and used for the development of competitive single-analyte indirect microplate-based ELISAs. The ability of the immunochemical assays to detect AIPs has been evaluated in the analysis of S. aureus isolates from patients infected by strains belonging to different agr genotypes. The assays are all performed in ½ broth dilution, without the need of any additional sample treatment

The antibodies produced show little or non-cross-reactivity among the four AIP types, regardless of the structural similarities, allowing a reliable detection and quantification of the AIPs with high specificity and sensitivity. All AIP types have been easily detected in clinical isolates obtained from patients suffering from S. aureus respiratory infections, reaching limits of detection (LODs) in the low-nM range. As a first approach, the AIP allelic profile of four different strains genotyped as agr I and agr IV has been studied, showing correlating results with the corresponding agr genotype.

Once properly validated, the technology developed in this work could be implemented as a method for a rapid and reliable diagnosis of *S. aureus* infections, demonstrating the potential of AIPs as biomarkers of infection. The applicability of this method directly in biological samples, would noticeably shorten the time of diagnosis, thus guiding the treatment and avoiding antimicrobial resistance. On the whole, the present technique could increase diagnosis efficacy, improving clinical outcomes.



	AIP1	AIP2	AIP3	AIP4
IC_{50} (nM)	28.16 ± 8.10	7.81 ± 3.95	6.68 ± 3.38	3.18 0.95
Slope	-1,02 ± 0.04	-0.62 ± 0.08	-0.86 ± 0.11	-0.80 ± 0.12
LOD (nM)	**3.12 ± 1.08**	**0.41 ± 0.22**	**0.70 ± 0.59**	**0.30 ± 0.12**
R^2	0.99 ± 0.001	0.99 ± 0.004	0.99 ± 0.003	0.99 ± 0.01

Figure 1: *ELISA Calibration curves for the detection of each AIP and their analytical parameters*

Conclusions

The results shown in this communication bring to light the potential of the immunochemical technique developed to early diagnose *S. aureus* infections. Likewise, the specificity profile towards the different AIPs gives possibility of using this method in genotyping studies

Paper-based integrated device based on rGO-sensing film for direct determination of carbaryl

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Keywords: electrochemical sensors, nanomaterials, reduced-graphene oxide, CO_2 laser, pesticides.

Introduction

In this work, an enzyme-free strategy for the determination of carbaryl, a carbamate widely used in grains, is proposed. To this aim, a paper-based pop-up device able to integrate the sample pre-treatment with the electrochemical determination was developed; the device allows for avoiding external preconcentration and hydrolysis steps commonly required for the electroanalysis of this pesticide. The analyte detection was performed exploiting a reduced graphene oxide (rGO) based lab-made sensor fabricate onto nitrocellulose. The latter was produced using a CO_2 laser approach, starting from a graphene oxide film **[1]**; the strategy ensures a flexible design and useful electroanalytical performance. The electrochemical sensor and the pop-up device, take advantage of the unique and sustainable features of cellulosic substrates **[2]** and were entirely lab-manufactured employing low-cost bench-top equipment.

Results and Discussion

The device and its measuring setup are shown in **Figure 1**. The measure consists of (a) sample concentration on paper, (b) carbaryl hydrolysis on paper, (c) pop-up device folding, (d) loading of the neutralization/measure buffer, (e) measurement via DPV. The device allows micromolar LOD (0.4 µM) and LOQ (1.5 µM), ensuring the measurability of grain samples below the allowed maximum residue limits (MRLs), avoiding external evaporation steps. Despite being lab-made, the device allows reproducible carbaryl measurments (RSD≤10%, n=3), demonstrating the analytical reliability of the device and the production process. The device was used to analyze five different types of grains. Quantitative and reproducible recoveries were obtained (93-108%, RSD<6%; n=3) at 0.5, 1, and 1.5-fold the MRL (3.5 µM). Furthermore, the selectivity was sucesfully proved towards pesticides belonging to other classes and with very similar chemical structures. Summing up, the device is suitable for the direct analysis of carbaryl in grains.

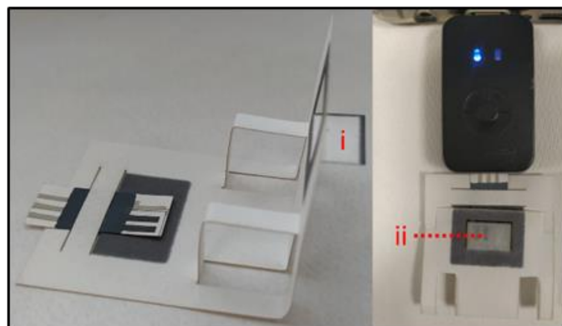

Figure 1: *Assembled device (left), device during the measurement step (right). (i) Holder for sampling and hydrolysis; (ii) configuration for neutralization and measure.*

Conclusions

Here, a complete paper-based device was produced and assembled using low-cost strategies. The device allows sample concetration, ensures the hydrolysis of the analyte, and detect the same at the MRL level in grain samples without suffering from interfering compounds.

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Acknowledgements

S.F. and D.C. acknowledge the Ministry of Education, University and Research (MIUR) and European Social Fund (ESF), act. I.1 "innovative doctorates with industrial characterization" for the PON R&I 2014-2020 (CCI 2014IT16M2OP005). This research was funded by the European Union – Next Generation EU. Project Code: ECS00000041; Project CUP: C43C22000380007; Project Title: Innovation, digitalization and sustainability for the diffused economy in Central Italy - VITALITY

Simultaneous determination of laccase activity in a cuvette assay: photometric assay versus oxygen dipping probe

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Keywords: bio fuel cell, laccase, enzyme activity, photometric assay, oxygen probe

Introduction

In bio fuel cells, a typical reaction at the cathode is oxygen reduction to water. This reaction can be catalysed by the enzyme laccase. Enzyme activity is often determined in a photometric cuvette assay via monitoring the consumption/ formation of a respective reactant/product of the enzymatic reaction. However, when the enzyme is adsorbed on particulate material in a suspension problems arise due to light scattering.

In an alternative approach, laccase activity can be determined via monitoring oxygen depletion. Here, oxygen is quantified by its role as fluorescent quencher using a dipping probe [1] or by a Clark-type oxygen electrode [2,3]. In this case, no free light path is needed in contrast to the photometric assay above. Thus, suspensions could also be used as samples.

In this contribution, we compare the two approaches (photometric assay vs. oxygen dipping probe) by measuring the respective laccase activities simultaneously in a cuvette assay.

Results and Discussion

The experimental set-up shown in figure 1 integrates a commercial oxygen dipping probe within a cuvette used in a photometric assay.

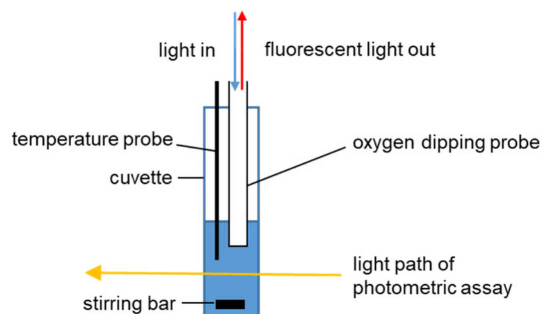

Figure 1: *Experimental set-up. Simultaneously measuring laccase activity in a cuvette: photometric assay vs. oxygen dipping probe.*

The measurement with the oxygen dipping probe within the cuvette is challenging. There is only a very small amount of oxygen dissolved within the assay solution due to the low solubility of oxygen in water and the small solution volume. The fluorescent signal of the dipping probe is strongly temperature dependent. Furthermore, there can be a substantial influx of oxygen from the headspace above the assay solution during the measurement. This counterbalances the consumption of oxygen by the enzyme reaction.

Thus, measures have to be taken to address and mitigate these effects. Preliminary results are depicted in figure 2.

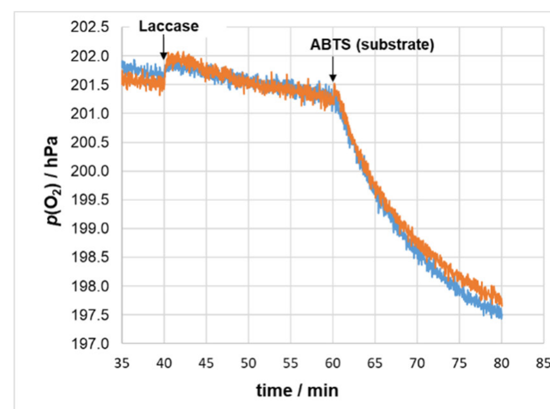

Figure 2: *Two consecutive measurements obtained with the oxygen dipping probe.*

Conclusions

Benchmarking enzyme activity obtained with an oxygen dipping probe versus a photometric assay is challenging. However, it could open the way to measure laccase activity in demanding samples such as suspensions.

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Development of an aptamer based assay for the detection of N-linked glycosylation pattern variations

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Keywords: aptamer, SELEX, N-glycans, molecular beacon, fluorescence detection

Introduction

Multiple studies have shown that altered IgG glycosylation plays a crucial role in various autoimmune and inflammatory diseases, including rheumatoid arthritis **[1]**. As biomarkers aberrant glycosylation patterns (G0 type N-glycans) could be essential for early diagnosis and further insight into the pathomechanisms **[1]**. However, standard methods for glycosylation analysis, such as HPLC and MS, are often complex and time-consuming **[2]**. This project aims to develop a fast and simple detection system for glycan pattern variations using anti-glycan aptamers integrated into a molecular beacon-like fluorescence assay.

Results and Discussion

To generate DNA aptamers, an in-vitro semi-automated selection process (SELEX) was performed. Initial attempts using a single-stranded DNA pool and G0-type N-glycan did not yield glycan binders, possibly due to the glycan's small size and low interaction strength **[3]**. A new approach using IgG with G0-type glycan on magnetic particles was used, and a counter selection step with aglycosylated IgG was added to remove protein-only binders. The first few selection rounds showed enrichment, hence the selection will continue.

In parallel, a detection system using literature aptamers was designed and tested. The combination of molecular aptamer beacons (MAB) with primer displacement strategies showed promising first results of this proof of principle. When the fluorophore and quencher labelled MAB was incubated with a complementary primer, the hairpin structure opened leading to an increase of the fluorescence signal. When incubating the MAB with the target before the primer, the target blocks the primer from binding, preventing a full opening of the hairpin structure (Figure 1).

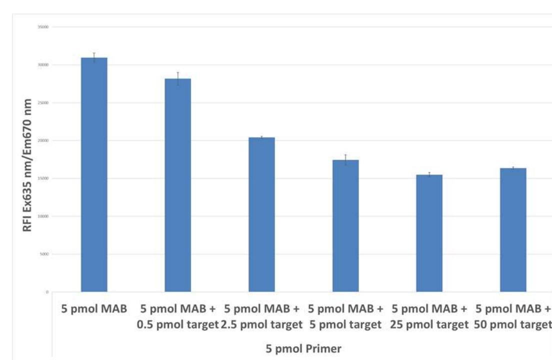

Figure 1: *Cy5 measurement after incubation of 5 pmol molecular aptamer beacon (MAB) with target and 5 pmol complementary primer in 96-wellplate. Target added in different concentrations from 0.5 pmol to 50 pmol. MAB and target incubated from 60 min at 23 °C, afterwards addition of primer and incubation for 10 min at 23 °C.*

Conclusions

This results display promising steps in generating glycan binding aptamers. The integration of this aptamers into the established MAB detection system and validating it with patient samples, would ensure the successful development of a fast and simple to use assay for the early diagnosis of rheumatoid arthritis.

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Acknowledgements

This work was supported and funded by the Fraunhofer Cluster of Excellence Immune-Mediated Diseases (Fraunhofer CIMD).

Self-assembly of DNA origami - gold nanoparticle hybrids as optical sensing element of biomolecular sensors

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Keywords: DNA origami, nanoparticle dimer assemblies, SERS

Introduction

Self-assembly of DNA origami structures was first demonstrated by Rothemund [1]. Since then, its impact to fields like biosensing [2] continuously increased due to the unique capability of precisely positioning of nano-scopic elements, such as semiconducting [3] or metallic [4] particles as well as conducting polymers [5]. Here we present a novel approach for the development of a SERS sensor, where precise positioning plays an important role: (1) Gold nanoparticle (AuNPs) dimers are positioned in very close proximity to each other, creating an enhanced electromagnetic field in the gap between them. (2) A recognition element (RE) is placed into this gap. (3) Functional elements are placed on the DNA origami to enable its site-specific immobilization on solid or flexible substrates.

Results and Discussion

A 2D DNA origami was designed with caDNAno. It includes a symmetry break for determining the immobilization side. Its strain relaxation was proven with CanDo. The AuNPs are immobilized by hybridizing complementary oligonucleotides, one type attached on the AuNP surface and the complementary one protruding from the DNA origami. The RE is also protruding from the DNA origami and its sojourn time in the hotspot region between the particles was determined with oxDNA. Other protruding oligonucleotides are implemented into the opposite DNA origami side to enable hybridization to substrate-attached oligonucleotides, thus facilitating immobilization on either solid or flexible supports. All sequences of the protruding oligonucleotides were determined using EGNAS [6] to reduce the possibility of unwanted hybridizations. The successful DNA origami synthesis and purification was verified by agarose gel electrophoresis and AFM (Figure 1A). The hybridization of AuNP dimers to the DNA origami and their subsequent purification was verified by STEM (Figure 1B).

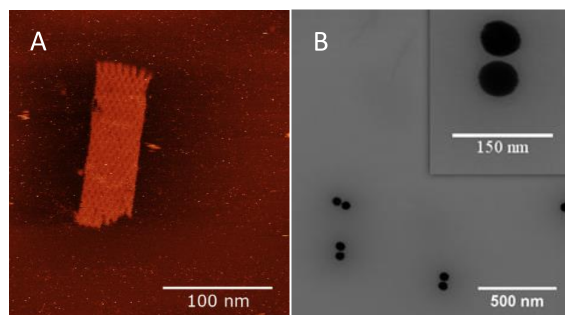

Figure 1: A: Liquid-AFM image of a DNA origami; B: STEM images of AuNP dimers positioned on DNA origami templates.

Conclusions

DNA origami proves its outstanding versatility in terms of precise immobilization of different kind of nano-objects. In combination with a DNA sequence generation algorithm, the amount of side products in all stages of the assembly can be significantly reduced. DNA origami, ultra-smooth AuNPs and the placement of the hybrid structures on substrates will pave the way to quantitative SERS measurements.

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Acknowledgements

This work is supported by funding from the European Union's Horizon 2020 research and innovation (FETOpen) under grant agreement No 964248 (DeDNAed). We like to thank Gunnar Klös and Aitziber Cortajarena from CIC biomaGUNE for AuNP synthesis and functionalization.

Generation and detection of pH gradients inside microfluidic channels

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Keywords: light-addressable electrode, light-addressable potentiometric sensor, lab-on-a-chip system

Introduction

In lab-on-a-chip systems, multiple miniaturized components such as sensors, liquid-handling devices, or actuators are usually integrated into a microfluidic channel to perform a chemical or biological analysis on a single chip. For most applications, these devices use rigid sensor and actuator structures such as electrodes fabricated by microsystem technologies. These processes are typically costly and time-consuming. On the contrary, semiconductor-based sensor and actuator technologies can adapt their “active area” spatially resolved during runtime through illumination. With a light-addressable electrode (LAE), photoelectro-chemical reactions (e.g., changes of the pH value) can be triggered **[1]**. Complementary, a light-addressable potentiometric sensor (LAPS) allows monitoring of these pH changes **[2]**. In this work, the joint implementation of LAPS and LAE into a microfluidic channel will be presented.

Results and Discussion

To fabricate the combined microfluidic system, an Al/n-Si/SiO_2/Si_3N_4-heterostructure was used for LAPS. On top, a double-sided microfluidic tape, cut by a laser, defines the microfluidic channel geometry, while the channel is closed with a glass/SnO_2:F/TiO_2-LAE structure (figure 1).

First, LAPS and LAE were characterized individually inside the microfluidic channel. In the next step, combined measurements were performed. The LAE induced pH changes were studied using current-voltage curves and chemical images obtained from LAPS. Finally, to demonstrate the flexibility of the system, LAE parameters were varied, and the effects were monitored by the LAPS.

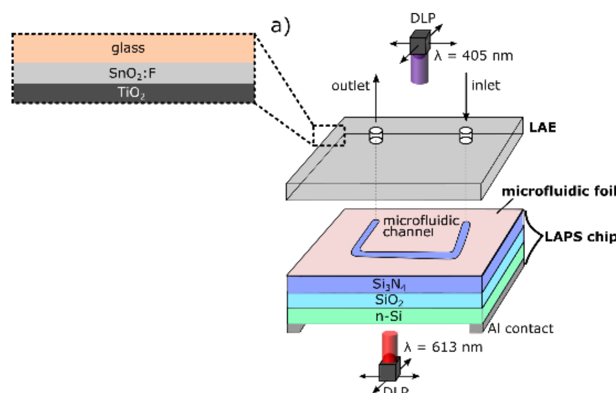

Figure 1: Schematic structure of a combined LAPS (bottom) – LAE (top) setup, combined with a microfluidic channel. a) Cross-section of the LAE.

Conclusions

This work describes a microfluidic platform enabling time- and spatially resolved pH measurements and manipulations. The successful application and flexibility show the potential for lab-on-a-chip systems without the need for predefined electrode structures.

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Acknowledgments

This work was supported by the German Federal Ministry of Education and Research (BMBF) within “NanoMatFutur“ (13N12585).

Surface Imprinted Polymers for the Detection of Fungal Spores

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Keywords: Fungal spores, *Botrytis cinerea*, Biomimetic sensing, Surface Imprinted Polymers

Introduction

The FAO estimated that about 123 million tons of tomatoes were produced in 2019 but spoilage by *Botrytis cinerea* is a problem that causes substantial losses of this harvest [1, 2]. In the context of improving energy efficiency in greenhouses, producers minimize energy losses by keeping the windows closed. As a result, the relative humidity increases, giving fungal spores the opportunity to germinate [3]. To prevent fungal contamination by *e.g. Botrytis cinerea* it is interesting to monitor the aerial spore count in the greenhouse so that measurements can be taken when a threshold value is crossed before widespread infections and economic losses occur. To that end, this research will focus on the development of fungal spore sensors based on imprinted polymers.

Discussion

This work is a continuation of previously obtained results by Arreguin-Campos *et al.*, who made SIP's for the *E. coli* bacterium [4]. The imprints are prepared by allowing free assembly of the template on a pre-cured PDMS polymer (Figure 1). Further curing and subsequent removal of the template produces the surface imprinted polymer (SIP).

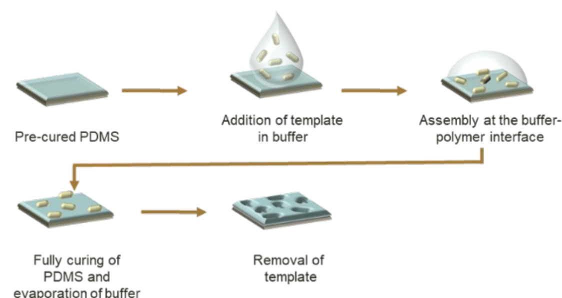

Figure 1: *Schematic representation of imprinting method, producing the SIP, reproduced from [4].*

Rebinding of fungal spores to the SIP layer will be studied using the so-called Heat Transfer Method (HTM). A heating element is placed below the SIP, while the temperature is monitored on the top. Upon binding of the target to the receptor, the thermal resistance of the system changes, leading to a change in temperature (Figure 2). To improve sensitivity of the setup, Graphene Oxide (GO) is added to the PDMS polymer before imprinting.

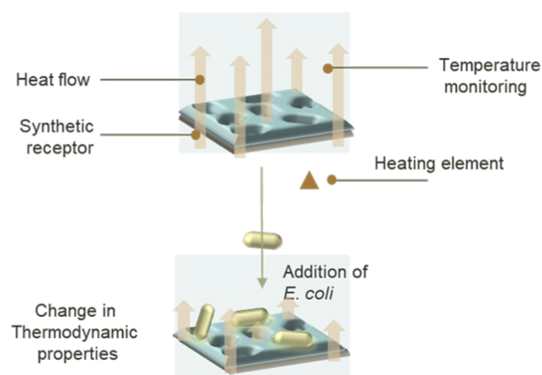

Figure 2: *Schematic representation of heat transfer method for measurements, reproduced from [4].*

Outlook

Future work will include the development of sensors for *Didymella bryoniae* and *Fusarium oxysporum spp.* to protect the harvests of cucumbers and paprika's respectively. In addition to the HTM, electrochemical readout technologies like impedance analysis and, if possible, potentiometry will be tested.

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Acknowledgements

The authors are grateful for funding of the project "ENERGLIK (Int6B003) by the European Regional Development fund of the E.U. through Interreg via Flanders-the Netherlands.

Locally Selective Immobilization of DNA origami for Raman Spectroscopy based biosensors

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Keywords: DNA origami, surface immobilization, nanotechnology, functional coatings

Introduction

In recent years, DNA origami has become more popular in biosensor applications due to its ability to arrange heterogeneous functional elements at high resolution in a self-assembly process. For applications using structures of millimeter range like plasmonic structures a locally selective and aligned surface deposition is necessary and a chemical contrast is used mostly based on SiO_2 as bond-attractive layer (BA) in combination with silane monolayers as hydrophobic bond-resistant layers (BR) [1,2]. However, the number of descripted material combinations for BA- and BR-layers is still very limited [3,4]. The purpose of this paper is to present a methodology for surface modifications that improve the selectivity of deposition and thus increase the number of technical relevant BA-/BR material combinations.

Results and Discussion

Parylene has a passivating effect due to its low surface energy. However, when used as a BR-layer in combination with SiO_2 as bond-attractive binding sites within a thin film system, only moderate selectivity for the selective immobilization of DNA origami could be observed. In particular, after an atmospheric plasma-based activation of the SiO_2 binding sites in order to improve the availability of the silanol groups to bind the DNA origami, an increased binding affinity of Parylene toward DNA origami was visible, reducing the selectivity of the BA/BR-system. To improve the BA/BR-system, surface modifications were tested to enhance both the binding to SiO_2 and the passivating effect of Parylene layer.

A treatment with Pluronics® F127 in combination with plasma activation increased the binding selectivity of DNA origami between SiO_2 and Parylene (see Fig 1).

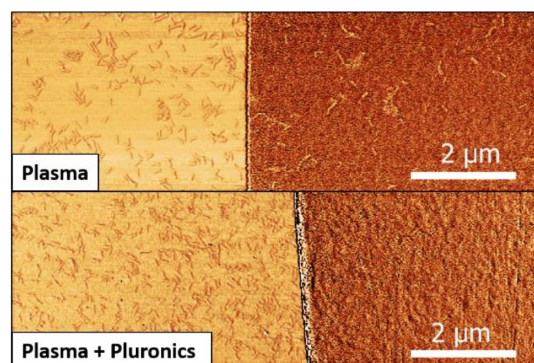

Figure 1: *AFM phase image of DNA origami deposition on SiO_2 (left) / Parylene (right); top: plasma treatment – non selective; bottom: with plasma and Pluronics® F127 – selective.*

Conclusions

We showed different surface treatments, with plasma and/or Pluronics® F127 in order to improve the selectivly binding of DNA origami and enable thereby further steps towards microtechnological surface integration of DNA origami.

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Acknowledgements

This work is supported by funding from the European Union's Horizon 2020 research and innovation FET Open programme under grant agreement No 964248. We like to thank F. Selbmann for providing Parylene layers and A. Heerwig and M. Mertig from KSI Meinsberg e.V. for DNA Origami synthesis.

Thermal pyocyanin sensor based on molecularly imprinted polymers for the indirect detection of *Pseudomonas aeruginosa*

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Keywords: *Pseudomonas aeruginosa* detection, molecularly imprinted polymer (MIP), infection control, pyocyanin, Heat-transfer method (HTM).

Introduction

Pseudomonas aeruginosa is an opportunistic ubiquitous bacterium capable of causing serious illnesses. Furthermore, it is particularly dangerous for people affected by Cystic Fibrosis, thus a point-of-care diagnosis is important to detect the presence of the bacterium in a fast and reliable way. This can be achieved through Molecularly Imprinted Polymers immobilized onto an aluminium chips. **[1]** The functionalised chips can measure the presence of pyocyanin, the main toxin secreted by *P. aeruginosa*, via thermal measurements at the liquid interphase, thus leading to the indirect detection of the bacterium itself.

Results and Discussion

In order to indirectly detecting *P. aeruginosa* without using laborious techniques, in this work we focused on the indirect detection of the bacterium using Molecularly Imprinted Polymers (MIPs), namely polymers presenting specific cavities able to selectively recognise the target. In this case, pyocyanin was used as a target, but due to its radical scavenging properties, phenazine was selected as a dummy template to resemble pyocyanin`s shape and interactions. MIPs were synthesised using (vinylbenzyl) trimethylammonium chloride as monomer, EGDMA as cross-linker, AIBN as the initiator and chloroform as solvent. To understand what MIP composition (ratio between template, monomer and cross-linker) was the best one, specific rebinding experiments were performed by exposing the MIPs powder to increasing concentrations of pyocyanin, in order to understand how much of the target molecule the MIP particles can be bound to the cavities. Once that the best composition was found, the particles were immobilized onto aluminum chips to perform thermal measurements by means of the Heat-Transfer Method (HTM) **[2]**. To study the sensitivity of the sensor both MIP and NIP (the Non-Imprinted Polymer as a negative control) were studied. The results confirm that the MIP is able to detect pyocyanin more selectively that the NIP, reaching a LoD of 0.347 ± 0.027 μM. Furthermore, selectivity measurements were performed using common interferents, such as glucose, riboflavin, *L*-ascorbic acid and phenazine methosulfate. After the selectivity studies assessed that the sensor was selective towards pyocyanin and it did not respond to the presence of interferents, a proof-of-concept was performed by using saliva spiked of pyocyanin. Once again, the study demonstrated that the sensor is able to detect pyocyanin inside a complex fluid, such as saliva, with a LoD of 0.569 ± 0.063 μM, thus opening the possibility to use this thermal sensor for on-site diagnosis.

Conclusions

In this work, we showed the development of a pyocyanin sensor, highlighting its potential for application in fast tests for the detection of infection in immunocompromised patients.

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Acknowledgements

This work was supported by the European Regional Development Fund through the AgrEU food project. funded by the Interreg VA Flanders-The Netherlands program, CCI grant no. 2014TC16RFCB046.

Activity determination of enzymes related to the treatment of Morbus Parkinson

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Keywords: monoamine oxidase B, catechol-O-methyl transferase, Prussian blue, fluorine tin oxide

Introduction

Morbus Parkinson belongs to one of the most severe disorders of the central nervous system in the world. Beside the intracellular accumulation of the protein α synuclein, Parkinson's disease (PD) is characterized by the loss of dopaminergic cells causing a dopamine deficiency. Due to the disturbed signal transmission at the synapses typical movement disorders occur.

The monoamine oxidase B (Mao B) and the catechol-O-methyl transferase (COMT) are involved in the metabolism of dopamine. Hence, they are important targets for the treatment of PD [2]. The administration of inhibitors for the Mao B and the COMT belongs to the therapy of PD. Direct measurements of enzymes activity could help to improve the individual adjustment of the drug application.

Results and Discussion

For the activity measurement of the Mao B which oxidises benzylamine to benzaldehyde, NH_3 and H_2O_2 the enzyme is captured by specific antibodies. The hydrogen peroxide production has been detected amperometrically at Prussian blue-carbon electrodes whith no interference by benzaldehyde and NH_3. Amperometric measurements with the captured Mao B over a period of 30 min show a linear increase of the detected H_2O_2 concentration. Here the measuring signal correlates clearly to the activity of the captured enzyme **[1]**.

For the activity determination of the COMT differential pulse voltammetry (DPV) has been used for the detection of the enzyme substrate dopamine. At fluorine doped tin oxide (FTO) a clear discrimination between dopamine and its conversion product methoxytyramine is feasible so that dopamine can be detected in the presence of the reaction product **[2]**. The linear dependency of the DPV signal on the dopamine concentration in the range of high substrate concentrations (which are necessary for enzyme activity detection) are essential for a reliable sensor. Furthermore, a high signal stability during consecutive measurements can be provided applying voltammetric surface regeneration protocol. After necessary adjustments of the assay composition and the DPV potential range the measuring signal correlates clearly to the substrate incubation time and to the activity of the captured enzyme.

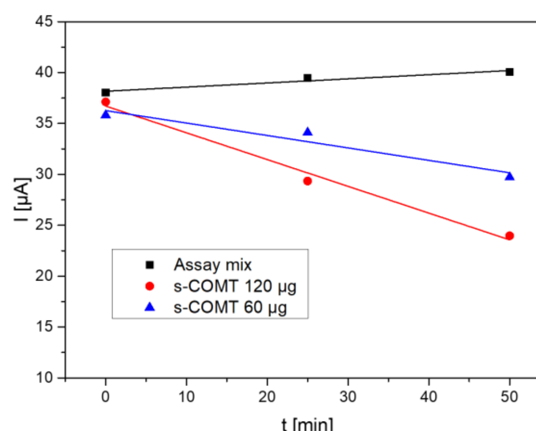

***Fig. 1** Voltammetric determination of dopamine concentration during COMT catalyzed conversion in dependency on the COMT concentration*

Conclusions

Two different electrode materials (Prussian blue for MaoB and FTO for COMT) allow via amperometry (MaoB) or DPV (COMT) the activity determination of Morbus Parkinson related enzymes. This opens up perspectives for an individualized treatment of PD patients.

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Acknowledgements

The financial support by the BMWi (project: 16KN041836) is gratefully acknowledged.

Photobioelectrochemical cells as potential energy source for sensing applications

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Keywords: monoamine oxidase B, catechol-O-methyl transferase, Prussian blue, fluorine tin oxide

Introduction

The combination of light-sensitive components with electrodes has gained increasing interest in the last decade. Light can be used to switch electrochemical reactions but also to change the energetic situation of the charge carriers which gives access to new reactions or establish reactions at more favourable potentials [1]. This is interesting with respect to bioenergetics, but also for sensing application. For the latter a photoelectrochemical cell can provide power for a sensor operation or the cell power itself becomes a measure for a certain analyte.

Results and Discussion

Two concepts of photoelectrochemical cells will be introduced here. The first system uses a combination of two semiconductor materials TiO_2 (high band gap) and PbS quantum dots (low band gap) and couple this with the photocatalytic activity of the biological photosystem II [2]. In order to interconnect both components an Os-based redox polymer has been applied [3]. This photobioanode is combined with a transparent cathode which exploits the catalytic activity of bilirubin oxidase for oxygen reduction on antimony tin oxide (ATO). This cell gives a high cell voltage of about 1V under illumination and a power density of 50µW/cm2 with only water as fuel. It is a sustainable system since the water which is consumed at the anode is formed by the oxygen reduction at the cathode. Furthermore, scalability in the production of both electrodes can be shown by a template-based approach resulting in 3D structures with good access for the biomolecules to be fixed.

The second cell is a tandem cell since here not only light is used as energy source, but also glucose. The photobioanode is again composed of TiO_2 with deposited PbS quantum dots, but coupled here to an enzyme which converts glucose – FAD-GDH (FAD-dependent glucose dehydrogenase). This light-sensitive electrode is combined with another light sensitive electrode working as photobiocathode. Here we have prepared a $BiFeO_3$ layer on top of a FTO electrode chip material. This semiconductor allows the generation of cathodic photocurrents already at rather high electrode potential and can be feeded by H_2O_2 as efficient electron acceptor molecule. Thus, here the enzyme glucose oxidase has been immobilised, which produces hydrogen peroxide during the glucose oxidation. Both electrodes interact with light, but illumination of the cell can be realised via the cathode since most of the light is absorbed here at lower wavelengths whereas the photobioanode exploits mostly the light at higher wavelengths. Both electrodes also consume glucose during operation, but the photobioanode will be feeded with electrons from the sugar conversion where as for the photobiocathode an acceptor molecule is formed during the biocatalytic sugar reaction. For this type of electrode combination a rather high cell voltage of about 1V can be achieved.

Conclusions

These two proof-of-concept studies may illustrate on the one hand the potential of such cell systems for applications with low power consumption and on the other hand the benefits of combining two light sensitive entities for the electrode construction.

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3D-printing resin as a convenient raw material for the creation of surface imprinted polymers

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Keywords: 3D Printing, Surface Imprinted Polymers, SIPs, Photopolymer

Introduction

Surface imprinted polymers (SIPs) are an innovative approach to the fabrication of recognition layers in biosensing [1]. They can be created, for example, by contact imprinting, where a stamp covered with the target analyte is pressed in a semi-cured polymer, which is consecutively crosslinked, creating imprints of the target on the surface. These imprints behave similarly to antibodies and can re-bind the compound they were imprinted with, which can be a wide range of biological material from proteins [2] to whole cells [3]. However, the fabrication of SIPs is time consuming and error prone, as the polymer has to be freshly synthesized from its precursors. This creates an entrance barrier for researcher with limited experience in organic synthesis, who then might stick to more common recognition elements such as antibodies. Here we present the creation of SIPs with commercially available 3D-printing photopolymer, simplifying and accelerating the process.

Results and Discussion

SIPs were created by spin-coating 200 µL of commercially available photopolymer (Formlabs Transparent V4) onto a glass slide, after initial pre-polymerisation a polydimethylsiloxane (PDMS) stamp covered in GFP-expressing E. coli was gently pressed into the precured polymer and post-cured for two hours. After curing the bacteria were removed by rinsing with 0.1% SDS.

SIP exposure to a suspension containing E. coli results in their rebinding to the SIP cavities, which can be verified by fluorescence microscopy (Figure 1). Several dilutions of photopolymer were used to test spin-coating efficiency, however only undiluted resin was able to form a confluent film. The spin-coating resulted in thin film formation, plateauing off at 1500 RPM to a film thickness of about 100 µm. AFM investigation revealed that pre-curing time directly correlates with SIP cavity depth and most efficient rebinding was achieved with cavities of 1/5 to 1/2 bacterial thickness.

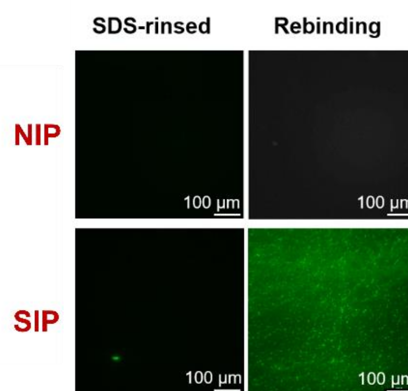

Figure 1: *SIP (Surface imprinted -) and NIP (Non-imprinted polymer) after template removal (left) and after E. coli rebinding (right)*

Conclusions

This proof-of-concept shows that commercial photopolymer can be used as a precursor for the fabrication of surface imprinted polymers. This reduces the entry barrier to SIP fabrication, due to the pre-formulation and wide availability of 3D-printing resins. Additionally, it opens up interesting future applications, like the use of 3D printers to manufacture imprinted, three-dimensional structures.

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Acknowledgements

We would like to acknowledge Rocio Arreguin-Campos for her contribution to the initial development of the process we have built upon in this study.

Detection of HMGB1 levels in serum with electrochemical immunosensor

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Keywords: immunosensor, electrochemical immunosensor, HMGB1, electrochemical impedance spectroscopy

Introduction

High Mobility Group Box 1 (HMGB1) is a nuclear protein stabilizing nucleosome formation and regulation of gene transcription, DNA replication and DNA repair.[1] The HMGB1 can be secreted by activated monocytes and macrophages or it is passively released from necrotic and damaged cells.[2] HMGB1 is one of the cancer markers since its extracellular forms have been associated with formation, progression, and metastases of tumours.[3] HMGB1 interacts with DNA, RNA, many proteins including antibodies, lipids and other molecules in serum. Pre-treatment of plasma with perchloric acid can overcome short half-life for HMGB1 isoforms if performed after serum preparation. Electrochemical impedance spectroscopy (EIS) can follow interaction of HMGB1 with capture antibody immobilized onto gold electrode as a change of impedance after formation of immunocomplexes blocking the electrode surface.[4]

Results

Initially, different types of sensors were tested – gold printed circuit board electrodes (PCB), gold screen printed electrodes (SPE), carbon SPEs modified with gold nanoparticles, and gold interdigitated electrodes; the PCB electrodes resulted as the best option.

The success of the immunosensor highly depends on specificity of the anti HMGB1 antibodies. In Figure 1 it is shown that only two (Ab1, Ab3) from selected antibodies provided the expected difference between blank and positive sample. Other antibodies showed fluctuating and non-specific responses.

Measured calibration using the Ab3 immobilized onto the gold PCB electrodes resulted with LOD 87 ng/ml. To possibly improve the LOD, the carbon SPE electrodes were modified with WS_2 nanoflakes and gold nanoparticles, and LOD 43 ng/ml was achieved.

Conclusions

Further, other nanoparticles will be tested for the improvement of the sensitivity of the immunosensor, and an advanced version will be constructed enabling simultaneous analysis of a few standards and samples.

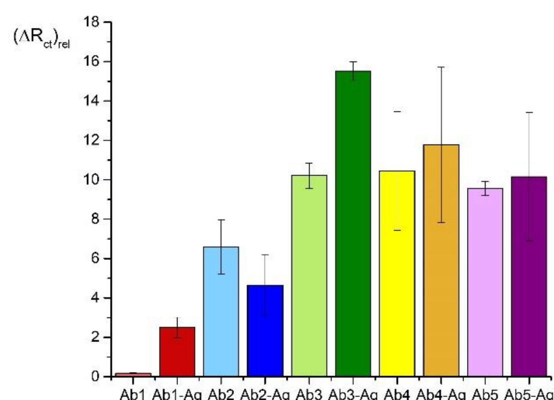

Figure 1: *Impedance changes depending on the choice of the capture antibody; light colour: blank, dark colour – positive samples of recombinant HMGB1 (Ab-Ag)*

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The work has been supported by AZV ČR, Czech Health Research Council grant number NU20-08-00106.

Detection of pathogenic bacteria in aquaculture systems using surface imprinted polymers (SIPs)

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Keywords: aquaculture, surface imprinted polymers (SIPs), *E. coli*, master stamp

Introduction

Over the last decade, increasing seafood consumption has raised public health concerns. This is due to the escalating contamination of aquaculture and farm products with pollutants such as antibiotics, pathogenic bacteria and etc. Therefore, evaluating the quality of aquaculture farms and products with regards to antibiotic and microbial pollutant dispersion throughout the water cycle is highly important **[1, 2]**. Consequently, we focus on developing a novel and specific biomimetic receptors called surface imprinted polymer layers (SIPs). These biomimetic receptors are able to detect specific bacteria in complex samples by identifying the morphological and chemical markers on their surfaces. Here, we aim to create a novel synthesis protocol using master stamp for serial production of these receptors without sacrificing their ability to bind their target bacteria selectively.

Results and Discussion

In this project, we have been creating a selective SIP layer for *E. coli* bacteria using a master stamp as the proof of concept. The master stamp mold was built by photolithography including *E. coli*-like cavities with different sizes. Filling the mold with PDMS and subsequent polymer curing results in a PDMS-based positive master stamp. Further imprinting by the master stamp on a pre-polymerized polyurethane (PU) layer creates *E. coli*-like pits subsequently after curing PU at 65 °C overnight that match the target cell type. However, previous studies using conventional cell imprinting technique with real cell templates suggested that cell membrane residuals in the imprints may play an important role in the SIPs selectivity **[3]**. Therefore, we drop casted the master stamps with *E. coli* cell extract, which were blended by an ultrasonic homogenizer to test the extract effect on the SIPs selectivity. All imprints were made on AU-coated SiO_2 chips, and both electrochemical impedance spectroscopy (EIS) and heat transfer resistance (R_{th}) measurements were performed, simultaneously.

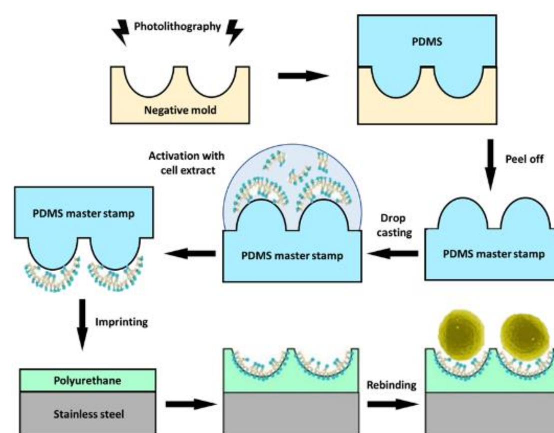

Figure 1: *The negative mold is made by photolithography, and is filled by PDMS. After curing the PDMS master stamp is peeled off that is covered with protrusions mimicking the size and shape of E. coli. The master stamp is loaded with filtered and diluted cell extract, transferring cell-membrane material to the imprinted cavities. The size of the imprints and their biochemical functionalization allow for rebinding of target bacteria from the sample.*

Conclusions

Our preliminary results indicates a higher selectivity for the SIP imprints with master stamp coated with cell extract. However, further investigation and tunning of the PU layer thickness and the cell extract cast on the master stamps are necessary.

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Acknowledgements

We gratefully acknowledge the support by the ARENA project titled “Antibiotic Resistance and Pathogenic Signature in Marine and Freshwater Aquaculture Systems.”

Exploiting Nanoparticle-Enhanced Surface Plasmon Resonance Imaging for better diagnostics

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Keywords: diagnostics, non-invasive, biomarkers, nanoparticles, SPR Imaging

Introduction

Advanced and robust methods to detect diagnostic biomarkers (i.e. proteins, cell-free DNA, tumor DNAs, and microRNAs) circulating at low concentrations in body fluids, hold great promise for the development of liquid biopsy approaches. **[1]** In this direction, there is a current need to improve the overall performances of the Surface Plasmon Resonance Imaging (SPRI) biosensor, in terms of sensitivity and selectivity enhancement, by guarantying in the meanwhile, not-complicated assay procedures, and the minimization of fouling by body fluid sample components. **[2]**

Results and Discussion

Here, we will present strategies that can converge to assure high sensitivity and selectivity for the detection of the target of interest directly in a clinical sample.

A PEG-pentrimer carboxybetaine modified surface has been described to achieve, with an outstanding antifouling activity, the SPRI biosensing of human Arginase 1 in plasma, a biomarker over-expressed in cancer patients. **[3]** By implementing a sandwich assay based on the use of Peptide Nucleic Acid (PNA) as surface-immobilized probes and biofunctionalized gold nanoparticles (NPs), it was possible the non-invasive prenatal fetal sex determination by detecting a Y-chromosome specific sequence in cell-free fetal DNA from maternal plasma, even at the earliest gestational age. **[4]** The assay doesn't require PCR amplification of the DNA sequence, therefore costly and time-consuming procedures have been avoided, minimizing errors and risks for sample contaminations. The Nanoparticle-Enhanced SPRI assay may be further simplified, skipping the need to isolate DNA from plasma, and ~1 attomolar KRAS mutated DNA circulating in 40 μL of plasma from colon cancer patients, was revealed demonstrating the potential for analysing liquid biopsy samples with a simplified workflow. **[5]** Possibilities offered in the quantification of microRNAs as cancer biomarkers circulating in serum of glioma patients will be also discussed.

Conclusions

It is expected that the proposed innovative diagnostic SPRI tool offers an efficient and straightforward analysis with multiplexing capabilities and high selectivity, to detect biomarkers in such a non-invasive and repeatable way, with ultrasensitivity (down to fM range) avoiding sample manipulation that may produce misleading results, and expanding the potential of personalized medicine.

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Acknowledgements

We acknowledge support from the European Innovation Council (EIC) under Horizon Europe program, grant agreement No 101046217 project VERSILIB, and from Università degli Studi di Catania, STARTING GRANT 2020, project PATmiREC.

A Planar Polymer Microelectrode for *in vivo* Bioelectronic Nose

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Keywords: *in vivo* bioelectronic nose, microelectrode, olfactory signal, polymer electrode

Introduction

Taking full advantage of the mammalian sense of smell, *in vivo* bioelectronic nose has been developed for the detection and quantitative evaluation of odorants. Aiming at *in vivo* detection of spatial distributing mitral/tufted cells in the olfactory bulb, a design and fabrication method of planar polymer microelectrode was proposed.

Results and Discussion

The planer microelectrode array consisted of three layers as shown in **Fig.1a,b**. We explored the effect of the size of electrode sites on the impedance value of polymer microelectrodes. As the diameter of the electrode site increased, the impedance of the electrode tended to decrease. PEG was used to wrap the shanks of the electrode. Through chronic experiments, the effective signals could be stably recorded within two months with gold as electrode material (**Fig.1c,d**), which confirmed that the electrode had well reliability.

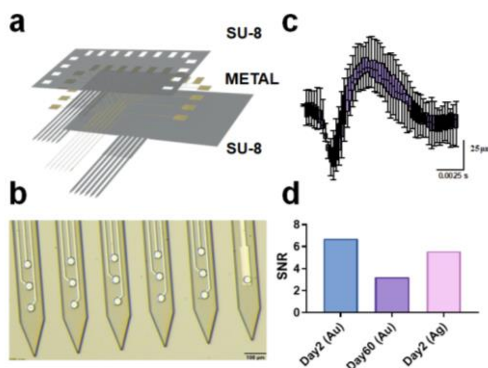

Figure 1: *Fabrication and testing of polymer-based planar microelectrode arrays*

Then an *in vivo* bioelectronic nose was constructed using the polymer microelectrode array for olfactory signals recording (**Fig.2a**) . The neural signal recorded by the planer electrode was shown in **Fig.2b**,which verified its odor detection ability. The output of in vivo bioelectronic nose was displayed by [1]a heatmap of the firing rate of neurons, which provided features for the spatiotemporal decoding of neurons (**Fig.2c**).

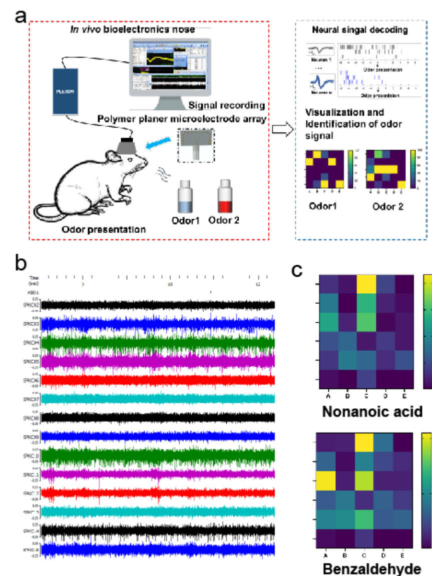

Figure 2: *Schematic diagram of in vivo bioelectronic nose and visual analysis of odor.*

Conclusions

A polymer-based planer microelectrode array was proposed in this study. It provided a novel tool for the acquisition of high spatiotemporal resolution neural signals of *in vivo* bioelectronic noses.

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Acknowledgements

This work was supported by the National Key Research and Development Program of China (No. 2021ZD0200405, 2021YFF1200803), National Natural Science Foundation of China (Nos. 62271443, 3225008)

Real-Time Interaction Cytometry reveals different binding kinetics of antibodies targeting membrane proteins on fixed versus living cells

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Keywords: biosensor, binding kinetics, antibodies, avidity, transmembrane receptor

Introduction

The development of therapeutic antibodies is a rapidly growing field since the approval of the first therapeutic monoclonal anti-CD3 antibody Muronomab in 1986 **[1]**. Many studies have shown that especially the dissociation rate of antibodies can be predictive for their clinical efficacy **[2,3]**. Thus, kinetic rate analyses are crucial to optimize the efficacy and safety of these therapeutic agents. Most common targets (PD-(L)1, CD3, HER2, etc.) are transmembrane proteins. Retaining them within their native cell membrane ensures physiologically relevant interaction kinetics with high *in vivo* predictability.

Results and Discussion

Here, we investigate the real-time binding kinetics of anti-CD3 and anti-PD-1 antibodies with their targets for the first time directly on T cells using Real-Time Interaction Cytometry (RT-IC). Single cells of an immortalized T cell line (Jurkat cells) are trapped on the surface of a biosensor, whereupon the association and dissociation of fluorescently labelled antibodies are measured in real time. We demonstrate the reproducible measurement of kinetic rates on cells and compare the binding behaviour of anti-CD3 antibodies to living *versus* fixed Jurkat cells. The interaction data shows different dissociation rates with a biphasic dissociation behaviour for fixed cells but a monophasic behaviour for living cells.

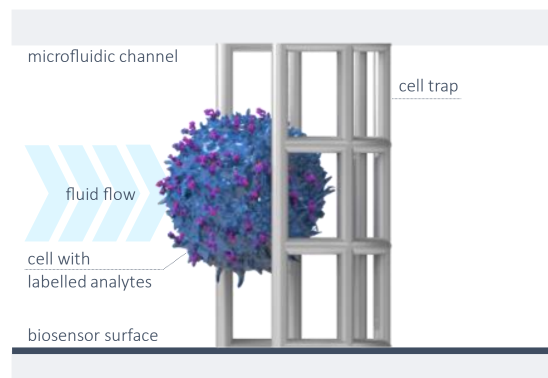

Figure 1: *The surface of the biosensor is equipped with flow-permeable cell traps to retain single cells. Fluorescently labelled analytes can be injected to measure their real-time association to targets on the cell membrane. Afterwards, a constant buffer flow induces the dissociation of the analyte from the cell surface.*

Conclusions

We presume that the differences in dissociation behaviour are caused by the preserved membrane fluidity in living cells in contrast to fixed cells. In live cells, the maintained receptor mobility within the membrane likely promotes bivalent interactions (avidity) by allowing the clustering of receptor molecules. These results demonstrate the importance of measuring binding kinetics directly on cell surfaces to predict the efficacy of therapeutic antibodies.

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Microbial Counting in a Multi-Chamber Microfluidic Device Combined with a Chemical Imaging Sensor

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Keywords: chemical imaging sensor, microfluidic device, microbial counting, *E. coli*

Introduction

We propose a new approach of microbial counting, in which (1) the suspension containing microorganisms under test is dispensed into a large number of tiny chambers for incubation; (2) if a chamber is loaded with at least one cell, the concentration of the metabolic product increases rapidly in the exponential growth phase in contrast to a chamber without cells, where the concentration remains the same; (3) the resulting concentration change in each chamber is compared with a threshold to judge if the chamber was initially loaded with at least one cell or not, and (4) the microbial concentration in the suspension is estimated by

$$c = \{\log(1 - n/N)\}/\{Nv\log(1 - 1/N)\},$$

where N is the total number of chambers, n is the number of chambers in which metabolic activity was detected, and v is the volume of a chamber.

A microfluidic device was developed, in which the above functionalities were implemented.

Results and Discussion

In this study, an array of 64 incubation chambers connected with a microfluidic channel for automatic dispensing [1] was constructed on the surface of a chemical imaging sensor [2, 3], as schematically shown in Fig.1. A pH change due to metabolism is detected as a change in the photocurrent.

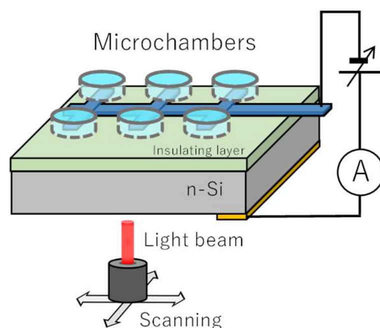

Figure 1: *Schematic view of the measurement system.*

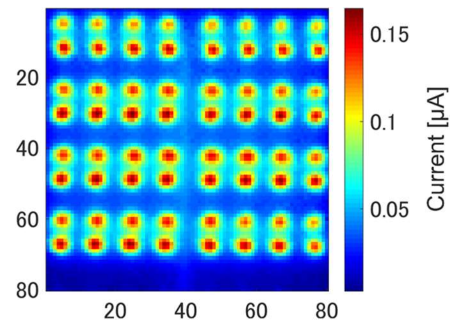

Figure 2: *Photocurrent image of microchambers. (The volume of each chamber was 0.1 μL.)*

Figure 2 shows a photocurrent image directly after dispensing *E. coli* suspension into all the chambers. It can be seen that the contents of the chambers are separated from each other, which guarantees independent incubation. The pH changes in all chambers are monitored in the course of subsequent incubation to detect and count the chambers loaded with cells.

Depending on the sensitivity and the noise level of the sensor, the threshold must be chosen appropriately to allow reliable discrimination between chambers with and without cells. A lower threshold enables microbial counting at a shorter incubation time.

Conclusions

A novel method and a microfluidic device for microbial counting based on a chemical imaging sensor were proposed. The results of preliminary experiments will be discussed in our presentation.

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Acknowledgements

Part of this work was supported by JSPS KAKENHI (Grant Number 22H02100).

A microfluidic system based on LAPS for real-time monitoring of organoid metabolism

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Keywords: organoid; metabolism; extracellular acidification; LAPS; microfluidic

Introduction

Organoids are 3D model proliferated and self-assembled from stem/progenitor cells, which exhibit similar structure and function with *in vivo* organs. The metabolic activities of organoids enable them to grow and maintain their own structures and respond to the external environment, so it is of great significance to detect organoid metabolism. Cell metabolism including glycolysis and respiration produce acid products, which can be reflected by extracellular acidification detection. Light-addressable potentiometric sensor (LAPS) is widely used in the field of biochemical detection due to its advantages of flexible detection area, high sensitivity and easy combination with microfluidic system [1,2].

Results and Discussion

Although LAPS is widely used in extracellular acidification detection of living cells, it is rarely used to investigate non-adherent targets directly in suspension. In this study, a microphysiometric system with a novel structure fabricated by Transwell insert and microfluidic LAPS chip was used to detect the pH change during the growth of organoids. Polydimethylsiloxane (PDMS) chamber was manufactured for organoid culture, and the microfluidic flow paths were designed for medium delivery. Transwell insert with 0.4 µm pore polycarbonate membrane was used innovatively to solve the problem that organoids are difficult to adhere to the surface of LAPS chip, which not only prevents organoids from being washed away, but also allows nutrients and metabolites to pass through. Mean-while, the existence of O-ring restricts the bacteria to the effective detection area and avoids contact between the Transwell and the LAPS chip. The pH change of the media during growth were determined and the results presented that this microphysiometric system performed well in real-time monitoring of organoid metabolism.

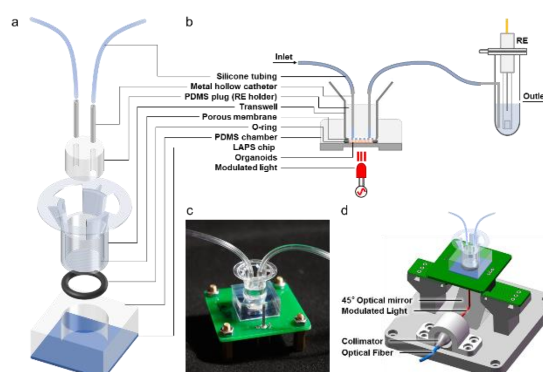

Figure 1: *Schematic of microphysiometer sensor unit with Transwell insert.*

Conclusions

In this work, a microphysiometer based on LAPS principle and microfluidic system combined with an innovative structure fabricated by Transwell insert was constructed for real-time and long-term monitoring of organoid metabolism. This system can be promoted as a model system for detecting metabolism of organoids and other non-adherent targets.

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Acknowledgements

This work was supported by the National Key Research and Development Program of China (2021YFF1200803, SQ2021AAA010032, and 2021YFB3200801), National Natural Science Foundation of China (No. 62120106004), Natural Science Foundation of Zhejiang Province (Nos. LBY21H180001, and LY21C100001), and Postdoctoral Research Foundation of China (Nos. 2020M671728, 2021M702859, and 2021T140605).

Nanomaterial-based high sensitive biosensors and systems for non-invasive disease screening in body fluids

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Keywords: biosensor, non-invasive diagnosis, biomarker detection, nanomaterials, body fluids

Introduction

Body fluids with non-invasive sampling such as urine and saliva contain a variety of biomarkers that can be used for disease screening and receive extensive interests. Recently, our group has special interests in urolithiasis and oral cancer screening by non-invasive biomarker detection considering their great significance. Urolithiasis is a prevalent urinary system disease affecting 10-15% of the world's population, with a high recurrence rate of 50% within 5 years of the initial episode and increased incidence worldwide. Oral cancer is the sixth most common type of cancer worldwide with a survival rate of less than 50% with five years. Conventional methods are based on imaging modalities such as computed tomography, requiring high cost, complicated operation and well-trained personnel. Moreover, the screening and recurrence in the early stage can be hardly achieved. Biomarkers in body fluids can provide effective information to indicate the occurrence and progression of diseases. In our studies, oxalic acid (OA) and citric acid (CA) in urine and Cyfra21-1 in saliva were used as the biomarkers for, respectively. To achieve trace and point-of-care detection of biomarkers, nanomaterial-based biosensors and systems were developed for biomarker detection in real body fluid samples.

Results and Discussion

Sodium dodecylsulfate (SDS) modified silver nanoparticles (AgNPs) were synthesized as the sensing probe for colorimetric and quantitative detection of citric acid by tuning the aggregation of SDS-AgNPs [1]. A self-developed portable CD-spectrometer was established for detecting CA featuring high simplicity, low time cost and good portability. Moreover, a colorimetric lateral flow strip was developed based on enzyme catalysis, and a color bar strategy was utilized for equipment-free and semi-quantitative determination of urinary OA by directly counting the number of color bars that is positively correlated with oxalate concentration [2]. To achieve more accurate screening, simultaneous detection of citric acid and oxalic acid was realized based on dual spectrum and biomimetic peroxidase for urolithiasis screening with fully automatic urine analyser (Fig. 1). Moreover, to achieve oral cancer screening, a multifunctional AuNPs@HRP@FeMOF immune scaffold and a fully automated saliva analyzer were developed and validated for Cyfra21-1 detection [3].

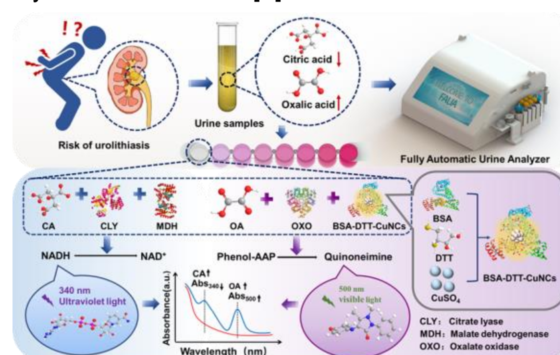

Figure 1: *Simultaneous detection of OA and CA for urolithiasis screening*

Conclusions

Different biosensors and systems were developed and validated for urolithiasis and oral cancer screening. These results provide a promising sensing strategy and platform for biomarker detection and disease screening.

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pH value and buffer capacity measurements from saliva samples: A new tool to denstist diagnosis

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Keywords: saliva, pH, buffer capacity, capacitive EIS sensor, SU-8 photoresist.

Introduction

Healthy saliva pH levels are between pH 6.7 and pH 7.4. Any deviation from this range, e.g., due to acid reflux, oral pathologies, or microorganisms, could cause oral health issues, such as dental caries (tooth decay), periodontal disease, or enamel thinning [1]. Thus, to measure the saliva pH value and the buffer capacity is crucial for timely intervention. In this work, we fabricated a miniaturized multiwell EIS sensor to measure pH value and buffer capacity of saliva samples. An electrolyte insulator semiconductor (EIS) sensor consists of several layers; tantalum pentoxide (Ta_2O_5) as a transducer, silicon dioxide (SiO_2) as an insulator, a p-doped semiconductor silicon layer, and an aluminum rear-side contact [2]. Wells (as shown in Figure 1) were photolithographically patterned on the sensor surface with a negative photoresist (SU-8 2150). Profilometer and contact angle measurements were performed to determine the thickness and hydrophobicity of the obtained photoresist layer.

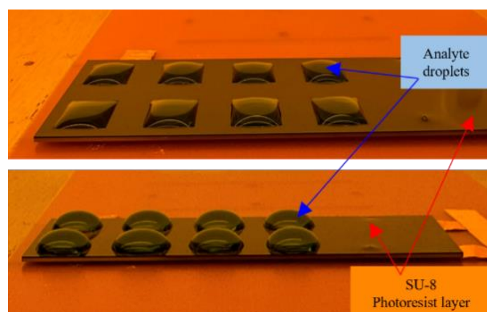

Figure 1: Two different volumes of analyte droplets inside the sensor's wells (size 8x8mm^2).

In order to individually contact the droplets in the wells with a reference electrode, a micro-agar-salt bridge was designed using a pipette tip filled with 3M KCl solution and a conventional glass-based reference electrode. Different samples, in the range of microliters, were added to the wells and measurements were carried out with an impedance analyzer to obtain the sensor signal, e.g., capacitance-voltage curves.

Results and Discussion

As shown in Figure 1, the droplets are limited to the wells, due to the difference in wettability between the transducer and the SU-8 layer. HCl solution was titrated onto the saliva samples, while the pH changes were recorded. Using the amount of acid added and the change in the pH value, the buffer capacity of the saliva was calculated.

Conclusions

This work presents the fabrication of a miniaturized multiwell pH sensor, that can quantitatively measure the pH value and the buffer capacity from small volumes (around 100 µl) of saliva.

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The authors would like to thank Heiko Iken for the help during the fabrication of the sensor.

Electrolyte-gated tellurium nanowire transistor devices towards biosensor applications

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Keywords: tellurium nanowires, electrolyte-gated transistor, biosensor, electrical characterization

Introduction

Nanowire devices exhibit superior sensitivity compared to macro-sized sensor devices, due to their enormous surface-to-volume ratio. The material tellur (Te) is a semi-metal, whereas crystalline tellurium (cTe) is an intrinsic, direct, p-type semiconductor material with a bandgap of 0.334 eV. Most of the time Te, is used in alloys or metal oxides for thermoelements, as phase change materials in modern storage devices and in waveguides. cTe nanowires recently attract attention in the field of sensors due to its facile preparation and excellent optoelectronic and electronic properties that stem from a special chiral crystalline structure [1-3]. Therefore, it is a promising candidate for sensors, optoelectronics and integrated circuits. In this work, we aim to develop a biochemical sensors based on cTe nanowires and characterize their performance in a liquid-gate configuration, which is to our knowledge one of the first studies about this topic.

Results and Discussion

Tellurium nanowires were prepared by a hydrothermal synthesis protocol as described before [1-2]. A series of characterization techniques including electron microscopy, Raman spectroscopy and X-ray diffraction were utilized to verify the presence and crystallinity of the cTe nanowires. Interdigital microelectrodes (IDEs) were fabricated by Ti/Au evaporation on Si/SO_2 wafers using photolithography and a lift-off technique. A parylene C layer was deposited to passivate the metal contact lines of the devices. In addition, a backgate contact was realized as well for thorough characterization of the devices. After that, cTe nanowires were spin-coated on IDEs followed by annealing at 80°C for 50 min.

Electrical characterizations indicated a good Ohmic contact between Te and Au. In subsequent stability tests, the cTe devices showed good stability after one month of storage in air. These results reveal that cTe nanowires have a promising potential to be applied in electronic devices. To perform electrolyte gating experiments, an Ag/AgCl reference electrode in contact with the buffer solution was used for the front-gated cTe nanowire transistors. Figure 1 exemplarily shows a p-type transistor behaviour. In the further step, cTe nanowire transistors will be used as chemical and biosensors for the detection of pH, proteins, DNA, etc.

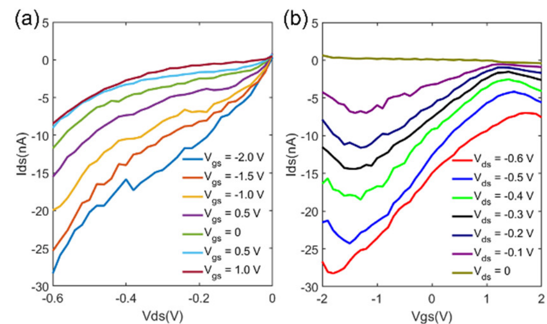

Figure 1: *The output (a) and transfer (b) characteristic curves of cTe nanowires electrolyte-gated transistors.*

Conclusions

Solution-synthesized cTe nanowires were successfully prepared and transferred on IDEs. Electrical characterizations exhibited good Ohmic contacts to Au and favourable stability in air after one month of storage. Additionally, p-type transistor behaviour was observed with liquid-gate modulation. In the future, we will develop cTe nanowire devices towards chemical and biological sensor applications.

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Heping Cui acknowledges the China Scholarship Council (Grant No. 202006050050) for her research fellowship. Financial support for this project came from RWTH Aachen University.

A biomimetic cardiomyocyte-based biosensor for the detection of medium chain fatty acids

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Keywords: cell-based biosensor, cardiomyocyte, fatty acid, microelectrode array, OR51E1

Introduction

Biomimetic cell-based biosensors have been developed for applications in many fields such as food [1], drug screening [2] and environment safety [3]. In this work, the biomimetic biosensor based on self-constructed integrated microelectrode array (MEA) system (Figure 1) was proposed for the detection of electrophysiological changes of cardiomyocytes culture *in vitro* induced by different medium-chain fatty acid (MCFA) stimulus for the first time.

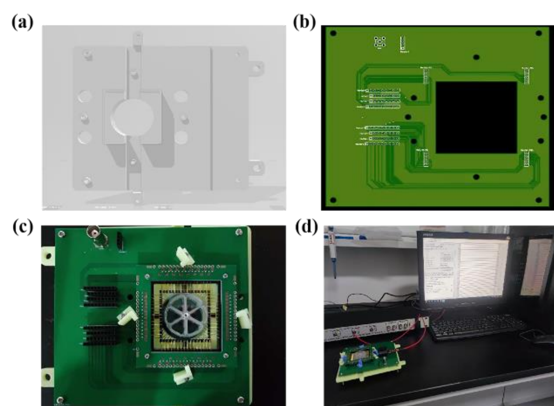

Figure 1: *The construction of multi-electrode arrays system.*

Results and Discussion

The extracted primary cardiomyocytes endogenously expressing the OR51E1 olfactory receptor were cultured on the MEA surface and formed syncytium for potential conduction and mechanical beating. The *Immunofluorescence staining for α-actinin and OR51E1* obviously demonstrated the extracted cardiomyocyte has highly-purity more than 99% and endogenously express OR51E1 as showed in (Figure 2). Then, nonanoic acid, orthogonic acid and methyl nonanoic acid, with different concentrations were added into the cardiomyocytes cultural chip and the real-time responsive signals were recorded with MEA system (Figure 3).

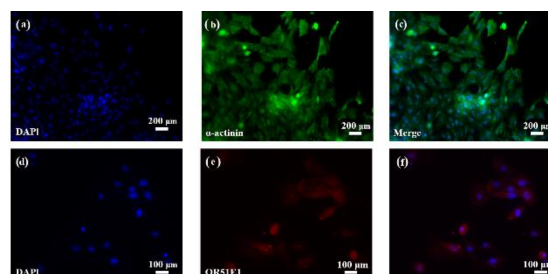

Figure 2: *Immunofluorescence staining for DAPI (blue) (a,d), α-actinin (green), OR51E1 (red) (e) in cardiomyocytes and the merged image (c,f).*

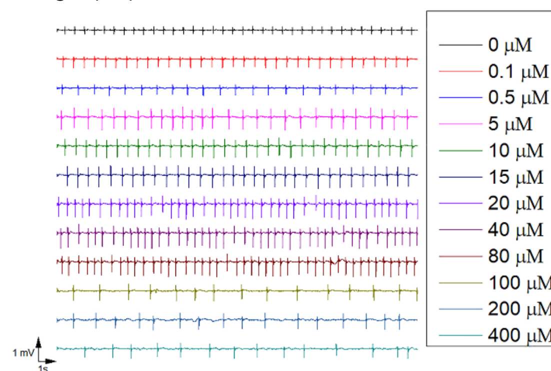

Figure 3: *Detection of nonanoic acid with different concentrations ranging from 0.1 μM to 400 μM.*

Conclusions

The cardiomyocyte-based biosensor was successfully developed and showed good detection performance towards MCFAs, which have regulating effect on the electrophysiological of cardiomyocytes.

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Acknowledgements

This work was funded by the National Natural Science Foundation of China (Grant No. 32271427, and 32071370).

3D multifunctional micro-trap biosensor base on GelMA microcarriers for anti-cancer drug assessment

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Keywords: 3D cell, Micro-trap, Microcarriers, Cell viability and electrophysiology, Drug assessment

Introduction

3D cell model featured more accurately response to drug effects due to the close resemblance of intercellular and extracellular matrix complexities in vivo. Microcarriers (MCs) were originally intended for producing large cell quantities in bioreactor culture systems due to the sufficient surface area for cells to attach and proliferate in tissue engineering and cell therapies[1]. In addition to its superiority of larger capacity, it can be served as the scaffold to minimize the limitations of 2D cell culture for 3D cell model construction. In the field of drug screening, real-time monitoring of 3D cell state in a label-free and non-invasive manner is most crucial process[2]. However, conventional sensors in bioreactors are unable to monitor cell viability and electrophysiological activity in real-time.

Results and Discussion

In this work, a novel 3D multifunctional micro-trap biosensor based on GelMA microcarriers was successfully developed for non-invasive and label-free monitoring of 3D cell viability, electrophysiological activities and cell beating. Then comprehensively evaluating the efficacy and cardiotoxicity of anticancer drugs (Fig1. A and C). A etched 300 um square hole[3] under the angle of 54.7 ° and the depth of 100 μm embedded with two pair of plate electrodes were fabricated on the sidewalls for the 3D impedimetric quantification and extracellular field potential signal recording (Fig. 1B and D). Through finite element analysis and experimental validation, the optimal excitation frequency for microcarriers model impedance measurement was located in the range of 50 KHz~100 KHz. A549 cells and cardiomyocytes of neonatal rats were cultured on this GelMA microcarriers, then be captured by this so-called “trap” to realize the coupling of 3D cell model with the sensor electrodes (Fig1. E). Anti-cancer and cardiovascular drugs were utilized to verify the performance of this platform. Electrochemical cell impedance spectroscopy and extracellular field potential were measured under our biosensor (Fig.1 F and G). The experimental results preliminarily verified the function of the biosensor chips.

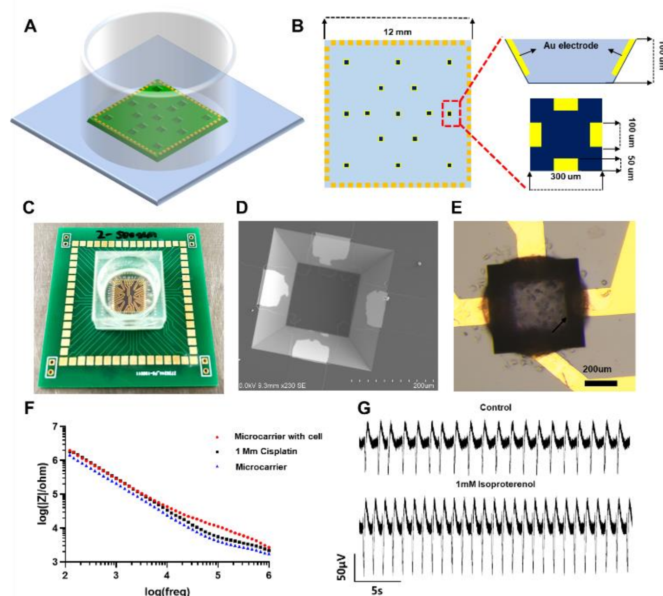

Figure 1. (A) Schematic diagram of multifunctional micro-trap biosensor chip. (B) Schematic diagram of chip and micro-trap structure dimensions. (C) The optical microscopy view of the biosensor chip. (D) Scanning electron microscopy (SEM) results of micro-trap structure. (E) The optical microscopy view of 3D cell model in the micro-trap structure based on GelMA microcarriers. (F) Impedance spectrum data measured by the biosensor. (G) Extracellular field potential data measured by biosensor

Conclusions

Owing to its simultaneously monitoring 3D cell viability and electrophysiological activity of cells in a real-time and dynamic way, the biosensors will be a utility platform to evaluate the efficacy and cardiotoxicity of candidate compounds in new drug development.

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Multi-microarray graphene field-effect transistor biosensor for rapid and ultrasensitive detection of miR208a in human serum

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Keywords: Multi-microarrays GFET, POCT, ultrasensitive biosensor, miRNA, portable GFET source meter

Introduction

Acute myocardial infarction (AMI) remains the world's leading cause of morbidity and mortality.[1] MiroRNA-208a (miR208a) is a cardiac-specific miRNA. Increased serum miR208a is associated with a high risk of AMI and can provide an earlier prediction for AMI onset than currently used cardiac biomarkers.[2] Establishing a POCT platform for rapid and ultrasensitive detection of miR208a is expected to effectively prevent premature death of AMI. As a promising candidate for next-generation biosensors, graphene (GR) field-effect transistor (GFET) biosensor has been demonstrated for real-time detection of a variety of bioactive molecules with ultrasensitivity from nM to fM level.[3] However, the rapid quantitation of DNA–miR208a hybridization is rarely achieved due to controllability and reproducibility across different devices and fabrication batches.

Results and Discussion

In this work, we developed a liquid-gated biosensor based on multi-microarray GFET for the sensitive detection of miR208a. As shown in Figure 1a, the single-stranded DNA (ssDNA) was immobilized onto the surface of GFET through a covalent linker PBASE. When DNA–miR208a hybridization occurs, the local Fermi level of GR is affected owing to the field effect caused by the introduced charges of targets, thus resulting in the shift of transfer characteristic curves. As GR possesses the rapid mobility and low noise of carrier transportation, the GFET biosensor has a promising to achieve ultrahigh sensitivity and very low detection limits. A portable digital source meter integrating an intelligent reading system was then designed to realize the rapid signal readout of GFET (Figure 1b). The portable device provides output voltages to the GFET and collects the drain-source current of the sensor with fast response and high resolution. Compared to a traditional source meter, it features the advantages of low cost, small size, simple operation, and highly integrated, which can rapidly and sensitively identify the signal generated by hybridization. Figure 1c-e is the optical microscopy views of 16-channel GFET chips with three GR sizes, which aims at optimizing highly sensitive GFET biosensor because the ratio of width to length (W/L) of GR manipulates the sensitivity of biosensing. In terms of the designs of 16 microarrays with common source on GFET chip, it can greatly increase the current response and reduce the batches variation across different chips

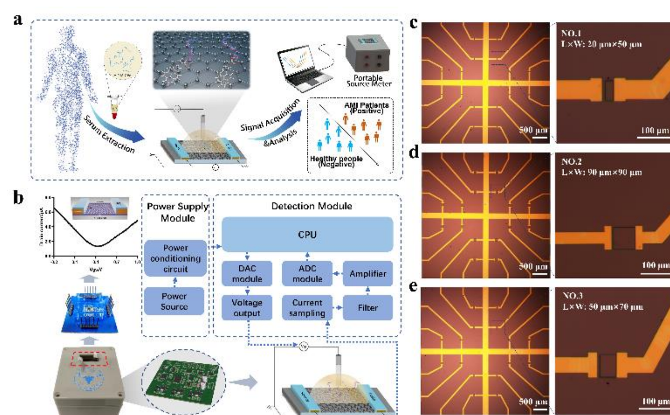

Figure 1. (a) Schematic diagram of multi-microarray GFET sensor for the detection of miR208a. (b) Design of portable digital source meter for acquiring drain-source current of GFET. (c-e) The optical microscopy view of 16-channel GFET chip and the optimization of GR sizes for developing high-performance GFET; No.1: 20 μm × 50 μm, No.2: 90 μm × 90 μm, No.3: 50 μm × 70 μm.

via parallel connection, which is advantageous to achieving good reproducibility in detecting miR208a.

Conclusions

Herein we optimized a high-performance multi-microarray GFET biosensor and designed a portable digital source meter to adapt with it for a fast readout of the electronic response readout. Our work provides a reliable strategy to rapidly and ultrasensitively quantify miR208a for screening AMI patients at high risk, which also holds great potential to be extended in a wide application of detecting other disease markers.

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Catheter-based sensors for the intestinal detection of molecular biomarkers in the context of functional bowel disorders

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Keywords: electro-polymerization, molecular imprinted polymers, impedance spectroscopy

Introduction

In this project, a catheter-based sensor is developed, which will be used to detect histamine (His) and serotonin (5-HT) in the duodenum of IBS patients. His occurs in allergies and is involved in chronical inflammations. 5-HT is a neurotransmitter, and its level in the blood is known to increase in blood in the case of certain IBS patients [1]. The sensor used in the catheter will have three pairs of gold microwires as electrodes. Two electrode pairs are functionalized to detect His and 5-HT, respectively. These electrodes are coated by molecular imprinted polymers (MIPs). To correct for false positive effects by electrode fouling, we will use a third electrode couple that is coated with non-imprinted polymer (NIP). Both MIPs and NIPs are synthesized by the electro-polymerization process. After the electro-polymerization, template molecules, which are trapped in the MIP layers, are removed by acid-base washing. The MIPs then result in a layer of cavities in which the His or 5-HT molecules can rebind through hydrogen bonding and π-π stacking (Figure 1a). Therefore, the impedance amplitude of the electrode pairs changes, that is corresponding to the concentration of the target molecules.

Results and Discussion

In preparatory work, an electro-polymerization process on titanium (Ti) wires resulted in well-functioning MIPs and NIPs to determine the His concentration *in vitro* (Figure 1b). The impedance of the MIP increased by 60% over a concentration range of 10 nM – 1 μM His in bowel fluid, while the NIP only increased by about 10% over the same concentration range [1]. Also, the results show a good selectivity of the MIP layer for His in comparison with the structurally similar molecule histidine [1].

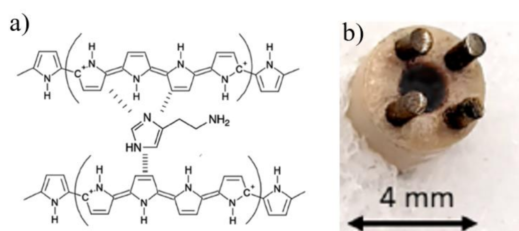

Figure 1: *a) His H-bonding and π-π stacking with a polypyrrole (PPy) MIP. b) PPy layer on Ti wires representing the MIP and NIP. [1]*

In the present project, we start with the *in vivo* measurements in healthy volunteers and in patients with functional bowel disease. The catheter will be inserted through the nose, therefore, miniaturization of the catheter to an outer diameter of 4 mm is necessary. Furthermore, an extra electrode pair is needed such that the catheter can detect not only His, but also 5-HT. We will use gold microwires as electrodes and are finalizing the process to polymerize MIPs on these wires using electro-polymerization. Polypyrrole can already serve for His [1], for 5-HT a new synthesis protocol is being developed, based on polymer blends.

Conclusions

Functional MIP layers were already developed for Ti wires. The same principle will be adapted to gold microwire electrodes to develop sensors that will be used in the nasopharyngeal catheter. The catheter has new dimensions and will undergo tolerability tests before being used on patient.

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Acknowledgements

Financial support by Research Foundation Flanders FWO (Project G0A6821N) is greatly acknowledged.

Deposition methods of biodegradable polymer as a future inflammation sensor for the cochlear implant

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Keywords: Molecularly imprinted polymer, deposition methods, corrosion, cochlear implant

Introduction

Patients might be suffering post-surgical inflammation (e.g. interleukin 6 or interleukin 21) after cochlear implant (CI) surgery. Inflammation can cause damage to the ear and leads to encapsulation of the CI by tissue, which impairs the CI stimulation [1]. While the inflammation can be treated by medication, the recognition can be too late. In the previous work from Wagner et. al. molecularly imprinted polymer (MIP) was deposited on the electrode acting as a sensor for histamine detection [2]. Such MIP sensors on a CI electrode are suitable for a duly detection of inflammation, where interleukin 6 can serve as an inflammatory marker [3]. Since the long-term use of CI electrodes is stimulation, biodegradability of the MIP layer is required. Moreover, the MIPs could be conductive for further sensitivity improvement. This work considers the deposition different polymers, copolymers, as well as nanoMIPs, with different properties in terms of biodegradability and conductivity. The deposition of the biodegradable polymer chitosan and the conductive polymer Poly(3,4-ethylenedioxythiophene) (PEDOT) by dip-coating and electrochemical polymerization were further investigated. Since interleukin and its epitopes are expensive, ibuprofen was used first as a template.

Methods and Results

In the dip-coating process, chitosan and ibuprofen were dissolved in an acetic acid-ethanol solution. A platinum electrode was first immersed in the coating solution and thereafter in a sodium hydroxide solution to precipitate the chitosan. PEDOT was deposited via cyclic voltammetry onto a platinum electrode. The MIPs were then washed in ethanol and an impedance measurement was realized to detect ibuprofen. Afterwards the PEDOT was corroded in phosphate-buffered saline (PBS) by additional impedance measurements, as the polymer is not biodegradable. Finally, the PBS solution was measured with a bio-drop spectrometer. With electrochemical polymerization the deposition a homogeneous layer with adjustable thickness could be achieved, but not with dip-coating. In ibuprofen detection, a small impedance change was measured using MIPs. During the corrosion of PEDOT an impedance shift could be observed (Figure 1). In the PBS solution, the corroded monomers and polymer molecules were detected.

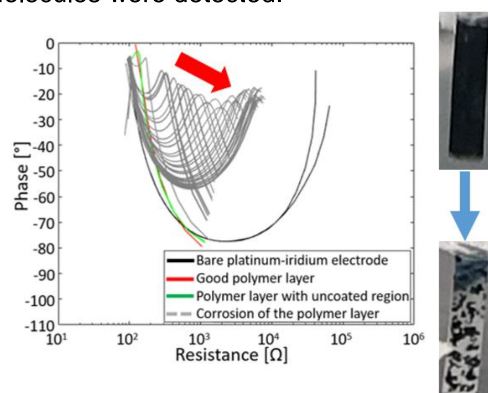

Figure 1: *Nyquist diagram (left) and polymer layer before and after corrosion (right)*

Discussion and Conclusions

The reason for the impedance change is due to the low solubility of ibuprofen in the deposition solution, resulting in small cavities in the MIPs. For a better distinctness in detection of ibuprofen, the production processes have to be optimized. The polymer corrosion needs further investigation to only release monomers, e.g. by lowering the voltage.

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Acknowledgements

This study is funded by the "Cluster of Excellence Hearing4All" (EXC2077)

Poly-3-thienylboronic acid: a chemosensitive derivative of polythiophene

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Keywords: chemosensitive conducting polymer, poly-3-thienylboronic acid, spectroelectrochemistry

Introduction

Extraordinary properties of conducting polymers (CP) [1] resulted in wide applications in different fields of science and industry. Among these CP, the polymers with polythiophene backbone belong to the most explored ones [2]. An application of polythiophene in selective chemical sensors requires the incorporation of a specific receptor. One of the most used artificial receptors is the boronic acid moiety. The perspective to use boronic acid as the receptor for diol-containing compounds was the motivation to incorporate this group into various conducting polymers.

Results and Discussion

In this work, we describe the structural, electrochemical, and spectroelectrochemical properties of films of poly-3-thienylboronic acid and demonstrate its applicability for the chemosensing of sugars. Electrochemical synthesis in the mixture of boron trifluoride diethyl etherate (BFEE) and acetonitrile allows to get a highly flexible polymer film with a thickness of ~100 nm (Fig. 1). The cyclic voltammetric curves during electropolymerization display typical behavior for the deposition of highly conducting materials.

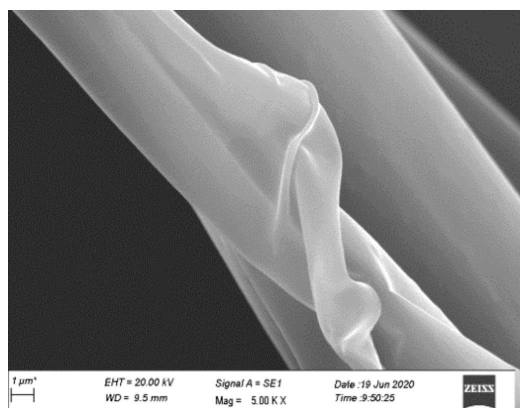

Figure 1: Scanning electron microscopy of poly-3-thienylboronic acid films polymerized on ITO from the BFEE / acetonitrile mixture (v/v): detached polymer film.

The polymer films deposited on ITO were used for optical spectroscopy and spectroelectrochemical measurements.

In acetonitrile, the spectroelectrochemistry of polythiophene and poly-3-thienylboronic acid is more similar. Both polymers show a strong negative solvatochromic effect, which is much larger for polythiophene.

The incorporation of boronic acid moiety into the polythiophene backbone leads to the small hypsochromic shift in the main absorption band, which is increasing with the polymer oxidation.

The electrochemical and spectral properties of poly-3-thienylboronic acid are close to polythiophene, but the incorporated boronic acid moiety provides its chemical sensitivity to diol-containing compounds.

The binding of sorbitol at fixed electrode potential leads to an increase in the absorbance in the short wave band and to a decrease in the long wave band, the effect depends on the electrode potential and pH.

Conclusions

A new chemosensitive material – poly-3-thienylboronic acid can be prepared by electrochemical synthesis in-situ, therefore it is well compatible with methods of surface design and preparation of sensor arrays or materials libraries based on electrically addressed polymerization. The ability to form stable films allows one to consider applications in support-free conditions, for example for the formation of chemosensitive electromechanical actuators.

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Benzoquinone-functionalized gold electrode for sensing applications

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Keywords: self-assembled monolayer, benzoquinone, electrocatalytic chemosensor, hydrogen peroxide

Introduction

Electrocatalytic chemical sensors are applied intensively for direct detection of various chemical species or as transducers in biosensors [1]. One of the mostly important chemical sensors in which the electrocatalytic effect is exploit is the sensor for hydrogen peroxide.

Results and Discussion

A novel electrocatalytical amperometric sensor for detection of H_2O_2 is reported. The sensor consists of a gold electrode covered by self-assembled monolayer (SAM) with immobilized *p*-benzoquinone. To provide highly stable immobilization of *p*-benzoquinone at the distance of effective electron tunneling, a new anchor compound - 1,3-dimercaptopropan-2-ol – was synthesized and used for the preparation of the SAM. Due to two thiol groups binding gold surface this compound provides a high stability of the SAM. The surface concentration of *p*-benzoquinone obtained from cyclic voltammetry is $2.5 \pm 0.2 \times 10^{-10}$ mol.cm^{-2} [2].
Cyclic voltammetry and chronoamperometry experiments proved that the immobilized benzoquinone exhibited high electrocatalytic activity towards the decomposition of H_2O_2. Depending on the used potential range, different sensing modes can be realized. For example, one can measure electrochemical response due to the oxidation of H_2O_2 at anodic potentials or due to the reduction of oxygen formed during oxidative decomposition of H_2O_2. Also amperometric response at fixed potential of +0.4 V vs. Ag/AgCl corresponding to the oxidation of benzoquinone to hydroquinone was studied. The sensor exhibited a linear response over a concentration range of 0.1-2 mM with a low detection limit of 4.24 µM. The reproducibility of three different electrodes prepared was examined at the H_2O_2 concentration range from 0.1 till 3 mM, which resulted in a relative standard deviation below 4.2% [3].

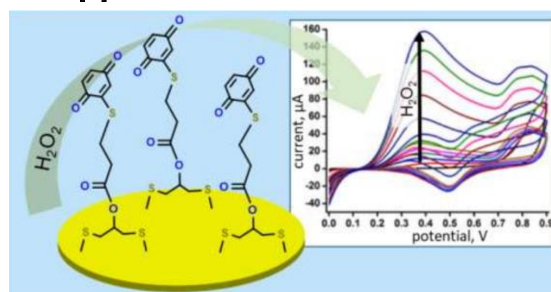

Figure 1: Au electrode modified benzoquinone terminated monomolecular layer and its CV response during successive additions of H_2O_2 to the media.

Conclusions

The fast and selective determination of hydrogen peroxide (H_2O_2) is of importance not only because of strong interest to this widely applied analyte but also because of the development of enzymatic biosensors for glucose or other metabolites where the sensor for H_2O_2 can be used as the transducer

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Acknowledgements

The work was supported by the German-Tunisian BMBF/DGRS funded project entitled «Organic redox responsive-based transducing systems for design of biosensing platforms».

Carbon Nanotube Biosensors Based on Covalent DNA-Anchors

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Keywords: Nucleic Acids, Aptamers, Biosensors, Carbon Nanotubes, Near-Infrared Fluorescence.

Introduction

Viral and microbiological infections are a major cause of death that burdens the infrastructure of health systems. To contain the spread of pathogens and assess available treatment options, a number of diagnostic methods have been developed. However, the development of diagnostic tests is typically time consuming and, in the light of current pandemics, the need for adaptable concepts has become apparent.

For sensor applications that require a minimally invasive read out, optical systems offer a rapid detection of biomolecules with a high spatial and temporal resolution. Due to the reduced autofluorescence, absorption and scattering, the near-infrared (NIR, 870 – 2400 nm) tissue transparency window offers an improved signal-to-noise ratio.[1] Biosensors that fluoresce in this region are therefore highly desirable. In this context, single-walled carbon nanotubes (SWCNTs) have emerged as versatile NIR fluorescent building blocks.[2] Most often, they are modified non-covalently. However, non-covalent chemistry has several limitations and prevents a consistent way to molecular recognition and reliable signal transduction.

Results and Discussion

We introduce a widely applicable covalent approach to create molecular sensors without impairing the fluorescence in the NIR (> 1000 nm).[3] To this end, we attach single-stranded DNA via guanine quantum defects as anchors to the SWCNT surface. A connected sequence without guanines acts as flexible capture probe allowing hybridization with complementary nucleic acids. Hybridization modulates the SWCNT fluorescence and the magnitude increases with the length of the capture sequence (20 > 10 >> 6 bases). Incorporation of additional recognition units via this sequence enables a generic route to NIR fluorescent biosensors with improved stability. To demonstrate the potential, we design sensors for bacterial siderophores and showcase sub-nanomolar sensing of the SARS CoV-2 spike protein.

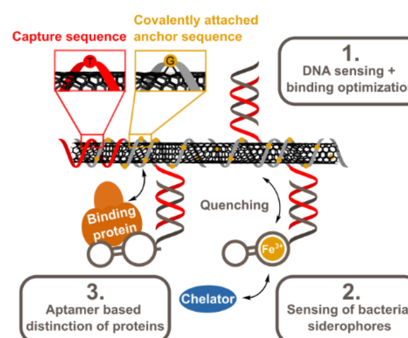

Figure 1. Covalent DNA anchors connected to capture sequences allow a rational design of near infrared fluorescent carbon nanotube-based biosensors. Guanine bases in the anchor sequence are pinned to the SWCNT as quantum defects. This approach enables DNA sensing, improves colloidal stability, and allows signal transduction.

Conclusions

We introduce covalent guanine quantum defect chemistry as a novel rational design concept for biosensors. Due to the enhanced selectivity and stability of these sensors, as well as the modular approach we anticipate that this technology can adapted in a time and cost-effective way. We envision broad usage in in fundamental research as well as next-generation biomedical applications.

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This work is funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy—EXC 2033–390677874—RESOLV. We are grateful for the support of the VW foundation. This work was supported by the Fraunhofer Internal Programs under Grant No. Attract 038–610097.

Automated manufacturing of integrated microfluidic devices for biosensing applications

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Keywords: integration, mass-manufacturing, microfluidics, biosensors

Introduction

There is an increasing need for automated mass-manufacturing of integrated microfluidics for biosensing applications. Various mass-manufacturing methods are available for discrete components and fluidic platform but often the integration of functional microfluidic system involves manual assembly steps. For example, the assembly of flexible and rigid parts on the microfluidics and the final sample singulation in a roll-to-roll (R2R) process are mostly done manually.
Here we report automated methods for microfluidics manufacturing and hybrid integration utilising 1) a conversion line with a robot system and 2) an integration line with a pick-and-place machine.

Results and Discussion

R2R conversion line with integrated robot system (VTT) was used to manufacture devices designed for viral pathogen detection. Device comprises a flexible multi-layer microfluidic and a rigid part, providing fluidic connections and mechanical support. Microfluidic channels were fabricated in a tape using a CO_2 laser integrated to the line followed by bonding of the processed tape layer with a plastic foil to create multiple layers of microfluidics. Integration of the rigid and flexible parts and device singulation from the roll were performed with a machine-vision guided robot system. Fig 1A) shows the robot placing the finalized samples on a tray.

Conversion line was also utilised in EU funded project MedPhab to manufacture and integrate a device for high-throughput drug screening. Device (Lucero Bio) comprises a microfluidic middle, well-plate top and glass bottom layers. UV-imprinted microfluidic structures in a roll format (Joanneum Research) were integrated with glass slides and well-plates. The glass slides were prepared for UV-curing based bonding (Joanneum Research). Fig. 1B) shows the robot picking up the singulated microfluidics and the integrated well plate from the line.

Integration line (VTT) was used to manufacture a flexible immunosensor (Fig. 1C). Microfluidic channels were R2R hot embossed on polymer film (COC). Plasma separation membrane for blood filtration and blister for sample actuation were integrated with a pick-and-place machine. Sensor was demonstrated to detect 2 µg/ml of C-reactive protein in the whole blood by using fluorescence based immunodetection. [1]

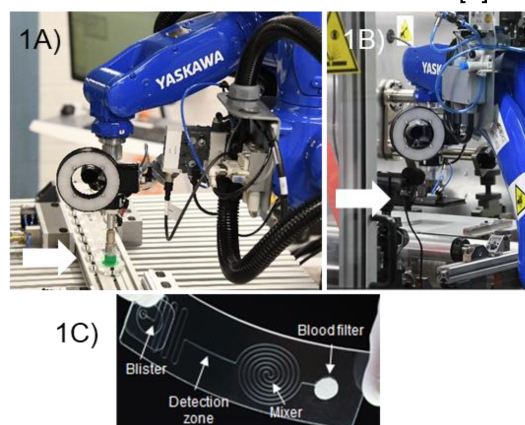

Figure 1: *Robot 1A) placing the viral pathogen detection devices on a tray and 1B) picking up drug-screening device 1C) Sample handling functionalities in an integrated immunosensor.*

Conclusions

We have reported here the use of automated manufacturing methods in the realisation of three types of sensors paving way towards mass-manufacturing and industrialization.

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Financial support acknowledged by the European Commission under the grant agreement No. 871345 “MedPhab” and European Regional Development Fund with the project “Developing production methods for rapid diagnostics and sample handling” and “CoronaRpTest”.

Influence of the Corona on the Redox Activity of Silver Nanoparticles in Stochastic Collision Electrochemistry

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Keywords: stochastic collision electrochemistry, silver nanoparticles, self-assembled monolayer, nanoparticle impacts

Introduction

Nanoparticle impact electrochemistry has recently gained attention in the field of single-entity sensing. Here, discrete signals stemming from nanoparticle collisions with a biased microelectrode are investigated. In particular, using impacts from functionalized silver nanoparticles has been suggested for new biosensor developments. Consequently, there is a need for profound understanding of the electrode–nanoparticle interaction, where the tailored particle corona plays a crucial role. **[1-3]**

Results and Discussion

In our recent study, we investigated the influence of the length of ligands in the nanoparticle corona, as well as the presence of carboxyl and hydroxyl functional groups on the redox activity of silver nanoparticles.

In doing so, we modified silver nanoparticles of two different sizes (20 nm and 40 nm in diameter) with n-alkanethiolates of various chain lengths, n ϵ {3,6,8,11}, and end groups (-COOH / -OH). Differently functionalized nanoparticles were then used for stochastic collision experiments.

We found that the length of the ligands has a significant effect on the redox activity of silver nanoparticles. Specifically, we observed that with longer ligands, the redox activity of the nanoparticles decreases.

We also found that the presence of carboxylic and hydroxyl groups as end groups on the ligands can influence the redox activity of silver nanoparticles. Specifically, we observed that the redox activity is higher when the ligands have carboxylic end groups compared to hydroxyl end groups. We speculate that this observation is caused by different electrostatic interactions or steric effects.

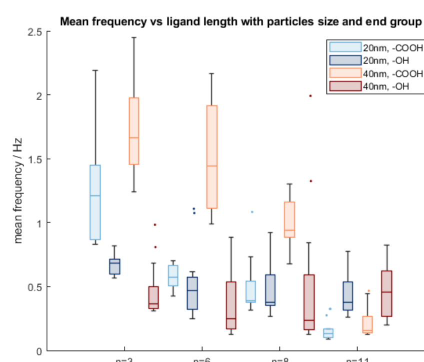

Figure 1: *Mean impact frequency of 20 nm and 40nm particles functionalized with different ligand length and end groups; oxidizing potential of 800 mV*

Conclusions

Overall, our study provides new insights into the complex relationship between the surface properties of silver nanoparticles and their redox activity, including the effects of ligand length and the presence of carboxyl and hydroxyl groups. These findings could have important implications for the design and optimization of silver nanoparticles for various applications, from catalysis to biosensors.

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Acknowledgements

We acknowledge funding from the DFG and funding from BMBF within the Indo-German Science & Technology Centre (IGSTC).

Real time in-situ monitoring of in-flow electroporation using impedance cytometry

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Keywords: Intra-cellular delivery, Impedance Cytometry, Electroporation

Introduction

Electroporation has become a widely adopted method for the intracellular delivery of exogeneous cargo. Its integration with microfluidics and further miniaturization has further consolidated its usefulness, offering more control, efficiency, and viability by performing electroporation on individual cells [1].

The characteristics of the applied electric pulse define the pore formation and are therefore of critical importance for the efficient delivery of cargo while avoiding negative side-effects (e.g. cytotoxicity) [2]. One possible method to further improve electroporation is through the implementation of a feedback loop, adjusting the pulse parameters based on electroporation outcome. However, current evaluation methods, like fluorescence microscopy, do not allow for such dynamic optimization scheme.

This works aims to monitor the in-flow electroporation process in real time using the impedance cytometry principle. The combination of electroporation and impedance cytometry has been shown before using macroscopic wire electrodes and a constriction channel geometry [3]. Our device further miniaturizes this process by using embedded electrodes inside a microfluidic channel, offering more control and sensitivity, paving the way for a dynamic optimization process.

Results and Discussion

The device consists of a straight microfluidic channel casted from PDMS (⌀ = 50 µm). The bottom of the channel is sealed with a (plexi)glass substrate which is outfitted with gold electrodes. Multiple electrode sets are included along the channel length to test the influence of electrode spacing (20 – 50 µm) and electrode widths (20 – 50 µm).

The layout of the electrode follows a typical coplanar impedance cytometry setup (Figure 1), while the excitation signal is a bipolar square wave typically used for electroporation. Using the property that a square wave is build-up of sinusoidal waves, lock-in amplifiers are used to extract the frequency components of interest.

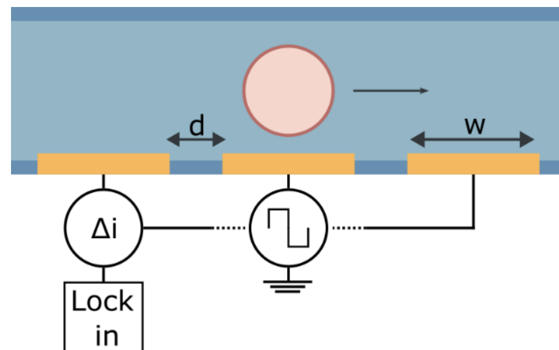

Figure 1: *Coplanar impedance cytometry setup with an electrode spacing d and electrode width w, and a square wave excitation signal. The current signals from the outer electrodes are subtracted from each other and outputted to a lock-in amplifier.*

The setup allows us perform impedance cytometry while simultaneously electroporating a cell and assessing electroporation performance in real time.

Conclusions

This work aims to develop a technique to simultaneously perform electroporation and impedance cytometry. The technique will provide the basis for a dynamic electroporation optimization scheme and enable in-situ monitoring to study the in-flow electroporation process.

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Ni-based MOFs for electrochemical sensor toward detection of lead and copper ions

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Keywords: Ni-MOF, pamam, electrochemical, SWASV, heavy metal ions

Introduction

Heavy metal ion pollution has become an unavoidable environmental problem[1]. Heavy metals mainly refer to chemical elements with potential biological toxicity, such as mercury (Hg), cadmium (Cd), lead (Pb), copper (Cu), zinc (Zn), and arsenic (As). Due to its high toxicity, non-degradability, and ability to enrich and amplify through the food chain[2], it has caused significant pollution to water , soil, and human health. Therefore, there is an urgent need to develop highly sensitive and selective methods for the determination of various complex component samples. In this study, conductive Na(2,3,6,7,10,11-hexaiminotriphenyl)2 MOFs and polyamide dendritic polymer (PAMAM) were used as sensing materials to construct a conductive chemical sensor with high enrichment and sensitivity.

Results and Discussion

We performed electrochemical characterization of the sensor construction process. Compared with the bare electrode, the current increases significantly after modifying Ni-MOF, indicating that the porous structure of Ni-MOF improves conductivity (*Figure 1*). In addition, GCE modified Ni-MOF/Pamam combines the dual advantages of high conductivity and multiple active sites. At the same time, we detected Pb and Cu at 40ug/l based on square wave anode dissolution voltammetry, and the results showed that Ni-MOF/pamam nanocomposites can be highly enriched with heavy metal ions and have high current intensity (*Figure 2*). The dissolution peak of pb at -0.82 mV and the peak at 0.17 mV is the dissolution peak of Cu.

Conclusions

An electrochemical sensor for heavy metal detection was developed, which can be used in point-of-care testing (POCT) in environmental monitoring to enable immediate detection of lead and copper ions in the water environment.

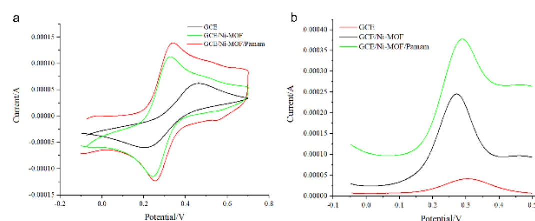

Figure 1: *CV (a) and DPV (b) for bare GCE, GCE/Ni-MOF and GCE/Ni-MOF/Pamam in the solution of 5 mM $[Fe(CN)_6]^{3-/4-}$ containing 0.1 M KCl. Potential scan rate: 0.1 V s^{-1}.*

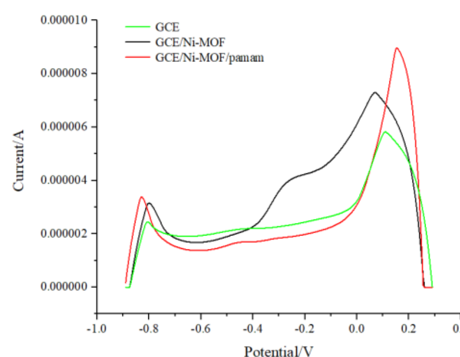

Figure 2: *Stripping voltammograms of GCE, GCE/Ni-MOF, GCE/Ni-MOF/Pamam in acetate buffer solution (0.2 M, pH 5.0) containing 30 μg L^{-1}Cu(II) and Pb(II) ions. Deposition potential: −1.2 V. Deposition time: 200 s.*

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Acknowledgements

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Diamond-based quantum biosensing of heterogeneous isothermal nucleic acid amplification reactions

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Keywords: NV center diamonds, quantum sensing, recombinase polymerase amplification, label-free

Introduction

Nitrogen vacancy (NV) center is a point defect in diamonds formed when two nearest neighbour carbon atoms are replaced by a substitutional nitrogen atom and the neighbouring lattice vacancy. The spin triplet ground state of the NV center, which results from the electronic structure, is largely responsible for quantum mechanical properties of the vacancy due to its spin-dependent fluorescence. The center can be optically initiated, read out and manipulated by means of microwaves. It was shown in previous experiments that phosphonic acid can be detected if a monolayer on a diamond is formed and ^{31}P is measured [1]. This effect can be used to measure ^{31}P from the sugar phosphate backbone of DNA. An increase of ^{31}P should be detected if DNA strands on the diamond surface are amplified. The principle of a heterogeneous isothermal asymmetric recombinase polymerase amplification (haRPA) assay which was established for chemiluminescence microarrays [2] were conducted the first time for a label-free diamond-based quantum biosensing approach.

Results and Discussion

The diamond surface was functionalized according to protocols used for glass surface chemistry. The diamond was etched by piranha solution and afterwards was silanized by (3-glycidyloxypropyl)trimethoxysilane. Jeffamine ED 2003 was used forming a polymer brush monolayer to immobilize DNA primer on the diamond surface. DSC was used as crosslinker to conjugate the amino groups of Jeffamine ED 2003 to the amino modified primer. First, it was shown by chemiluminescence that haRPA is able to amplify DNA on diamonds. Second, the created double stranded DNA is detected by the quantum sensor approach. A ^{31}P peak at 3 MHz with an intensity of 0.0029 a.u. was measured which was a result of heterogeneous amplification of double stranded DNA.

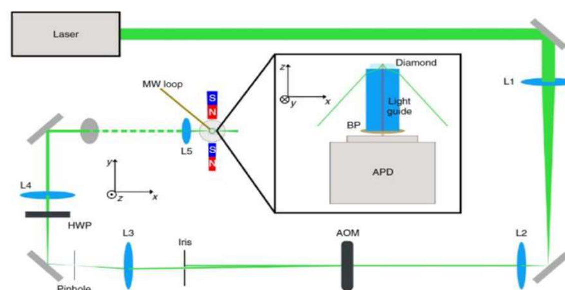

Figure: *Applied structure of the quantum sensor method for the detection of nuclear spins of ^{31}P from sugar phosphate backbone of DNA.*

Conclusion

The standard surface chemistry used for chemiluminescence-based haRPA could be performed on diamonds. Immobilized primer in presents of the enzymes recombinase, polymerase and single stand binding proteins were able to generate double stranded DNA on the surface of a diamond. The surface NV-NMR technique has been successfully demonstrated to detect microscopic NMR signals down to sub-monolayer coverages. The low density of DNA molecules on the diamond surface was sufficient to measure ^{31}P in the NMR spectrum. Quantum biosensing of DNA is a new label-free technique. Other applications of quantum biosensing would be miniaturized magnetometer which are able to detect magnetic nanoparticles. In contrast to fluorescence, no background noise should arise.

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Microgels in tandem with enzymes: Beneficial combination for advanced biosensing

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Keywords: microgel; stimuli-sensitivity; surface modification; adsorption; enzyme biosensor

Introduction

Efficient immobilization of enzymes on sensor surfaces is in the focus when developing various biosensor systems. Polymers are recognized to be advantageously applied as matrices for non-destructive immobilization of enzymes. In this context, stimuli-responsive copolymer microgels based on poly(*N*-isopropylacrylamide) (PNIPAM), which exhibit dual (pH- and temperature) sensitivity, are of considerable interest as they are capacious hosts for enzymes and provide to them favorable (highly hydrated) microenvironment [1-5].

Results and Discussion

We highlight benefits of microgel's stimuli-responsiveness for surface modification and engineering of microgel-enzyme biosensor constructs. Accordingly, the adsorption behavior of the pH- and temperature-sensitive cationic PNIPAM-based microgels was examined by atomic force microscopy and quartz-crystal microbalance with dissipation monitoring. Enhanced adsorption of the microgels onto relatively hydrophobic surfaces was observed at high pH and elevated temperatures as manifested by a high surface coverage and a large adsorbed mass. This is a consequence of pronounced hydrophobization of the microgels under the above conditions as revealed by dynamic light scattering and turbidimetry (cloud points). The subsequent electrostatic loading of the surface-bound microgel with enzymes was shown to result in highly-capacious integration of biomolecules into the formed microgel films. Finally, the biosensor responses of the microgel-enzyme films fabricated onto screen-printed graphite electrodes were electrochemically assessed to demonstrate important biorelated applications of such constructs and possible regulation of enzymatic activities by stimuli-dependent properties of microgel matrix [6].

Conclusions

In this work, a comprehensive conceptual understanding was attained on the following key points: (1) how the stimuli-responsive properties of the microgel correlate with its amount deposited onto the surface, (2) what determines the highly-capacious loading of the surface-bound microgel with the enzymes, (3) how the tandem of the stimuli-sensitive microgel and enzymes can be used for easy engineering of biosensor systems, and ultimately (4) how stimuli-sensitivity of the microgel can regulate enzymatic activities of the immobilized enzymes.

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Acknowledgements

This work was supported by the DFG within SFB 985 “Funktionelle Mikrogele und Mikrogelsysteme”, the RSF (project no. 22-24-00424), and the Lomonosov Moscow State University Programm of Development.

Microgel-enzyme complexes for engineering of improved electrochemical biosensors

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Keywords: electrochemical biosensor, enzyme immobilization, microgel

Introduction

Efficient and non-destructive immobilization of enzymes on sensor surfaces is one of the most important challenges when engineering enzymatic biosensors. Gentle immobilization of enzymes can be achieved with polymers, wherein a polymer is exploited for surface modification and a formed polymer coating can act as “glue” keeping an enzyme at the surface [1]. Among various polymers, which can be applied for this purpose, are stimuli-responsive copolymer microgels exhibiting pH- and temperature sensitivity as they are capacious and can host considerable amounts of enzymes, providing to them favorable (highly hydrated) microenvironment [2]. Up to date, adsorption first of a microgel and then of an enzyme (two-stage sequential adsorption) was used to construct microgel-enzyme films on the sensor surface [3]. This work highlights a yet unexplored approach to efficient surface modification by such films, which consists in adsorption of microgel-enzyme complexes pre-formed in solution (Figure 1).

Results and Discussion

A cationic poly(*N*-isopropylacrylamide-*co*-*N*-(3-dimethylaminopropyl)methacrylamide) (P(NIPAM-*co*-DMAPMA)) microgel containing about 9.2 % (mol.) of the DMAPMA units and glucose oxidase (GO) were used as a polymeric component and a biocomponent, respectively. The loading of the enzyme into the cationic P(NIPAM-*co*-DMAPMA) microgel was performed at pH 7 where GO bears an overall negative charge.

Adsorption of the microgel-enzyme complexes was examined by means of quartz crystal microbalance with dissipation monitoring. By contrast with the pristine P(NIPAM-*co*-DMAPMA) microgel, the pre-formed microgel-enzyme complexes at pH 7 exhibit remarkable adsorption onto a gold-coated quartz crystal, resulting in the well-adhered films that appear to be stable against subsequent flush off treatment.

Further, the microgel-enzyme complexes were adsorbed onto the modified (by a mediator) screen-printed graphite electrodes, which results in the electrochemical (amperometric) glucose biosensors with the LOD as low as 5 μM. By contrast with the microgel-enzyme films fabricated via two-stage sequential adsorption, their amperometric responses are notably more stable against manifold-repeated measurements, thereby indicating enhanced operational stability.

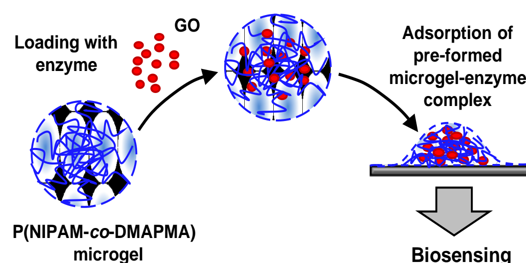

Figure 1: *The strategy exploited for the preparation of the microgel-enzyme biosensor coatings.*

Conclusions

Adsorption of the pre-formed microgel-enzyme complexes, wherein the enzyme is loaded into the microgel by the electrostatic interaction, is a successful approach to engineering of (electrochemical) enzymatic biosensors with enhanced operational stability.

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Acknowledgements

This work was supported by the DFG within SFB 985 “Funktionelle Mikrogele und Mikrogelsysteme” and the RSF (project no. 22-24-00424).

Flexible neural interface for reading the sense of smell

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Keywords: olfactory biosensing, biohybrid system, flexible neural electrode

Introduction

Humans perceive the world through five senses, of which olfaction is the oldest evolutionary sense that enables the detection of chemicals in the external environment. Recent progress in bioinspired electronics has boosted the development of artificial sensory systems. Here, we propose a biohybrid olfactory system by integrating living mammals with implantable flexible neural electrodes, to employ the outstanding properties of mammalian olfactory system (**Figure1**). The initial event of olfactory perception occurs in the olfactory epithelium (OE) inside the nasal cavity, which is the peripheral organ for the sense of smell. The olfactory bulb (OB) is the first relay station of the central olfactory system. It receives input from OE and sends output to the olfactory cortex. The olfactory information encoded in the neural activity was recorded from both OE and OB simultaneously using flexible neural electrodes.

Results and Discussion

The spontaneous neural signals of the olfactory epithelium and olfactory bulb in mice at different behavioral states were recorded by flexible neural electrodes (**Figure2**). The olfactory neural activity patterns related to behavioral states were studied by combining EEG and nasal respiratory signal. Two typical odorants, isoamyl acetate with banana smell and trimethylthiazoline with fox feces smell, were selected as stimuli to deliver to mice. The results indicated that the local field potential signal extracted by flexible electrode has a specific response change in beta (15-35 Hz) and gamma (35-120 Hz) frequency bands. The support vector machine algorithm can distinguish the corresponding stimulus odor using the response activites of the OE and OB (**Figure3**).

Conclusions

The current work improved the applicability of flexible neural interfaces and provided a new approach for exploring the information contained in neural activities at different regions of the olfactory system.

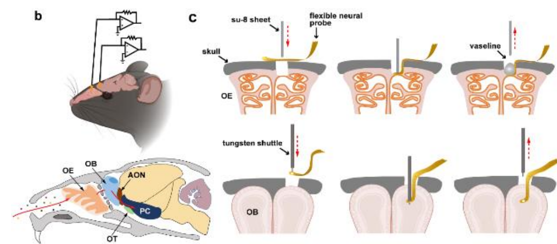

Figure 1: *Schematic configuration of biohybrid olfactory system.*

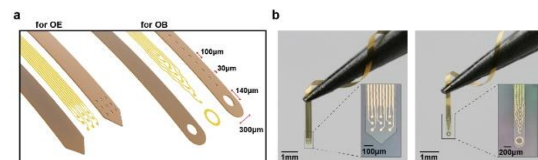

Figure 2*: Flexible neural electrodes for OE and OB recording.*

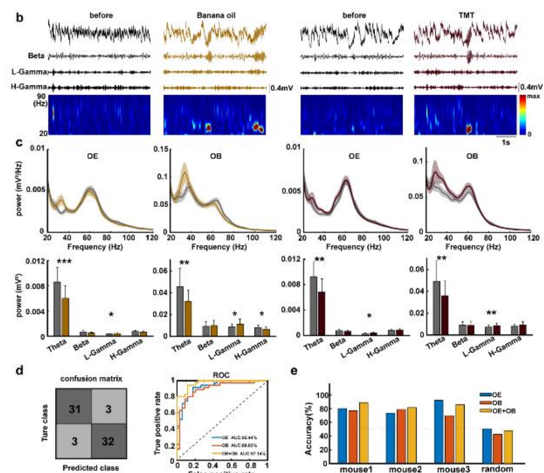

Figure 3*: Odor-induced responses and odor classification.*

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Acknowledgements

This research was funded by the National Natural Science Foundation of China (No. 62271443, 3225008); Natural Science Foundation of Zhejiang Province (No. LBY21H180001, LY21C100001).

Electrodeposited Gold Nanoparticle based Electrochemical Aptasensor for Cortisol Detection

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Keywords: Electrochemical, cortisol, gold nanoparticle, saliva

Introduction

Cortisol, a steroid hormone and major glucocorticoid in human body is the key biomarker for stress and hence also known as stress hormone. There are laboratory-based techniques to detect cortisol levels but they are time consuming, require large sample volume and skilled personnel to operate the techniques. Hence, the biosensor development is the need of the hour

Owing to the various properties such as simple synthesis, tuneable electrical and optical properties, and strong binding to bio-recognition probes through amino, thiols, and disulphide groups, gold nanoparticles (AuNPs) have been explored for the sensing of the stress hormone cortisol using DNA aptamers[1]. In the present work, gold nanoparticles were electrodeposited on the screen-printed electrode and a 5'-thiol modified aptamer against cortisol was immobilized. Practical applicability of the sensor was tested in the artificial saliva matrix and real saliva samples.

Results and Discussion

AuNPs electrodeposition on the electrode was confirmed using FESEM, EDX and electrochemical characterization. Aptamer immobilization on the gold-nanoparticle modified electrode was confirmed using Raman spectroscopy, contact angle measurement and XPS measurement. Additionally, real electroactive area of the bare (AuSPE), gold nanoparticle modified electrode (AuNP/SPE) and aptamer-immobilized electrode (Apt/AuNP/SPE) was calculated using the Randle-Sevick equation for quasi reversible reaction[2]. The calculated % Real with respect to the geometrical area for AuSPE, AuNP/SPE and Apt/AuNP/SPE is 23%, 195.2% and 142.86%, respectively.

Further, different cortisol concentration in the range of 0.1 pg/ml-100 ng/ml were detected in the artificial saliva matrix. A detection limit of 0.28 pg/ml with 1.33 μA (pg/ml)-1 sensitivity. Selectivity of the sensor was evaluated using the structural analogues of the cortisol and sensor was found to be selective towards the cortisol.

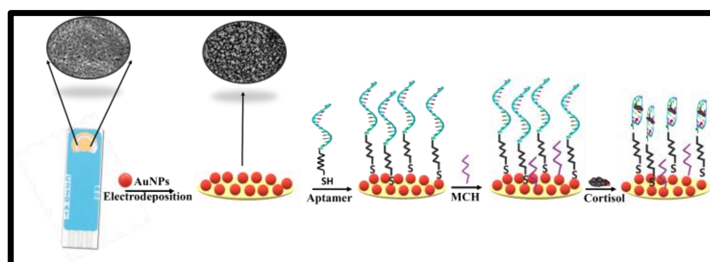

***Figure 1**: Representation of aptaelectrode fabrication steps and cortisol sensing*

Conclusions

In current work, electrodeposited gold nanoparticle has been employed as a transducer to improve the electron transport for the development of the electrochemical-based aptasensor and hence, leading to an amplification in the electrochemical signal. The proposed cortisol aptasensor has achieved good performance with the detection limit of 0.28 pg/ml in artificial saliva matrix, reproducibility, and enhanced sensitivity with good specificity. These types of aptamer-based electrochemical sensors are robust, highly sensitive and can be readily applied in determining the cortisol level non-invasively and may have potential clinical diagnostic value.

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Acknowledgements

The author Vipasha Sharma acknowledges Department of Science and Education for INSPIRE fellowship.

Nanoparticle-assisted paper based assays with optical and electrochemical detection

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Keywords: paper based sensor, phenylalanine detection, pathogen detection, SERS, colorimetry

Introduction

During the last decade, paper-based diagnostic has become a special field of quantitative analysis, especially, in the biological sciences with simple equipment, portability, low manufacturing cost, a minimum consumption of sample and time. The low manufacturing cost and the ease of application makes paper-based diagnostic devices a viable option for use in low-resource settings. However, some improvements are still required regarding the accuracy and precision in quantitative applications. Since electrochemical and optical methods such as colour or surface enhanced Raman Scattering (SERS) measurements have been used for detection, the construction of sensitive and reproducible substrate is still a challenge. Especially, the paper microfluidics or microchip-based measurement is a novel approach and will be a powerful alternative to the expensive conventional techniques that necessitate the consumption of excess amount of sample and materials [1].

Results and Discussion

Our research group demonstrated several paper-based sensor applications such as gold nanoparticles decorated paper electrodes to obtain the electrochemiluminescence signal of $Ru\text{-}(bpy)_3^{2+}$ in the presence of NH_3. The phenylalanine (L-Phe) detection was performed by converting L-Phe into ammonia using phenylalanine ammonia-lyase (PAL) enzyme and the ECL signal of $Ru\text{-}(bpy)_3^{2+}$ was produced in combination with ammonia as a co-reactant. A linear increase of the ECL signals with the logarithms of varying L-Phe concentrations between 1.5×10^{-2} and 1.2 mM was observed with a correlation coefficient (R^2) of 0.9898 and a limit of detection (LOD) of 8.4×10^{-3} mM. The proposed method was efficiently applied for L-Phe detection in reference blood samples and the average recovery was calculated as 96.8% [2].

This presentation is also devoted to the tag-based immunoassay technique without the need for multiple washing process and SERS label was placed in the system prior to analysis. In this work, we have synthesized magnetic nanoparticles which are suitable for immunomagnetic separation of microorganism. The surface of nanoparticles is modified with controlled orientation of antibodies. After immunomagnetic separation of bacteria from matrix, labelled nanoparticles were immobilized to the target bacteria on the surface of chip surface. Then, SERS and colorimetric measurement was performed on the test line.

Conclusions

The design, preparation and surface modification of paper-based platform could be useable for the detection of different target analyte from complex matrices with a with a total quantitative analysis time of less than 30 minutes. The optimization strategies and the analytical performance of the chip-based assays will be presented.

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The author acknowledges Gazi University, BAP with the project no: TPD-2023-8630 for funding.

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