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Real time optical immunosensing with flow-through porous alumina membranes

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ABSTRACT

Through the presentation of analytical data from bioassay experiments, measured by polarimetry, we demonstrate for the first time a real time immunoassay within a free standing macroporous alumina membrane. The 200 nm nominal pore diameter of the membrane enables flow-through, thereby providing an ideal fluidic platform for the targeted delivery of analytes to bioreceptors immobilized on the pore walls, enabling fast sensing response times and the use of small sample volumes (<100 μL). For the immunoassay, the pore walls were first coated with the functional copolymer, copoly(DMA-NAS) using a novel coupling process, before immobilization of the allergen protein, β-lactoglobulin, by spotting. The immuno-assay then proceeded with the binding of the primary and secondary antibody cognates, rabbit anti-β-lactoglobulin and anti-rabbit IgG respectively. Through the use of streptavidin coated quantum dots as refractive index signal enhancers, a noise floor for individual measurements of 3.7 ng/mL (25 pM) was obtained, with an overall statistical, or formal assay LOD of 33.7 ng/mL (225 pM), for total assay time below 1 h.

1. Introduction

Due to an increasing demand for rapid, reliable and economical near patient or field testing, over the last few decades there has been a strong trend toward in vitro point of care (PoC) testing in clinical diagnosis, food safety, environmental monitoring, safety and security [1,2]. Significant segments of PoC testing are based on bioassays where extremely low concentrations of markers (infectious agents, pesticides, cardiac markers, allergens etc.) need to be identified and distinguished from other matter within small volumes of complex matrices (e.g. whole blood, sputum swabs, feces, cell lysate...). To meet these needs, PoC in vitro diagnostic devices are required to provide a fast, sensitive and selective analysis of assays, ideally a simultaneous analysis of multiple assays, and therefore new approaches being explored are often based on highly integrated sensors within a Lab on Chip format [3]. Furthermore, for low cost and practical application by non-technical users, optimal designs negate the need for lengthy off-chip sample preparation.

At the core of these devices is the biosensor [4] and those based on optical interrogation offer important advantages such as: (1) non-invasive, safe and multi-dimensional (intensity, wavelength, phase, polarization) detection; (2) well-established tools from communication and Micro-Nano technologies (MNT) industries (lasers, detectors, waveguides) and (3) optical frequencies that coincide with a wide range of physical properties of bio-related materials.

Many optical biosensors are based on refractive index (RI) sensing, where the effective refractive index of the surface of an optical structure is modified by the presence of a target analyte [5]. For the development of photonic biosensors, nanostructured materials like porous silicon (PSi) or porous alumina (AAO) have gained special attention, as they have higher surface areas than planar biosensors for capturing analytes and thus permit lower detection limits [6]. To date, several label-free photonic biosensors have been successfully developed on both PSi [7,8] and AAO [9,10] porous membranes, using reflectometric interference spectroscopy (RIfS). However, this approach produces slow responses and long

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sensing times, due to the narrow (<100 nm) closed end pores of the membranes used, resulting in analyte delivery being mainly governed by the stationary flux produced by electrostatic interactions. The problem of analyte delivery has been obviated in a fluorescent flow-through assay using a fibrous filter as an improved method for protein capture [11], however this was as an ex situ end point assay which after the analyte was bound required the substrate to be dried and transferred to a reader for measurement under index matched liquid. In a recent approach using optical polarimetry [12], we demonstrated that free standing macroporous AAO membranes with 200 nm diameter pores when functionalized with an epoxysilane allow analytes to flow-through them. Due to the pore size, the analyte molecules flow-through no more than 100 nm from the assay surface (the pore wall), breaking the mass transport limitations and so effectively targeting their delivery, for real-time biosensing responses, an approach which has also been proposed elsewhere with similar porous optical structures [13,14].

Here, we report on the use of this approach for immunosensing, by coating the membrane with a functional copolymer, copoly(DMA-NAS), through a novel procedure that has demonstrated less non-specific binding, and therefore greater selectivity, and more stability over time for immobilized allergens than epoxysilane [15]. In this paper, we first detail the procedure for immobilizing the bioreceptors inside the pores of the alumina membrane, including the novel process for coating the pores with the copolymer. After demonstrating the stability of the immobilized allergens, we show a 5 times signal enhancement through the use of streptavidin coated quantum dots (SA-QDs) compared to the secondary antibody used. Finally, we show the concentration performance of the immunoassay by using solutions of the first antibody at different concentrations, followed by a fixed concentration of the secondary antibody and streptavidin coated quantum dots.

2. Materials and methods

2.1. Materials

The free-standing macroporous alumina membranes were purchased from Whatman (Anodisc™ membranes, 13 mm diameter, 200 nm nominal pore diameter, 60 μm thickness and 0.5 porosity) and several were characterized morphologically by SEM to verify the approximate pore dimensions. PBS (Phosphate buffered saline) tablets, Tween 20, toluene, dimethylformamide (DMF) *N,N*-dimethylacrylamide (DMA), [3-(methacryloyl-oxy)propyl]trimethoxysilane (MAPS), tetrahydrofuran (THF), azoisobutyonitrile (AIBN) and absolute ethanol, were acquired from Sigma-Aldrich (St. Louis, MO, USA). *N*-acryloyloxysuccinimide (NAS) was synthesized as reported elsewhere [16].

The primary antibody employed rabbit anti-β-lactoglobulin, was purchased from Bethyl Laboratories (Montgomery, TX, USA). Antigen protein β-lactoglobulin B, the secondary antibody, biotinylated anti-rabbit IgG, and BSA (bovine serum albumin), were purchased from Aldrich (St. Louis, MO, USA). Strepavidin conjugated CdSe quantum dots (Qdot® 800 Streptavidin Conjugate) were supplied by Invitrogen (Life Technologies, NY, USA). All buffers were filtered through 0.2 μm filters before use.

2.2. Bioreceptor immobilization

One of the key factors for the development of optical biosensors for point-of-care diagnostic devices is the immobilization of the bioreceptors on the sensor surface in a way that retains a high degree of activity and low levels of non-specific binding. A polymeric coating introduced by Pirri and co-workers in 2004 [17] was shown to possess favorable characteristics for performing an optimal microarray analysis. This coating is obtained by adsorption on solid surfaces of a diluted aqueous solution containing a co-polymer, which provides a stable and hydrophilic film. This polymer, poly(DMA-NAS-MAPS) is obtained by random radical polymerization of three monomers: *N,N*-dimethylacrylamide (DMA), which self-adsorbs on glass by weak, non-covalent interactions with silanol groups, 3-(trimethoxysilyl)propyl methacrylate (MAPS), which stabilizes these interactions by the formation of stable, covalent bonds and *N*-acryloyloxysuccinimide (NAS), which represents the chemically reactive groups with the aim to bind biomolecules on the surface. Thanks to its monomer composition this copolymer provides a low fluorescence background for surfaces and a highly selective binding chemistry whilst retaining the native configuration of immobilized probes. This polymer has been previously demonstrated to immobilize allergens on different materials such as glass, nitrocellulose and silicon slides [18] and more recently on a SiOxNy Dual Polarization Interferometry chip [15] allowing the efficient measurement of their interactions with allergen-specific Immunoglobulin E (IgEs) in the complex matrix of serum, proving its suitability as a non-fouling coating and that it is robust to allergen storage.

For this work it was not possible to coat the surface with poly(DMA-NAS-MAPS) using the usual dip and rinse method, due to the instability of AAO toward some of the reagents used in the procedure, therefore, a functional coating of DMA and NAS was obtained via a novel two step procedure involving modification of the surface with MAPS followed by the *grafting* of poly(DMA-NAS) formed by in situ radical polymerization. This coating was used for the first time to immobilize allergen proteins on a porous surface (Fig. 1).

2.2.1. Pore surface coating

Before the pore surfaces were coated, the membranes were cleaned with an oxygen plasma for 10 min in a Harrick Plasma cleaner (Ithaca, NY, USA) and then immersed in a solution of MAPS in toluene (10% v/v). A vacuum was applied for 10 min in order to assure that the pores were completely filled with this solution, before the reactor was sealed and left overnight at room temperature. After silanization, the membranes were first washed on a Buchner funnel, under vacuum with toluene and then with THF and finally cured under vacuum at 80 °C for 30 min. Thereafter, a 20 mL solution of 10% DMA and NAS in DMF (molar ratio 90–10; 1.85 mL, 17.95 mM and 340 mg, 2.01 mM respectively) was prepared. The silanized membranes were then immersed in this solution and an argon purge was used to remove oxygen from the solution for 15 min. AIBN (6.5 mg, 2 mM) was then added and a vacuum applied for 10 min to assure that the pores were completely filled with the degassed monomer solution and then the reactor was sealed and heated with a silicon bath, at 65 °C overnight. Finally, the membranes were washed on a Buchner funnel, under vacuum, first with DMF and then with THF, before being dried under vacuum at room temperature.

2.2.2. Allergen immobilization

The allergen β-lactoglobulin was spotted onto the coated membranes using a piezoelectric spotter (Scienion SciFlexArrayer S5). Several droplets of a solution of β-lactoglobulin (1 mg/mL in PBS) were spotted in the central area of the membranes so as to form a square of 5 mm × 5 mm. Membranes were layered on nitrocellulose slides wetted with 10 μL of the same antigen solution, so to assure complete filling of the pores by capillary action. The spotted membranes were then left overnight in a moisture chamber before being rinsed with PBS, on a Buchner funnel, and then treated with

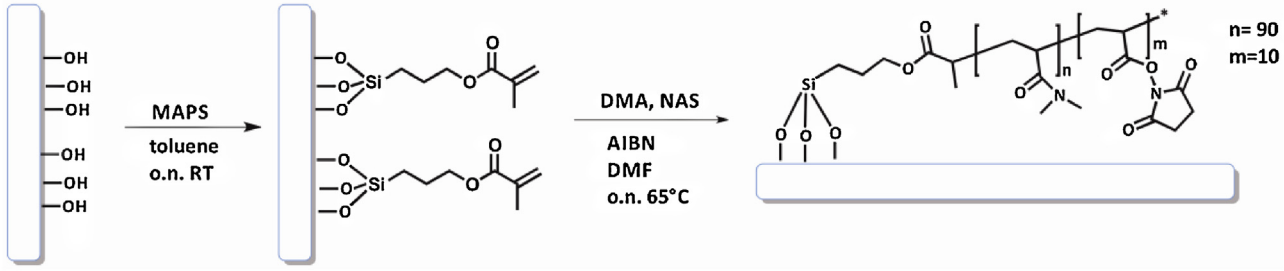

Fig. 1. Scheme of the grafting process of poly(DMA-NAS) onto an AAO membrane silanized with MAPS that bears an allyl group which allows polymerization with DMA and NAS.

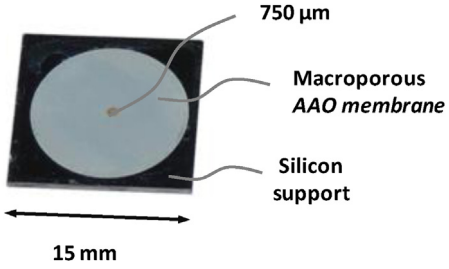

Fig. 2. Picture of a free standing membrane mounted on a silicon support.

a solution of BSA (0.1 mg/mL) to block any remaining active sites of the coating.

2.3. Membrane transfer to supports

After having immobilized the bioreceptors on the functionalized free-standing membranes, in order to conduct flow-through sensing experiments, the circular membranes were first transferred onto mechanical support chips (Fig. 2). The support chips chosen were 15 by 15 mm pieces of single side polished silicon wafer, 500 µm thick, due to their stability in an aqueous environment as well as the flat interface they provide for a good mechanical contact between them and the membranes. The support chips had a 750 µm diameter opening in the center which was made using a CO_2 laser, and the edges were chamfered on the reverse side in order to allow the laser light to readily pass through the hole without being occluded by the walls of the support chip. A 1 µm thick layer of PMMA (Poly(methyl methacrylate)) resist was used as the adhesion layer between the alumina membranes and the silicon supports. Support mounted membranes were then stored in a desiccator at 4 °C until they were used in the biosensing experiments. This set-up forms a 750 µm diameter active spot area and is compatible with a multispot format which would allow multiplexed assays. Smaller spots are also be possible as the measurement sensitivity is in principle not spot size dependent.

2.4. Readout platform

The readout platform (Fig. 3a) used a polarimetric setup [19] to interrogate the changes in the optical anisotropy of the macroporous free-standing membranes, the sensing mechanism for the bioassay. Analyte molecules within the pores modify the birefringence which is detected within the setup as a change of phase retardation between the components of light polarized along the main axis directions. Briefly the setup is: the output light from a 980 nm laser diode is first collimated and then directed to a linear polarizer with the resulting linearly polarized light then entering a photoelastic modulator (PEM; Hinds Instruments PEM-100). The modulated light exiting the PEM is then incident at 45° to the planar surface of the alumina membrane, which is mounted on the support chip within a flow-cell. The light exiting the membrane, after passing a second polarizer, is detected by a photodiode which is connected to a lock-in amplifier (SR-830). The lock-in amplifier demodulates the detected signal extracting the amplitudes of its first and second harmonics, which are related to the phase retardation.

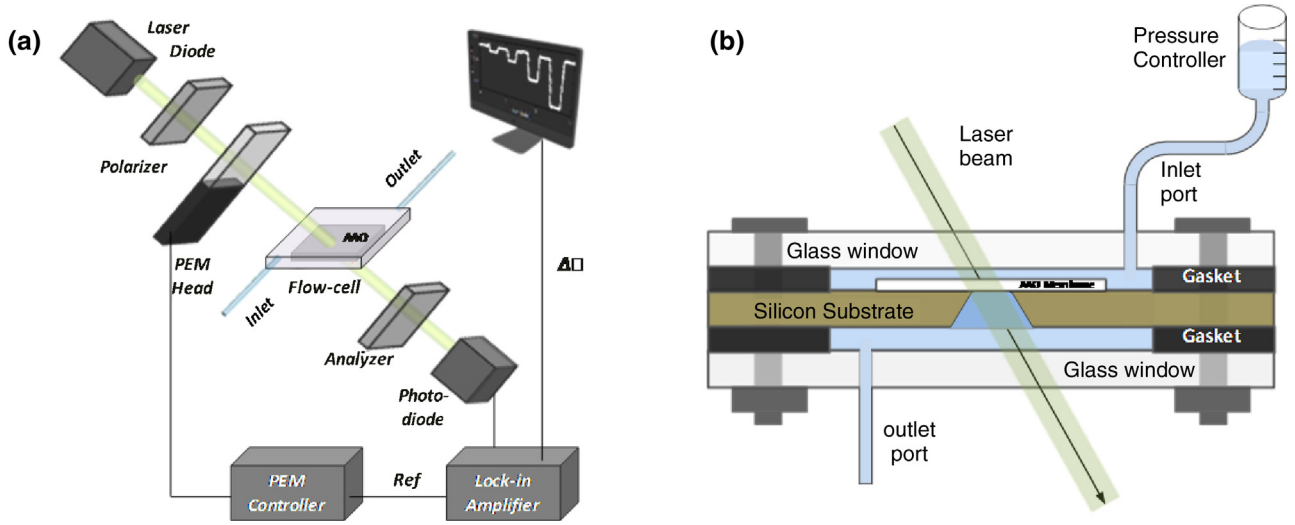

Fig. 3. (a) Layout of the optical polarimetric readout platform used for measuring the phase retardation within the membranes. (b) Scheme of fluidic setup integrated within a flow-cell where the mounted membrane is placed and whose inlet port is connected to a pressure controller providing a constant pressure flow.

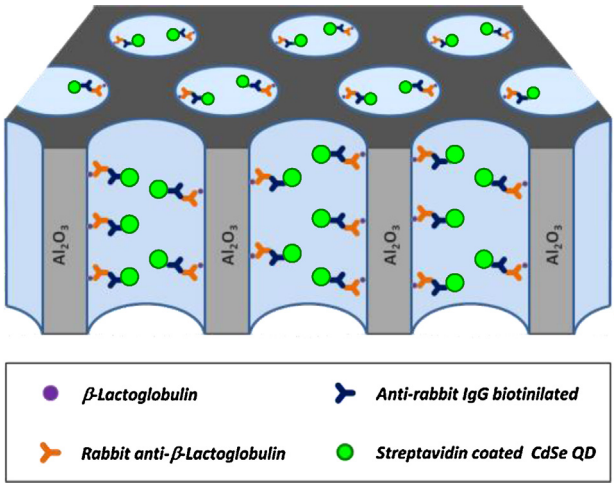

Fig. 4. The immunoassay carried out in a macroporous alumina membrane. β-lactoglobulin protein was used as the immobilized antigen for the detection of rabbit anti-β-lactoglobulin. Biotinylated secondary antibody (anti-rabbit-IgG) and streptavidin coated CdSe quantum dots were used to increase the signal produced by the primary antibody.

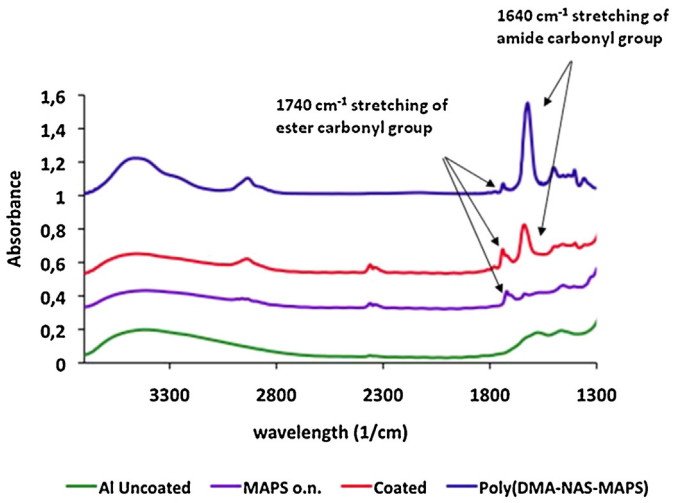

Fig. 5. FT-IR analysis of a functional polymer coated membrane. Peaks corresponding to DMA and NAS carbonyl moities are easily observed when compared to an uncoated membrane.

The fluidic setup used within the readout platform allows the analytes to be pumped through the flow cell encased and mechanically supported membrane by use of a pressure controller connected to a syringe where the running buffer is stored (Fig. 3b). Before filling with liquid, the system is purged with CO_2 for 5 min to ensure complete filling and to reduce the incidence of bubbles. The purging consists of the CO_2 displacing the air in the system and any that is not displaced on filling with solution is then dissolved in the water or buffer.

2.5. Immunosensing experiments

After mounting a membrane with immobilized allergen into the flow-cell and purging with CO_2, a running buffer of PBS-T (PBS, 0.02% (v/v) Tween 20) was introduced for 15 min before the behavior of the immobilized allergen was tested by injecting its cognate antibody rabbit anti-β-lactoglobulin. The membrane was then rinsed again with the running buffer and the secondary antibody anti-rabbit-IgG (biotinylated) was then introduced into the system followed by streptavidin conjugated quantum dots (SQ-QD) as signal enhancers, which increased the sensitivity for low concentrations of the primary antibody. A schematic representation of this bioassay is depicted in Fig. 4.

3. Results and discussion

3.1. Characterization of AAO membranes

The membranes were analyzed by FT-IR after each step of the coating process, to confirm that the chemical modification was taking place as intended. The signals from uncoated membranes (blank samples) were subtracted from the spectra of modified membranes (using Spectra Manager software 1.52 from Jasco, MD, USA) so as to highlight the peaks corresponding to the coating reagents. The subsequent FT-IR spectra of the polymer coated membranes, Fig. 5, show a peak around 1740 cm^{-1} corresponding to the stretching of carbonyl group of MAPS which increases when NAS is added onto the surface. On coated membranes, as well as the NAS peak, it is possible to observe a peak at 1640 cm^{-1} which corresponds to the stretching of the DMA carbonyl group. For comparison, an FT-IR (poly(DMA-NAS-MAPS) was also recorded, so as to confirm the presence of the coating on the membrane.

3.2. Immunosensing results

The activity of the immobilized allergens was tested by running the immunoassay explained in Section 2.5. Fig. 6 shows the measured phase retardation when concentrations of 1 μg/mL (6.7 nM) are used for the first and secondary antibody and a concentration of 2.5 nM is used for the SA-QD. After placing the alumina membrane in the flow-cell and prior to the antibody injections, the baseline obtained during the buffer rinse showed good stability which demonstrated that the antigen is stably immobilized on the polymer coated surface. After recording a stable baseline during 6 min we then injected the first antibody rabbit anti-β-lactoglobulin for a period of 10 min. Following a 6 min rinse with the running buffer the secondary antibody anti-rabbit IgG was then injected for 10 min. As the secondary antibody is polyclonal, a larger response is observed for this, compared to the binding of the initial primary antibody. After further rinsing, the SA-QDs were added as a signal enhancer at a concentration of 2.5 nM, which was sufficient to saturate the captured secondary antibodies. Due to the size of

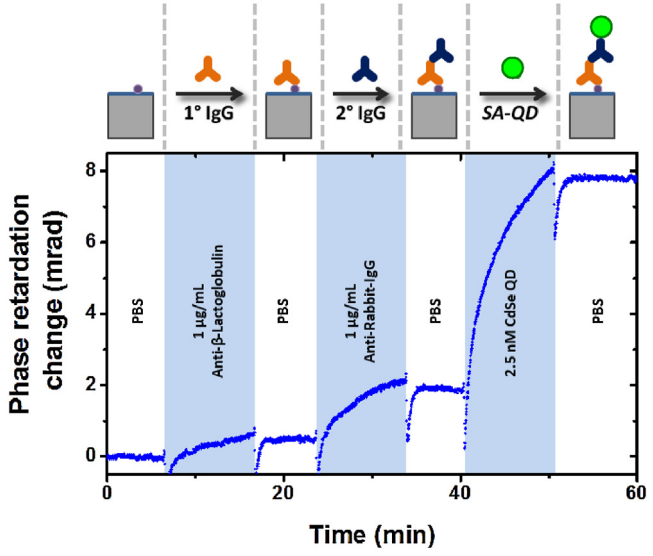

Fig. 6. A sensorgram showing the signal response due to the binding of the first and secondary antibodies, followed by the enhancement produced by the SA-QD.

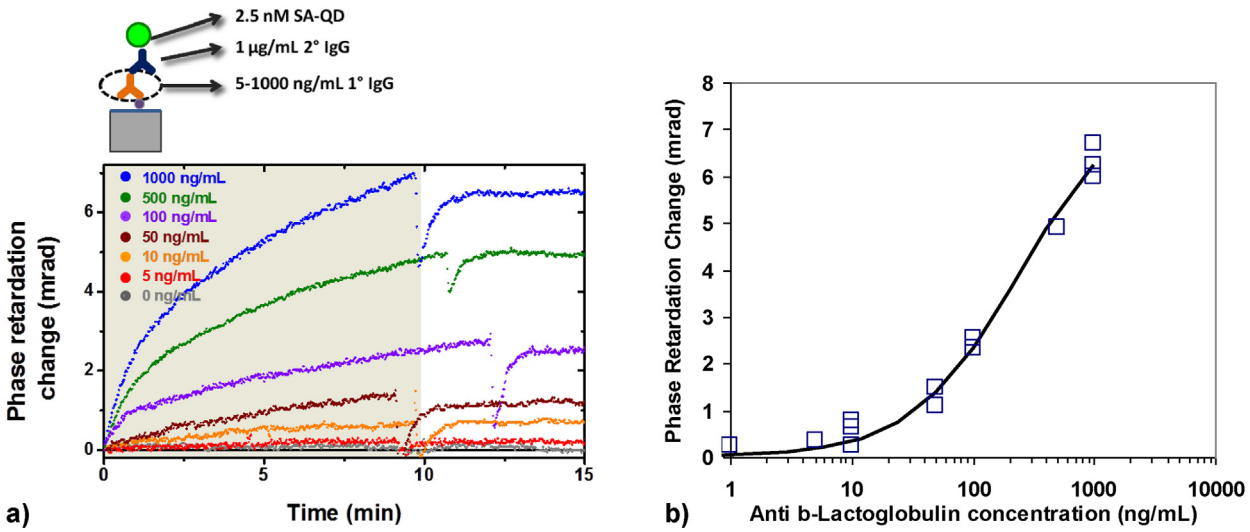

Fig. 7. (a) An overlay of the signal responses produced by the SA-QD when the concentration of the first antibody is increased from 5 to 1000 ng/mL. (b) Phase retardation change upon injection of 2.5 nM SA-QD over biosensing experiments using a range of primary antibody concentrations (5–1000 ng/mL). The fitted line corresponds to a 1:1 binding model of K_D 228 ng/mL and R_{max} of 7.7 mrad.

the SA-QDs an enhancement of 5 times is observed in the signal over the response produced by the secondary antibody.

After this experiment a series of experiments was run using 12 different membranes with the same immunoassay where the concentration of the first antibody was varied between 5 and 1000 ng/mL in order to estimate the detection limit of the system. Fig. 7(a) shows the overlaid response obtained for the different membranes.

In Fig. 7(b), we present the phase retardation change produced by the signal enhancement quantum dots as a function of the concentration of the first antibody. The limit of detection of the system was calculated according to the formula given in [20], which defines the analytical limit of detection as the analyte concentration that can reliably be distinguished from the noise:

$$\mathrm{LOD} = \mathrm{LOB} + 1.645 \cdot \mathrm{SD}_{\mathrm{low\_concentration\_sample}} \quad (1)$$

where $\mathrm{SD}_{\mathrm{low\_concentration\_sample}}$ corresponds to the standard deviation obtained from a series of samples at low concentration and LoB is the limit of blank defined as the highest analyte concentration value expected to be obtained for a sample containing no analytes. LoB is equal to:

$$\mathrm{LOB} = \mathrm{mean}_{\mathrm{blank}} + 1.645 \cdot \mathrm{SD}_{\mathrm{blank}} \quad (2)$$

where $\mathrm{mean}_{\mathrm{blank}}$ corresponds to the mean value obtained from a series of blank samples and SD corresponds to the standard deviation of the samples.

In our case, using β-lactoglobulin as the immobilized antigen, the limit of detection of our system was 33.7 ng/mL. This value is chiefly limited by the reproducibility of the mechanical setup rather than the measurement signal noise level, which would establish a limit an order of magnitude lower, at 3.7 ng/mL. For application in clinical allergy diagnostics, a measurement range of 0.84–240 ng/mL (0.35–100 kU/L) is required by clinicians [21], and thus the presented proof-of-concept system already shows relevant performance for this application. With improvements in the mechanical design and further assay optimization, we expect to improve the detection limit to cover the full range.

Compared to previous work employing porous membranes in a flow-over configuration, the flow-through configuration applied here enables more efficient convective delivery of analytes to the immobilized recognition molecules. This results in a short total assay time of below 60 min for a sandwich assay employing both a secondary and tertiary binding, compared to flow-over assay times of 5 h for a two-step assay in [8]. Furthermore, in a flow-through configuration the entire sample passes through the membrane in close proximity to the immobilized recognition molecules, and hence the sample utilization is greatly improved. For example, the total analyte containing sample volume consumed during the 10 min injection in this work was 1 mL, while in [8] a total volume of 50 mL was consumed (100 min injection at a flow rate of 0.5 mL/min). In terms of analyte mass consumed, the contrast is even more stark, i.e. 1 μg (1 mL of 1 μg/mL Anti-β-lactoglobulin solution) in flow-through, compared to 5 mg (50 mL of a 0.1 mg/mL IgG solution) in flow-over [8]. This difference is particularly important for analysis of small and costly samples, such as in neonatal diagnostics and drug development, respectively.

4. Conclusions

We have demonstrated for the first time a real time immunoassay within a free standing flow-through macroporous alumina filter membrane, which provides a route toward rapid and low cost biosensing.

Both the stability and the functionality of the coating and spotting procedure were evaluated by injecting inside the pores the antibodies rabbit anti-β-lactoglobulin and anti-rabbit-IgG. The response produced by the binding between the allergen protein and its cognate antibodies was acquired in real-time, by using an optical polarimetric readout platform to measure the anisotropy change of the macroporous alumina membrane.

Finally, the use of streptavidin conjugated CdSe quantum dots was investigated as refractive index signal enhancers obtaining fivefold signal amplification compared with the signal produced by the secondary antibody alone. Using this signal amplifier a noise floor for individual measurements of 3.7 ng/mL (25 pM) was obtained, with an overall statistical, or formal assay LOD of 33.7 ng/mL (225 pM), for total assay times of under 1 h.

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References

[1] X. Fan, I.M. White, S.I. Shopova, H. Zhu, J.D. Suter, Y. Sun, Sensitive optical biosensors for unlabeled targets: a review, Anal. Chim. Acta. 620 (July (1/2)) (2008) 8–26, Available at: http://www.sciencedirect.com/science/article/pii/S0003267008009343
[2] D. Hill, Advances in nanophotonic sensing technologies during three international label-free lab-on-chip projects, Bionanoscience 1 (2011) 162–172, http://dx.doi.org/10.1007/s12668-011-0026-1.
[3] F.S. Ligler, Perspective on optical biosensors and integrated sensor systems, Anal. Chem. 81 (January (2)) (2009) 519–526, http://dx.doi.org/10.1021/ac8016289.
[4] A. Brecht, G. Gauglitz, Optical probes and transducers, Biosens. Bioelectron. 10 (9-10) (1995) 923–936.
[5] M.C. Estevez, M. Alvarez, L.M. Lechuga, Integrated optical devices for lab-on-a-chip biosensing applications, Laser Photon Rev. 6 (4) (2012) 463–487.
[6] T.D. Lazzara, I. Mey, C. Steinem, A. Janshoff, Benefits and limitations of porous substrates as biosensors for protein adsorption, Anal. Chem. 83 (14) (2011) 5624–5630.
[7] M.M. Orosco, C. Pacholski, M.J. Sailor, Real-time monitoring of enzyme activity in a mesoporous silicon double layer, Nat. Nanotechnol. 4 (2009) 255–258.
[8] C.K. Tsang, T.L. Kelly, M.J. Sailor, Y.Y. Li, Highly stable porous silicon–carbon composites as label-free optical biosensors, ACS Nano 6 (2012) 10546–10554.
[9] S.D. Alvarez, C.P. Li, C.E. Chiang, I.K. Schuller, M.J. Sailor, A label-free porous alumina interferometric immunosensor, ACS Nano 3 (2009) 3301–3307.
[10] T. Kumeria, M.D. Kurkuri, K.R. Diener, L. Parkinson, D. Losic, Label-free reflectometric interference microchip biosensor based on nanoporous alumina for detection of circulating tumour cells, Biosens. Bioelectron. 35 (1) (2012) 167–173.
[11] R.M.L. van Lieshout, T. van Domburg, M. Saalmink, R. Verbeek, R. Wimberger-Friedl, M.P. van Dieijen-Visser, C. Punyadeera, Three-dimensional flow-through protein platform, Anal. Chem. 81 (2009) 5165–5171.
[12] J. Álvarez, L. Sola, G. Platt, M. Cretich, M. Swann, M. Chiari, D. Hill, J. Martínez-Pastor, Real-time polarimetric biosensing using macroporous alumina membranes, in: Proceedings of the SPIE 8765, Bio-MEMS and Medical Microdevices, 87650I, 28 May, 2013.
[13] A.A. Yanik, M. Huang, A. Artar, T.Y. Chang, H. Altug, Integrated nanoplasmonic–nanofluidic biosensors with targeted delivery of analytes, Appl. Phys. Lett. 96 (2) (2010) 021101.
[14] Y. Guo, H. Li, K. Reddy, H.S. Shelar, V.R. Nittoor, X. Fan, Optofluidic Fabry–Perot cavity biosensor with integrated flow-through micro-/nanochannels, Appl. Phys. Lett. 98 (4) (2011) 041104.
[15] G.W. Platt, F. Damin, M.J. Swann, I. Metton, G. Skorski, M. Cretich, M. Chiari, Allergen immobilisation and signal amplification by quantum dots for use in a biosensor assay of IgE in serum, Biosens. Bioelectron. 52 (2014) 82–88.
[16] M. Mammen, G. Dahmann, G.M. Whitesides, Effective inhibitors of hemagglutination by influenza virus synthesized from polymers having active ester groups – insight into mechanism of inhibition, J. Med. Chem. 38 (1995) 4179–4190.
[17] G. Pirri, F. Damin, M. Chiari, E. Bontempi, L.E. Depero, Characterization of a polymeric adsorbed coating for DNA microarray glass slides, Anal. Chem. 76 (5) (2004) 1352–1358.
[18] M.M. Cretich, D. Breda, F. Damin, M. Borghi, L. Sola, S.M. Unlu, M. Chiari, Allergen microarrays on high-sensitivity silicon slides, Anal. Bioanal. Chem. 398 (4) (2010) 1723–1733.
[19] J. Álvarez, C. Serrano, D. Hill, J. Martínez-Pastor, Real-time polarimetric optical sensor using macroporous alumina membranes, Opt. Lett. 38 (7) (2013) 1058–1060.
[20] D.A. Armbruster, T. Pry, Limit of blank, limit of detection and limit of quantitation, Clin. Biochem. Rev. 29 (2008) S49–S52.
[21] B.I. Fall, B. Eberlein-König, H. Behrendt, R. Niessner, J. Ring, M.G. Weller, Microarrays for the screening of allergen-specific IgE in human serum, Anal. Chem. 75 (2003) 556–562.

Biographies

Jésus Álvarez has a degree in telecommunications engineering from the Polytechnical University of Valencia (UPV), with a final year project 'Design and simulation of photonic sensors based on resonant rings' in the FP6 SABIO project. He then successfully completed a masters in technology, systems and communication networks (UPV) with the thesis 'Modeling and assessing an energy-aware power-supply wireless sensor nodes for the TUHH (Hamburg University of Technology)'. In 2013 he successfully defended his PhD thesis 'Development of a polarimetric based optical biosensor using a free standing porous membrane' at UMDO (University of Valencia) from work performed in the FP7 Positive project.

Laura Sola received the M.S. degree in chemistry and pharmaceutical techniques in 2008 and the PhD in drug chemistry in 2012 from the University of Milano. She is currently a post-Doc fellow at the Institute of Molecular Recognition Chemistry of the National Research Council of Italy where she works on the design, synthesis and characterization of new polymers for bioanalytical applications in the framework of several international collaborative projects. Her research activity is reported in more than 15 papers on international peer-reviewed journals.

Marina Cretich graduated in biological science, specialty molecular biology at University of Milano in 1998. In 2003 she has been appointed research scientist at the Institute of Molecular Recognition Chemistry of the National Research Council of Italy where she works on the development of new biochemical research methods in the microscale for diagnosis and monitoring. She has been responsible of national research contracts and staff scientist in several EC funded projects. Her scientific activity, documented by more than 40 articles on peer reviewed journals, covers the field of microarrays, microchip electrophoresis and microfluidics.

Marcus Swann (CChem, MRSC) is chief scientific officer for Farfield Group Ltd., having originally joined as a post-doctoral researcher in 1999. Marcus is a physical chemist, obtaining his PhD from the University of Bristol in 1991, studying conducting polymers with the electrochemical Quartz Crystal Microbalance. Since then he has undertaken research at the Universities of Durham (Physics) and Glasgow (Bioelectronincs, Electrical Engineering) and has worked with a wide range of surface analytical methods. In the last 13 years he has developed the application of the Dual Polarization Interferometry waveguide technique in areas ranging from surface science through to protein characterization and molecular interactions.

Kristinn B. Gylfason is an assistant professor of micro and nanosystems at the KTH Royal Institute of Technology, Stockholm, Sweden. He received the PhD degree in electrical engineering from KTH in 2010, and the BS and MS degrees in electrical engineering from the University of Iceland in 2001 and 2003, respectively. From 2003 through 2005 he was a research engineer at Lyfjathroun Biopharmaceuticals, Iceland, and from 2010 until 2014 he was a researcher at KTH. During spring 2013 Kristinn was a visiting post-doctoral scholar at the Photonics Group, Ghent University, Belgium. His research involves photonic sensors for biomedical applications.

Tormod Volden received the Ingénieur Diplômé degree in engineering physics from Institut National des Sciences Appliquées, Toulouse, France in 1995 and worked as a staff engineer at the Norwegian Radium Hospital, Oslo, Norway. From 2000 he was a research assistant at the Physical Electronics Laboratory of ETH Zurich and in 2005 received the PhD degree on CMOS-integrated sensors for atomic force microscopy and biochemical detection. He is since developing microfluidic actuators for the company Osmotex and from 2010 for CSEM (both in Alpnach, Switzerland) in the field of liquid handling and microfluidics.

Marcella Chiari graduated in Chemistry and Pharmaceutical Techniques at the Istituto di Chimica Organica, Facoltà di Medicina, Università di Milano in 1982. She received the Diploma in Clinical Biochemistry, Università di Milano in 1990. Since 1992 she has been Senior Researcher at the ICRM, CNR, where she leads the laboratory "Development of Analytical Microsystems". Her research activity is documented by more than 100 publications and several patents. She has been a contractor of the EC in the framework of different projects and responsible for several national research programs.

Daniel Hill gained silicon fabrication experience at Philips Semiconductors following a PhD in semiconductor materials (University of Liverpool) before returning to research. Since 2006 he has worked in biophotonics, being awarded the FP7 InTopSens, FP7 Positive and FP7 CanDo projects as well as coordinating the FP6 SABIO project. In 2011 he joined the UMDO group (University of Valencia) focussing on the nanostructuring of photonic and plasmonic materials for novel matter and electromagnetic wave interaction controls to add functionality in optical biosensors. A member of the IEEE, SPIE, and IOP, and a chartered physicist, he regularly contributes to International Journals and Conferences.