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Optimisation synthesis and biosensing performance of an acrylate-based hydrogel as an optical waveguiding sensing film

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ABSTRACT: The metal-clad leaky waveguide (MCLW) is an optical biosensor consisting of a metal layer and a low index waveguide layer on a glass substrate. This label-free sensor measures refractive index (RI) changes within the waveguide layer. This work shows the development and optimisation of acrylate based-hydrogel as the waveguide layer formed from PEG diacrylate (PEGDA, Mn 700), PEG methyl ether acrylate (PEGMEA, Mn 480), and acrylate-PEG_{2000}-NHS fabricated on a substrate coated with 9.5 nm of titanium. The acrylate-based hydrogel is a synthetic polymer, so properties such as optical transparency, porosity and hydrogel functionalisation by a well-controlled reactive group, can be tailored for immobilisation of the bioreceptor within the hydrogel matrix. The waveguide sensor demonstrated an equal response to solutions of identical RI containing small (glycerol) and large (bovine serum albumin; BSA) analyte molecules, indicating that the hydrogel waveguide film is highly porous to both sizes of molecule, thus potentially allowing penetration of a range of analytes within the porous matrix. The final optimised MCLW chip was formed from a total hydrogel concentration of 40% v/v of PEGMEA - PEGDA (M_n 700), functionalised with 2.5% v/v of acrylate-PEG_{2000}-NHS. The sensor generated a single-moded waveguide signal with a RI sensitivity of 128.61 ± 0.15° RIU^{-1} and limit of detection obtained at $2.2x10^{-6}$ RIU with excellent signal-to-noise ratio for the glycerol detection. The sensor demonstrated RI detection by monitoring changes in the out-coupled angle resulting from successful binding of d-biotin to streptavidin immobilised on functionalised acrylate hydrogel, generating a binding signal of $(12.379 \pm 0.452)x10^{-3}$°.

INTRODUCTION

Optical waveguide biosensors form an emerging technology for environmental and safety control. The sensitivity and specificity of the sensor depend on the properties of the bioreceptor combined with the engineered design of the transducer.[1] This paper describes how a synthetic hydrogel can be used as a waveguide sensing layer in a metal-clad leaky waveguide (MCLW) biosensor, in which the light confining mechanism at the waveguide-sample boundary is total internal reflection (TIR) and Fresnel reflection at the waveguide-substrate interface.[2] The majority of conventional optical waveguide-based devices, are fabricated from rigid and solid materials like silicon or silicon nitride, and so restrict interaction of bioentities to the evanescent field at the interface, and thus do not permit biomolecular access to the energy-rich core field of the optical waveguide.[3-5]

For MCLW based optical waveguide devices, the sensitivity of the device was improved by incorporating a 3D-porous structure of hydrogel material to serve as the waveguide layer, to enable larger protein molecules to diffuse into the waveguide and interact with the optical mode propagating in that layer.[6-10] Hydrogels are considered as smart materials in that they are biocompatible and can be designed to respond to, or sense, different external stimuli including pH, temperature or light.[11-15] Moreover, the volumetric change in the hydrogel is reported through modulation of optical properties such as reflection, diffraction, refraction, Surface Plasmon resonance (SPR) or emission upon interaction with the analyte, thus allowing the optical changes to correlate with concentration and binding of analyte. In addition, hydrogels have a highly open structure and large inner surface, thus increase loading capacity of active binding sites modified with a bioreceptor or biorecognition element (BRE) to specifically recognise the target analytes compared to evanescent wave sensors. If attached to a metallic surface, these transparent films can serve as optical waveguides that support additional waves propagating along the sensor surface with lower losses than surface plasmons employed in regular SPR biosensors.[16-18] For example, measurement of analyte binding-induced refractive index (RI) changes in the hydrogel-based optical waveguide biosensor enable increased LOD by a factor of 5 with respect to regular SPR due to the hydrogel enhanced figure of merit (FOM) and resolution.[18]

Natural hydrogels with a RI value lower than the substrate, can improve the sensitivity of the MCLW device by two to ten-fold compared with structures previously reported.[2,19-21] However, natural hydrogels such as agarose[2] and chitosan[22] are generally chemically cross-linked using glutaraldehyde,[7,23-25] which tends to solidify the gel before completing the fabrication of the chip device. Human error such as delays in hydrogel spin coating from one chip to another can cause unreliable and uncontrolled quality of the fabricated natural-based hydrogel thin film. Gel hardening causes increased gel viscosity, thus increasing the thickness of the waveguide layer, and so affecting its waveguide properties. The properties of natural hydrogels are also difficult to modify. For instance, chitosan is hydrophobic, causing poor solubility in both water and organic solvents.[26] Consequently, it is challenging to use chitosan as a waveguide material in MCLW sensors, since it requires a longer time for each buffer to diffuse and stabilise within the waveguide. Also, the natural polymer quality is not consistent across each commercially supplied batch, thus affecting the quality of the hydrogel produced. Quality inconsistency will affect the biocompatibility of the hydrogel, due to the functional group (*e.g.* $–NH_2$) within the hydrogel. This functional group links the hydrogel matrix with the BRE so that the biomarker can be covalently immobilised within the biosensing structure. Since the functional group quantity cannot be measured from batch to batch, it's variation will significantly affect the specificity and sensitivity of the biosensor. For example, if the active functional group is presented in excess, the process to block the remaining active surface will not occur under optimal conditions, causing false-positive detection of disease.

This work aims to overcome the limitations of naturally occurring hydrogels by substituting them with a new formulation of synthetic acrylate-based hydrogel as a waveguiding sensor material. A method to improve the sensitivity of the MCLW sensor by incorporating an amine-reactive functional group to bind proteins in a low index acrylate-based polymer is presented. The optimised hydrogel properties include its light-guiding properties such as optical transparency and porosity behaviour. These can be tailored to different RI values, by controlling the polymer type, structure and composition.[27] The functional group used to activate the hydrogel can be manipulated to match the BRE needed for the biosensor. Ultraviolet (UV) light is used to crosslink the polymer, ensuring consistent viscosity of the acrylate solution deposited on the substrate, thus reducing human error during fabrication of the MCLW chip, and ensuring a simple, fast step especially for mass production of the device. The synthetic polymer quality is consistent from batch to batch, using chemicals easily available on the market, ensuring consistent waveguide properties in the MCLW biosensor.

EXPERIMENTAL PROCEDURES

Instrumentation. The instrumentation used to probe the MCLW devices was previously described in detail.[2] The optical arrangement is contained within a light-tight enclosure, to shield external light from the detector. In order to carry out the RI sensitivity measurements of the MCLW chip, a 650 nm superluminescent light-emitting diode (SLED EXS210035-02, Exalos, Schlieren, Switzerland) was used. The light emitted from the SLED was collimated then TE-polarised using a polariser (32 CA 25, Comar Instruments, Cambridge, UK) and passed through a cylindrical lens to form a wedge beam. This beam of light was then coupled into the MCLW device through a glass prism (N-BK7, $n \approx 1.52$, Qioptic Photonics, UK). A drop of index matching fluid (Series A, Cargille Labs, New Jersey, USA) with RI of 1.517 was placed between the MCLW chip and surface of the prism to optimise the optical coupling between them. The chips to be characterised were mounted under a CNC machined flow cell, made of 3 mm thick black acrylic plate. The detail of system design used is described in detail in Supporting Information illustrated by Figure S1. Later, the flow cell was placed on the MCLW chip and was held in place using a water-cooled fixture (Pharmacia Biotech) maintained at 20°C clamped on top. The incident angle (θ_i) of the light source was fixed at 64° to obtain the peak reflected light signal at the centre of the image sensor. The optical signal output from the MCLW chip (the white line corresponding to the coupling angle at the effective RI in the reflected light) versus time was measured as a function of RI (Δn), by introducing different RI buffer solutions into the flow cell, and detection using a camera (USB2.0 6.6-megapixel monochrome PL-B781, PixeLink Inc., Ottowa, Canada). The data was transferred to a host PC, and the angular position of the peak in reflected light was monitored in real-time using customised in-house software written using Embarcadero C^{++} Builder (Embarcadero Inc, Austin, Texas, USA). The software enabled controlled exposure time (ms), to optimise the contrast (peak height and width at half height) of the image captured by the camera. The image area was divided into several regions, each converted to a one-dimensional profile by integrating intensities along the x-axis and plotting them as a function of pixel values along the y-axis (where the x- and y-axis represent the position across the width of the chip and the angular position of the reflected light peak respectively), to display the waveguide out-coupling profile.[28] The position of peaks in the one-dimensional intensity profile was estimated using a centre of gravity algorithm,[9] where the threshold was set at 50% of peak height. The camera pixel dimensions were converted to an angular position in degrees, using a calibration set-up of 1 pixel ≈ 0.0015°, to display the optical peak position change, $\Delta\theta$. The waveguide modelling package (LPRO v4.1) was used to theoretically obtain the waveguide profiles of TE_0 mode as well as plotted reflectivity against coupling angle for the acrylate-based MCLW chip.

Chemicals and Materials. 1 mm thick glass slides (ECN 631–1550, VWR, Leicestershire, UK), titanium (>99.99%, 433667), PEG diacrylate; PEGDA (M_n 700, 455008), PEG methyl ether acrylate; PEGMEA (M_n 480, 454990), 2–Hydroxy–4′–(2-hydroxyethoxy)–2–methyl propiophenone; HHEMP (98%, 410896), 3–(Trimethoxysilyl)propyl methacrylate; APTES (≥98%, A3648), NaOH (306576), HEPES sodium salt (H8651), fluorescein (98%, F245–6), Phosphate buffer saline; PBS (P3813), streptavidin (S0677), serum albumin from bovine; BSA type V (05479), d-biotin (B4501), (all: Sigma-Aldrich Ltd, Dorset, UK). Acrylate-PEG_{2000}-NHS ester; ACLT-PEG-NHS (ZZ236P009) (JENKEM Technology, Plano, Texas, USA), Decon 90 (Decon Laboratories, Hove, UK) and methanol, glycerol (>99.5%, G10650108) and absolute ethanol (Fisher Scientific, UK) were used as received.

Preparation of MCLW chips. The glass slides were washed with three types of solutions; Decon 90 (0.5% v/v), NaOH solution (0.1 M) and ethanol (50% v/v) in an ultrasonic bath, for 30 mins each, after which the glass slides were dried in the oven at 110°C for 1 hour. The cleaned slides were coated with 9.5 nm thick of titanium (Ti) at a rate of 0.2 $nm.s^{-1}$ and pressure of 10^{-6} mbar using an e-beam deposition system (Auto500, Edwards Ltd, Crawley, UK). The thickness of Ti was selected using the previous publication[24], which the Ti thickness was established at 9.5 nm (n = 2.11 – 2.88i at 2 eV/620 nm) to select single-mode operation of the TE_0 waveguide profile. A diamond scribe was used to cut the glass slides into 25 x 25 mm squares, which were air-cleaned with nitrogen to remove debris caused by the cutting process. The detail of the cutting process is described in Supporting Information of Figure S1. The selected composition of the acrylate-based polymer was deposited at the appropriate spin speed and time on the top of a Ti-coated glass substrate using a spin coater (CHEMAT Technology KW-4A, Sigma-Aldrich Ltd, Dorset, UK). The chips were then cured under UVA light of 105 $mW.cm^{-2}$ (Model 2000-EC, Dymax Corp., Torrington, Connecticut, USA) for 10 mins under a nitrogen blanket to avoid oxygen inhibition of the curing process.

The synthesis of PEGDA (M_n 700) hydrogel. Synthesis of PEGDA involved optimising its properties such as transparency by controlling the composition and conditions to cure the solution. PEGDA (M_n 700) contained 100 ppm MEHQ as an inhibitor, which required removal from the monomer prior to use, using inhibitor removers (Aldrich, 306320). The glass vial containing the solution was then ultrasonicated (Ultrasonic, 230V, Hilsonic, Bromborough, UK) for 10 mins at room temperature. After that, the solution was degassed with nitrogen gas within a sealed environment and crosslinked under a UV lamp for 10 mins. The optimisation of PEGDA M_n 700 was performed by increasing the concentration of PEGDA from 2.5% to 5%, 10% and 20% v/v with 1% w/v of HHEMP in a total volume of 3 ml in deionised (DI) water. Then, the 10% and 20% v/v of PEGDA Mn 700 were further spin-coated onto the MCLW chip at 5000 rpm for 30 seconds and cured under the UV lamp system for 10 mins with a nitrogen blanket.

The synthesis of PEGMEA–PEGDA hydrogel. The procedure was similar to that of forming hydrogel from pure PEGDA (M_n 700), except that PEGMEA (M_n 480) was added during the mixing process. The total polymer concentrations were varied at 20%, 40% and 60% v/v with ratio composition of 4:1 of PEGMEA to PEGDA with 1% w/v of HHEMP. The solution was then ultra-sonicated in the water bath for 10 mins prior to deposition on the glass substrate. The polymer mixtures of PEGMEA-PEGDA were spin-coated at 3000 rpm for 30 seconds, following the same procedure as used for agarose-based MCLW chips.[7]

At 40% v/v of total hydrogel concentration, the concentration of PEGDA and PEGMEA was varied at composition of 4% - 36%, 6.25% - 33.75% and 8% - 32%; respectively with 1 ml of 1% w/v of HHEMP to form a total volume of 3 ml with DI water. Then, the polymer compositions at 40% v/v of total polymer concentration were spin-coated at 6000 rpm for 3 mins. Finally, the deposited hydrogel on the Ti coated slides was UV-cured under nitrogen for 10 mins. The completed MCLW chips were stored at room temperature under darkroom conditions prior to testing using the optical waveguide instrument.

Synthesis of PEGMEA–PEGDA–NHS ester hydrogel. A hydrogel containing NHS groups was synthesised by adding ACLT-PEG-NHS to the solution to activate the hydrogel waveguide layer. BREs containing available amine groups added to the activated hydrogel would then react with the immobilised NHS groups. 2.5% v/v of ACLT-PEG-NHS in 40% v/v of total polymer concentration was added during the mixing process. HHEMP and DI water were added to keep the same overall concentrations as the previous hydrogels. The hydrogel was deposited at a spin speed of 5500 rpm for 3 mins on the silanized substrate. As longer detection time was needed to immobilise the BREs, the substrate of the chip was modified via silanization in order to form a stable bond between the substrate and the sensing layer. The primary purpose of silanization is to form bonds across the interface between the substrate and the deposited hydrogel by the adsorption and covalent binding of silane molecules onto substrate surfaces.[29-31] The details of an optimisation procedure of surface-modified for the MCLW chip used via silanization was previously reported.[32] The silanized substrate was freshly prepared immediately before the spin coating step. Silanization was carried out by treating the Ti-coated side of the chips using 5% v/v APTES prepared in absolute ethanol. The chips were immersed for 1 hour at room temperature, and then thoroughly rinsed with ethanol and DI water, dried under nitrogen then further activated with 1% v/v ACLT-PEG-NHS. The NHS linker was introduced to the treated substrate to improve the hydrophilicity of the treated substrate via $–PEG_{2000}$ compound and link with hydrogel by acrylate functional group. The activation was performed by incubated the surface of the silanized chips with 1% v/v ACLT-PEG-NHS for 2 hours. The chips were rinsed with copious amounts of with DI water to remove the unbound acrylate linker, dried under nitrogen and then placed in an oven at 110°C for minimum 1 hour prior to use.

Optical Reflectivity Measurements. The optimum thickness of the different hydrogel solutions was selected to support a single sharp reflectivity peak indicating single-mode behaviour. If two modes were detected, the waveguide layer was too thick, and if no optical mode or a distorted mode was detected, the waveguiding layer was too thin to support waveguiding.[33,34] The determination of the number of guided modes was an established procedure used for the evaluation of the layer thickness and the RI of the waveguide. The number of guided modes will increase as the thickness of the waveguide layer increases.[35] If the peak intensities of the waveguide reflectivity profiles are not the same as each other, this indicates that the hydrogel is not uniformly distributed with equal thickness throughout the substrate, causing non-uniform penetration of the evanescent field coupled light into the waveguide layer. The uniformity of the hydrogel waveguide thickness is crucial when designing the MCLW chip with multiple channels, to ensure identical behaviour across the channels. The transparency of the hydrogel was measured at 650 nm using UV-Vis spectroscopy (6715 Jenway, Staffordshire, UK).

Pore Size Determination. The pore size of the hydrogel-forming the waveguide layer within the MCLW chips was studied by measuring the $\Delta\theta$ (as a function of measurement time response) when two types of the buffer with equal RI

value (n=1.3346) were injected into the flow channel. Firstly, deionised (DI) water was injected, to form a baseline measurement at RI of 1.33 followed by 1% w/v of glycerol in HEPES buffer and then 0.75% w/v of BSA in HEPES buffer. Finally, the DI water was flushed through, to ensure that all BSA was flushed out from the hydrogel waveguide layer, preventing BSA from being entrapped inside the structure. The change in peak angle indicates a change in RI, Δn. The diffusion study was performed to observe the porosity behaviour of the hydrogel when the ACLT-PEG_{2000}-NHS was added inside the composition. The diffusion analysis was done on the 40% hydrogel composition with 2.5% v/v of NHS ester by diffusing a dye (0.5 mM of fluorescein solution prepared in PBS, pH 9).

RI sensing and binding kinetics of BRE species. The MCLWs chip made of 40% v/v PEGMEA-PEGDA functionalised with 2.5% v/v of NHS linker and spun on silanized Ti-coated substrate was used to demonstrate sensor performance when real-time monitoring of a change in Δn. Sequences of different RI solutions were injected into the flow cell channel starting with DI water as a baseline, followed by PBS and a series of glycerol-PBS solution (0.25%, 0.5% and 1% w/v glycerol) and finally rinsed with DI water.

The immobilisation of protein inside the functionalised hydrogel matrix was investigated using streptavidin and d-biotin as a BRE species by monitoring the signal ($\Delta\Theta°$) in real-time measurement. All the solutions used were prepared in PBS (0.01 M, pH 7.4). The chip with dual flow cell channels (see
Figure S1, (a)) was used to perform this testing, and all buffers were pumped at a flow rate of 0.2 $ml.min^{-2}$. Firstly, the DI water was pumped across both flow cells channels following with PBS. For the test channel, a series of proteins were pumped-in started with the streptavidin, blocked with BSA followed by d-biotin. The reference channel was continuously circulated with PBS buffer as a control, blocked with BSA and followed by d-biotin to observe if there is any unspecific binding. The channels were washed with PBS for every change of the protein to remove any unbound proteins from the hydrogel matrix. In order to obtain the differential binding signal of the d-biotin to the streptavidin, the differential angle measured after washing procedure of the blocking BSA was subtracted to the differential signal measured at the final washing step.

RESULTS AND DISCUSSION

Optimisation synthesis of the acrylate-based hydrogel. For PEGDA (M_n700), a series of concentrations were used, ranging from 2.5% to 20% v/v with 1% w/v of HHEMP. Figure 1(a-b) shows the visual appearance of synthesised PEGDA (M_n 700) hydrogels made of at concentration of 10% and 20% v/v. The hydrogel sensing layer was required to be transparent to the light used to interrogate the waveguide, to optimise the light intensity collected at the sensing layer.[36] The correct choice of molecular weight of PEGDA is required to form the transparent hydrogel, since lower molecular size of macromers contains an increased concentration of acrylate group, which cause increased crosslinking density. Thus, an opaque hydrogel will be formed, since less water is absorbed into the structure during the crosslinking reaction.[37] PEGDA (M_n 700) generated a transparent film of the crosslinked product with a transmittance value above 90%. However, only 20% v/v of PEGDA was used to prepare a hydrogel waveguide film since a lower concentration, the viscosity of the solution was too low, thus causing problems in spinning the coating evenly on the substrate. The waveguide profile (see Figure 1(c)) of the measured reflected light intensity across the chip. The signal generated, formed by the white contrast line was broad and distorted with a noisy background (see the inset image), indicating that the thickness of the hydrogel distributed across the chip was non-uniform.

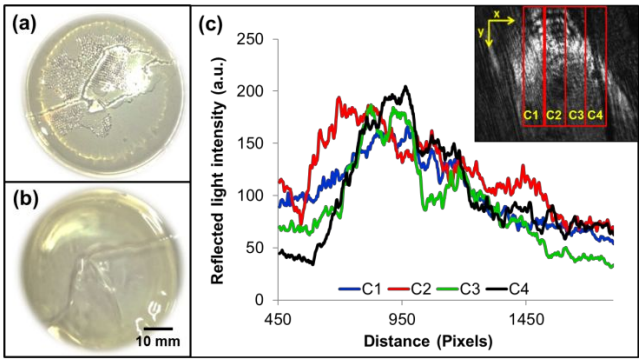

Figure 1: Photograph of crosslinked PEGDA (M_n 700) hydrogel for (a) 10% and (b) 20% v/v. (c) The reflected light intensity of 20% v/v PEGDA (M_n 700) with insert shows the waveguide profile observed on the camera.

Nevertheless, the main challenge for using pure PEGDA (M_n 700) as a hydrogel waveguide film, involved low adhesion properties of the hydrogel onto the substrate, thus preventing the PEGDA solution from forming a homogeneous thin film on the substrate during the spin coating process. For this reason, PEGMEA (M_n 480) was introduced into the hydrogel to increase the adhesion of the hydrogel to the Ti-coated glass substrate. PEGMEA is a group of polymer brushes which were selected to induce strong interaction between the brush-surface structures and the substrate, by providing higher surface tension through dipole-dipole interactions.[38] In addition, PEGMEA has excellent solubility and wettability to enable homogenous dispersion onto the substrate surfaces.[39] Moreover, the hydrogel formed by PEGMEA and PEGDA was non-toxic and protein compatible.[40]

The total composition of PEGMEA and PEGDA hydrogel on the MCLW chip, was varied between 20%, 40% and 60% v/v. All hydrogels formed were transparent (see Figure S2(a)) with the transmittance values above 92%. The measured waveguide modal properties indicated that a guiding waveguide mode was obtained for samples with hydrogel concentrations at 40% (Figure S2(b)) and 60% v/v (Figure S2(c)) of PEGMEA-PEGDA with a spin speed of 3000 rpm for 30 seconds. At low hydrogel concentrations (20% v/v); the film thickness was too thin to support a waveguiding optical mode within the layer. The solution became viscous when the hydrogel concentration was increased to 60% v/v, and formed a thick film layer, which displayed a double-moded output intensity profile as seen in Figure S2(c). At 40% v/v hydrogel composition, the waveguide exhibited single-mode operation. Because no reflectivity peak was observed for 20% v/v hydrogel at 3000 rpm spin speed, the waveguide was below

the cut-off thickness. The "cut-off-thickness" for a mode 'm' is obtained when the m^{th} mode order no longer exists in the film's smallest thickness. When the waveguide is below the cut-off for the 0^{th} mode, then no mode can propagate. This means that at this film thickness, the field's propagation angle in the film drops below the critical angle at the waveguide film-cover interface, thus the light escapes from the waveguide.[5] From Figure S2(b), the single-mode operation of the waveguide was not uniformly supported over its surface, showing that the speed and time used to spin the hydrogel should be increased to generate a thinner, homogenised hydrogel film. Consequently, at 40% v/v of total concentration of PEGMEA-PEGDA should be used for further investigation, to find the optimum condition for the waveguide layer of the MCLW device.

Figure S3 shows the output profile of the hydrogel waveguide formed when each element of 40% v/v PEGMEA-PEGDA hydrogel, was varied between 4%, 6.25%, and 8% v/v of PEGDA spun at 6000 rpm for 3 mins. The result indicated that all the hydrogel compositions used on the MCLW chips, supported a single-moded waveguide, and longer spin time (3 mins) at higher speed (6000 rpm) is needed to form a thin layer for the waveguide. Figure S3(b) shows a sample; 33.75% v/v PEGMEA with 6.25% v/v, with the optimum distribution of hydrogel material, demonstrated by a sharp, uniform peak in each column of the testing regions (see Figure S3(b)). Furthermore, the signal-to-noise ratios (*S:N*) and peak width at half height (*P50*) of the selected testing region (C1, C2, C3 and C4) from the Figure S3 were calculated using Origin 8.5 software and the combined information was plotted in Figure S4. The *S:N* was calculated from the highest reflected peak light intensity signal divided by the average background noise.

From the graphs (Figure S4), the *S:N* value is maximised for the signals from the chip made of 6.25% v/v of PEGDA, while the highest noise is observed when 4% v/v of PEGDA was used. The distribution of the hydrogel is defined by the *P50* value, since a low *P50* value indicates a more homogeneous hydrogel distribution.[41] Figure S4 shows that for the MCLW chip made of 6.25% v/v of PEGDA, each testing region has the lowest *P50* value between 133 to 270 pixels, indicating that the substrate surface is completely covered by the hydrogel. In contrast, the *P50* values for the chip of 4% v/v of PEGDA ranging between 271 to 1065 pixels indicate non-uniform distribution of hydrogel over the substrate.

To recap, the plotted *S:N* and *P50*, indicates the optimum composition of hydrogel to ensure a uniform coating over the substrate. The presence of PEGMEA improved the adhesion to the substrate.[42] Increasing the PEGDA concentration in the total hydrogel concentration indicates that a more crosslinked structure is formed, but the concomitant reduction of PEGMEA concentration means that less hydrogel will attach to the substrate. Meanwhile, if insufficient PEGDA concentration is used, an inhomogeneous hydrogel network is formed, causing non-uniform distribution of the hydrogel network across the waveguiding film. Overall, this affects the reflected light of the film, due to non-uniform scattering across the film.[43]

Pore Size Determination. The RI of BSA was matched with the RI of the glycerol solution to compare their effect on the peak angle shift of the device. The RI of 0.75% w/v BSA in HEPES buffer was measured at 1.3346 which is equal to the RI of 1% w/v glycerol, to provide a valid comparison between small (glycerol) and large (BSA) analytes. Figure 2(a) shows a graph of peak shift measured when the MCLW chip was tested with buffers; 1% v/v glycerol and 0.75% w/v of BSA. The sensor signal output changed by the same value in response to the solutions, demonstrating a similar peak shift, $\Delta\theta$=0.15°, indicating that the hydrogel was highly porous to small and large molecules, indicating its potential to be used for biosensor applications.

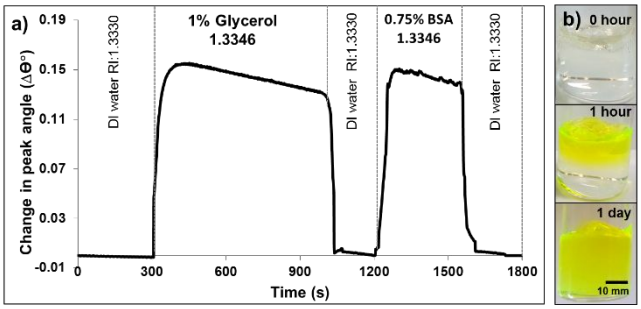

Figure 2: (a) Sensor response to peak angle of the MCLW device for RI measurement of 1% v/v of glycerol vs. 0.75% w/v of BSA. (b) Diffusion of 40% v/v hydrogel with 2.5% v/v of ACLT-PEG-NHS linker using fluorescein solution.

The porosity of the functionalised hydrogel was investigated by diffusion of a dye; fluorescein solution. Here, the comparison approach of small and large analytes using BSA and glycerol at the same RI value cannot be performed; since BSA will tend to attach to the functionalised hydrogel through its surface amines. Consequently, the diffusion of the dye in the hydrogel was studied when 2.5% v/v of ACLT-PEG-NHS was added to the composition. As a result, the dye diffused inside the hydrogel as seen in Figure 2(b) thus confirming that the linker presence did not affect the density and porosity of the hydrogel for small molecules. This diffusion study is an innovative approach to study the porosity of the wet hydrogel system[44], in which the imaging technique is difficult to perform as the sample preparation step could damage the sample and not represent the real condition of the hydrogel structure in thin-film condition on the MCLW device.

The silanized Ti-coated substrate for MCLW chip was further functionalised with the linker; 2.5% v/v of ACLT-PEG-NHS to bind the BRE for biomolecule detection. The transparency of the hydrogel film was measured to give a transmittance value of 94%. The single TE_0 mode of waveguide profile plotted using LPRO software is shown in Figure S5 (Supporting Information). Figure 3(a) shows the device signal converted from a change in the pixel number Δx to change in peak angle $\Delta\theta^o$ using a single-channel flowcell. To demonstrate real-time monitoring of Δn detection, the sensor generated a rapid response when different buffers were introduced to the cover medium, by causing a rapid change of $\Delta\theta^o$ in Figure 3. The result indicated that the chip fabrication using 5500 rpm spin speed for 3 mins produced a waveguide thickness which is sufficient to support a single mode in the waveguide. Figure 3 shows that the device gave a uniform response to Δn in the ten areas across the width of the chip (columns C1-C10) and returned to baseline when irrigated with DI water. Since the sensing peaks returned to their initial

$\Delta\Theta°$ for all tested columns following the final DI water injection, this indicates that the glycerol molecules completely diffused out from the hydrogel matrix. The relationship between the $\Delta\Theta°$ and RI of glycerol solutions (n_{gly}) is given by $\Delta\Theta° = (128.61 \pm 0.15)n_{gly} - 171.43$ (r^2:0.9988) is shown in Figure 3 (b). The signal of the change in a peak angle of Figure 3 (a) was also observed in a stable state (as a horizontal straight line) throughout the detection. Note that this is different compared to the same substance tested in Figure 2(a) due to the improvement made resulted from the surface modification of the chip. This shows that the covalent bonding between the acrylate hydrogel and the silanized substrate is improved, thus leading to the generation of a steady signal.

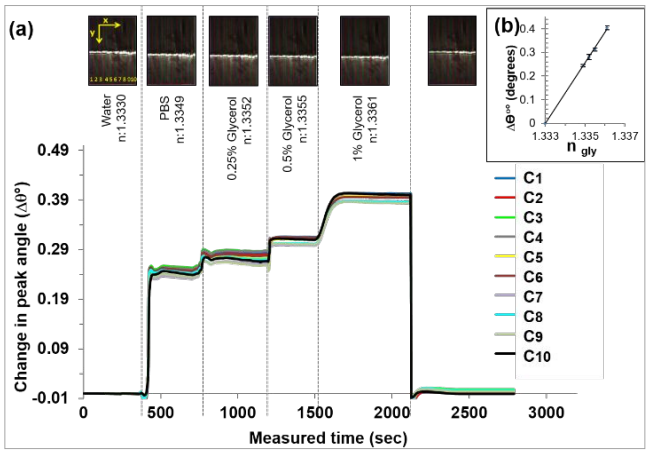

Figure 3: (a) The detection of Δn for different glycerol concentrations of MCLW chip made on silanized substrate, 40% v/v hydrogel with 2.5% v/v of ACLT-PEG-NHS measured the $\Delta\Theta°$ vs. time (where inset shows the monitoring of the waveguide mode under each buffer) and (b) The linear relationship between $\Delta\Theta°$ and RI of glycerol with the error bars (some are too small to be visible).

Thus, the RI sensitivity of the MCLW is 128.61 ± 0.15° RIU^{-1}, which exceeded previously published results[7] whereby here, the $\Delta\Theta°$ for 1% v/v of glycerol in PBS (n=1.3361) generated a signal $\Delta\Theta$ = 0.40° which is 44% better than previous work ($\Delta\Theta$ = 0.176°) reported. Moreover, the RI limit of detection was 2.22×10^{-6} RIU calculated from three times the standard deviation of the angular peak position (9.5×10^{-5}°) divided by the RI sensitivity (128.61° RIU^{-1}). In addition, this sensitivity approaches that of SPR devices (*e.g.* 6×10^{-5} RIU for glycerol detection)[45]. This experiment also verified that MCLW devices made from synthetic acrylate-based hydrogel are more sensitive than natural agarose-based hydrogel for Δn detection. The explanation behind this may be that the synthetic hydrogel consisted of a more porous structure with larger pore sizes, thus enabling the samples rapidly to diffuse through the hydrogel. In addition, the uniform distribution of the hydrogel throughout the modified substrate improved the quality of the waveguide profile observed on the camera.

The performance of the activated hydrogel was investigated by first measuring the immobilisation of streptavidin through the reaction of its surface amines with the NHS groups on the hydrogel followed by blocking the remaining active surface with BSA and finally binding of d-biotin using optical waveguide detection. Figure 4 shows a real-time measurement of the immobilisation of streptavidin followed by binding of d-biotin by monitoring $\Delta\Theta°$ with the blue line representing the test channel, the red line for the reference channel while the black line for the differential angle measurement. The result showed that the sensor detected streptavidin reacting with the functionalised hydrogel waveguide layer, and then the interaction with d-biotin. The binding of d-biotin to streptavidin within the hydrogel matrix caused a permanent shift in the pixel position since $\Delta\theta°$ did not return to its baseline, after the channel was flushed with the washing buffer (PBS) as observed by the differential angle (double arrow, inset of Figure 4) measured at $(12.379 \pm 0.452)\times10^{-3}$°. The result indicated a 19% improvement compared with previously reported work[7] (differential angle ~0.01°), which used the same system to measure the biosensing activity. This demonstrated that the functionalised acrylate hydrogel in the active waveguide layer, successfully immobilised the protein through an amide bond with free amino groups (in this case streptavidin), and thus was able to bind the d-biotin with better sensitivity (19% improvement) than agarose-based hydrogel[7]. Since the specific binding between the protein and d-biotin occurs within the guiding mode layer, thus the results of Δn, within this region and consequent variation in the out-coupling of the TE_0 mode can be measured.

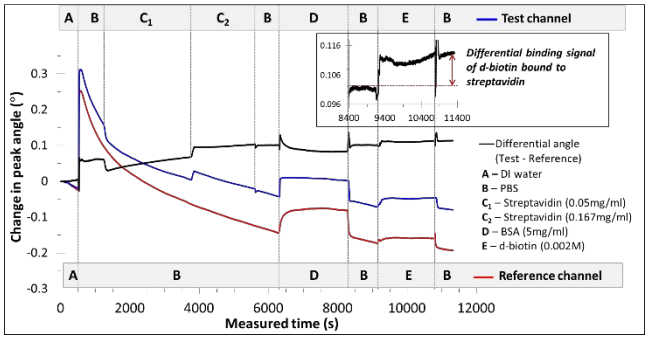

Figure 4: Real-time monitoring detection of protein immobilisation (streptavidin followed by d-biotin) using 40% v/v acrylate hydrogel functionalised with 2.5% v/v of ACLT-PEG-NHS linker of MCLW biosensor. The inset shows the closed-up measurement profile of the differential angle line between 8400 seconds to the end of detection; showing the differential binding signal of d-biotin to streptavidin.

CONCLUSIONS

This work demonstrates the optimisation of the parameters in the development of synthetic acrylate-based polymers (PEGDA and PEGMEA) as a waveguide thin film to achieve the superior analytical performance for the MCLW-based biosensors. The hydrogel made of PEGDA provides transparent gel, however, the fabrication process to deposit the hydrogel onto the substrate posed a challenge. The synthetic polymer; PEGMEA-PEGDA hydrogel was successfully fabricated as a waveguide layer, with the correct composition ratio of the PEGDA to PEGMEA of the hydrogel waveguide layer controlled the interrogating light signal as a single-mode waveform, verified by observation of a single sharp peak characteristic (S:N=16.20) of the TE_0 mode. The sensor demonstrated an equal response ($\Delta\Theta$=0.15°) to solutions of the same RI consisting of small and large analytes, indicating that

the hydrogel waveguide film was highly porous, enabling mobility of a range of analytes within the porous matrix. Overall, this new formulation of acrylate-based hydrogel demonstrated a promising performance as a sensing waveguide layer with the RI sensitivity being 128.61 ± 0.15° RIU^{-1}, which greatly enhanced the sensor sensitivity by a 44% improvement over previously reported devices. In addition, the device was demonstrated to successfully monitored a real-time interaction of protein and a small molecule (streptavidin and d-biotin) in which the binding signal was measured $(12.379 \pm 0.452)\times10^{-3}$°. Future work will focus on the application of the MCLW to detect bacteria and their secretions where several types of bioreceptor will be immobilised in the acrylate-based hydrogel waveguide. Included in the design will be multiple channels for simultaneous detection of two types of bacteria using single samples. This will be advantageous for environmental or medical/biological sensing, providing a low cost and faster procedure.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:

System design for characterisation of MCLW chips; Photographs of hydrogel for 20%, 40% and 60% v/v of PEGMEA-PEGDA; The waveguide profile of PEGMEA-PEGDA hydrogel with (a) 4% (b) 6.25% and (c) 8% v/v of PEGDA; Graphical display of signal-to-noise ratio (*S:N*; straight line) and peak width at half-height (*P50*; dashed line); Reflectivity plots versus coupling angle and mode profiles for the TE_0 for single-mode MCLW and clarification on difference pattern of a signal resulted in Figure 2, 3 and 4.

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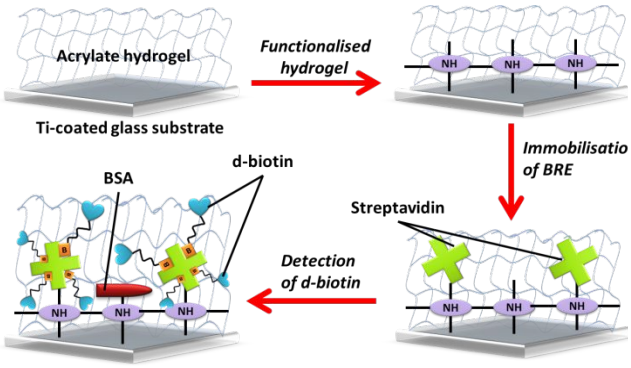
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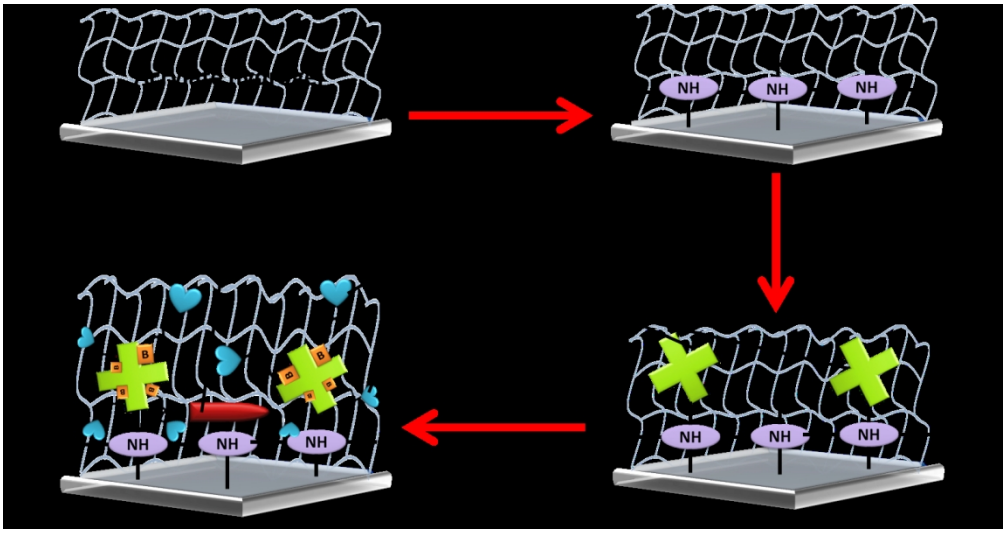

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