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Non-fouling microfluidic chip produced by radio frequency tetraglyme plasma deposition†

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This Technical Note presents the direct surface modification of a glass/PTFE hybrid microfluidic chip, *via* radio frequency glow discharge plasma polymerisation of tetraethlylene glycol dimethylether (tetraglyme), to produce hydrophilic, non-fouling, PEO-like surfaces. We use several techniques including X-ray photoelectron spectroscopy (XPS), direct enzyme-linked immunosorbent assays (ELISA) and immunofluorescent imaging to investigate the channel coatings. Our results indicate the successful deposition of a PEO-like coating onto microchannel surfaces that has both solution and shelf stability (>3 months) and is capable of preventing fibrinogen adsorption to the microchannel surfaces.

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Introduction

Microfluidic systems for medical and biological applications have rapidly evolved. The high surface to volume ratios associated with microfluidic devices is critical for their exploitation in many bioengineering applications,[1] due to the rapid adsorption of biomolecules, especially proteins from the surrounding biological environment.[2] Surface modification strategies capable of eliminating the non-specific adsorption of biomolecules are essential, if reliable and reproducible bioassays and analyses are to be operated. A vast range of methods for controlling protein adsorption and regulating electroosmotic flow (EOF) in microfluidic devices has been reviewed.[3] However, several difficulties arise due to large variations in microchip construction materials, and only a few of these techniques have the longer-term stability required in many electrophoresis and lab-on-a-chip applications.[4]

Polyethylene oxide (PEO)-based coatings are well-known for their abilities to reduce adsorption of proteins and cells onto biomaterials.[5] These surfaces can be prepared through various methods including plasma polymerisation. Plasma polymerisation, *i.e.* formation of polymeric materials under plasma conditions, offers several advantages over other coating techniques, the key one being that it can be applied to almost any substrate without altering its bulk properties. In addition, it is solvent-free, is a one-step coating procedure and due to the fact that there are a large number of monomers available for the process, it can produce a wide range of different surface functionalities.[6] Plasma polymerisation of tetraethylene glycol dimetyl ether (tetraglyme), CH_3–[O–CH_2–CH_2]$_4$–O–CH_3, has been previously shown to exhibit a PEO-like surface in planar and tubular substrates.[7–9] Since plasma polymer formation is a complex process (where repetitive fragmentation, rearrangement, crosslinking, ionization, and polymerisation of the monomers occur), the exact mechanism responsible for protein resistance of the coatings is unknown at present. At present it is postulated that their behaviour may mimic that of short ethylene glycol chains coupled to surfaces *via* self-assembly of alkane thiols on gold.[9] Chemically, it has been established that, the protein resistance of these coatings can be correlated with a number of surface chemical characteristics including high ether carbon content.[10]

In this study, the adaptation of plasma polymerised tetraglyme (ppTG) coating onto microchannels is investigated, where a glass/poly(tetrafluoroethylene) (PTFE) hybrid microfluidic chip is coated using a radio frequency (RF) glow discharge. X-ray photoelectron spectroscopy (XPS), direct enzyme-linked immunoassay (ELISA) and immunofluorescent imaging techniques were used to characterise the coating quality within the channel, as well as the surface non-fouling properties. The stability of the ppTG coatings under shelf storage, buffer immersion and protein-flow conditions is also demonstrated.

Experimental

Hybrid glass/PTFE microfluidic chip

Serpentine and linear microfluidic channels were prepared in glass (width × depth = 100 × 75 μm^2) by standard photolithographic wet-etching procedures.[11] The glass chip and PTFE sheet were placed in the chip holder and clamped together to form a sealed device.

Plasma polymerisation

Plasma polymerisation of the glass microfluidic chip and PTFE sheet was carried out in a home-built stainless steel

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plasma reactor (further details in the ESI†). Deposition power, time and monomer flow rate were fixed at 10 W, 25 min and ~2.5 standard cubic centimeters per minute (sccm) respectively. Due to the low volatility of tetraglyme monomer, both the reactor chamber and the monomer were heated to 55 and 90 °C respectively.

Immunofluorescent labelling and imaging

Static conditions. Human fibrinogen solution (1000 μg ml^{-1}) in phosphate buffered saline (PBS, pH 7.4, 150 mM) was incubated in both ppTG coated and uncoated glass chips for 2 h at room temperature. Blocking solutions (PBS with 3% (v/v) Tween 20 and 5% (w/v) bovine casein), and 0.025% (v/v) polyclonal sheep anti-human fibrinogen IgG (Binding Site Ltd., UK) in blocking solutions were incubated sequentially for 2 h at room temperature. 0.1% Alexa Fluor-546 donkey anti-sheep IgG (Invitrogen, UK) in blocking solutions were then introduced and incubated overnight at 4 °C. Thorough PBS rinsing was performed between each step. The microfluidic glass chip was then transferred to a modified 24-well plate and images were taken using an automated cellular imaging and analysis system (Axon ImageXpress) instrument. A Propidium Iodide filter was selected, with excitation and emission wavelengths of 535 nm and 645 nm respectively.

Dynamic conditions. Human fibrinogen solutions (1000 μg ml^{-1}) prepared in PBS (pH 7.4, 150 mM), were flowed through the microchannels at 0.5 μl min^{-1} for 2 h and 15 μl min^{-1} for 1 h for the low and high-flow rate experiments respectively. The flow rates were controlled using a syringe pump (ProSense BV, model NE-1000, USA). Immunofluorescent labelling identical to the static-experiment protocols were then performed.

Direct ELISA. Direct ELISA experiments were carried out using the protocols previously described.[2] Briefly, the microchannel was rinsed with PBS and sequentially incubated with 1000 μg ml^{-1} human fibrinogen solution, blocking solutions, and goat polyclonal anti-fibrinogen with horseradish peroxidase (HRP) conjugate. Each incubation was performed for 2 h at room temperature with thorough PBS rinsing between each step. Substrate solution was flowed through the chip at 3.0 μl min^{-1} for 17 min and collected into a single well in a 96 well microplate. The optical density(OD) of the well was read with a microplate reader at 405 nm. Negative control experiments without the addition of fibrinogen were also carried out. All the experiments were performed in triplicate.

X-ray photoelectron spectroscopy. XPS analyses were performed using a Kratos AXIS Ultra DLD instrument equipped with a monochromatic Al Kα X-ray source operated at a power of 150 W, with charge compensation on (further details in the ESI†).

The coating coverage and its affect on protein adsorption were investigated by XPS imaging. A section of the microfluidic channel was masked with a solution of polystyrene in toluene (20 wt%) prior to plasma deposition. The mask was removed from the surface prior to protein surface analysis, and the interface between treated and untreated channel analysed for the ppTG-associated C–OR component with monochromated Al Kα source at 160 eV pass energy using the medium resolution imaging mode available on the instrument. After analysing, the glass chip was then immersed in 50 μg ml^{-1} of fibrinogen in PBS for 2 h at room temperature, rinsed in PBS and water and air dried. XPS N 1s core level images were then collected under the same conditions in order to map protein adsorption across the masked and unmasked regions of the channel.

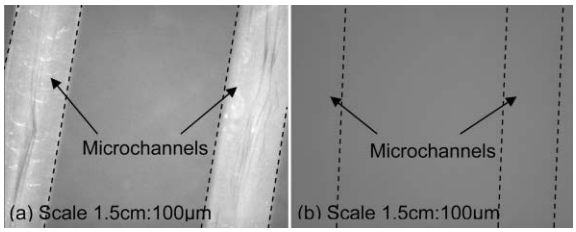

Fig. 1 Immunofluorescent imaging of the (a) uncoated, and (b) ppTG coated microchannels after contact with a 1000 μg ml^{-1} fibrinogen in PBS for 120 min under static flow conditions.

Results and discussion

Immunofluorescence imaging

The efficacy of the ppTG coating inside the microchannels was evaluated using an immunofluorescent imaging technique, where static and dynamic fibrinogen adsorption was assessed. Fibrinogen, due to its most 'sticky' nature, is an excellent candidate for evaluating efficiencies of surface bio-inertness.[12] The results are shown in Fig. 1 and 2. The bright areas indicate the presence of a fluorescent secondary antibody which was coupled to the adsorbed fibrinogen on the microchannel walls. In contrast, no fluorescent signal was observed in ppTG coated microchannels, indicating no fibrinogen adsorption. Negative control experiments were also performed, illustrating no adsorption of anti-human fibrinogen IgG onto the ppTG surface.

As most microfluidic devices require the flow of analytes through the system, it is important to analyse the stability and effectiveness of the coating under dynamic conditions. Both low (0.5 μl min^{-1}) and high (15 μl min^{-1}) flow rates of the fibrinogen solution were tested. From the results shown in

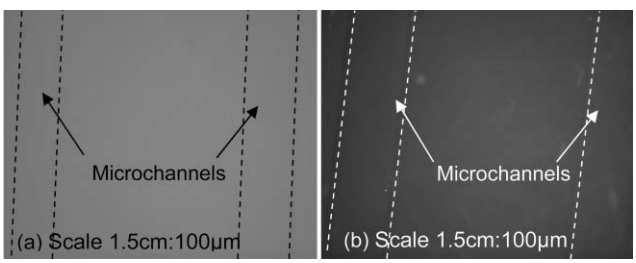

Fig. 2 Immunofluorescent imaging of the ppTG coated microchannels after contact with 1000 μg ml^{-1} fibrinogen in PBS in low flow conditions of 0.5 μl min^{-1} for 120 min (a) and high flow conditions, 15 μl min^{-1} for 60 min (b).

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Fig. 2(a) and 2(b), it can be deduced that no fibrinogen adsorption occurred on the microchannel walls, as represented by the absence of fluorescent signals. Thus, the ppTG coating was shown to be stable under flow conditions.

Direct ELISA

The immunofluorescent experimental results were confirmed using a direct ELISA that we have previously shown to be an effective measure of in-channel protein adsorption. In this colourimetric assay, the OD of the enzyme activated substrate solution can be directly correlated with the amount of protein adsorbed to the channel walls. In three separate experiments, the average OD values measured at 405 nm for the untreated glass channels was 1.50 ± 0.20 while values dropped to 0.05 ± 0.02 for the ppTG coated chips. This ppTG chip absorbance was similar to the background signal (0.05–0.07); confirming the presence of a low-fouling coating inside the channels. Together with the fluorescence imaging data, these results serve to highlight that the coating is continuous over of the surface of the channel as any discontinuity would result in protein adsorption.

X-ray photoelectron spectroscopy

In order to validate the immunoassay results, and confirm the coating chemistry within the channels, XPS analyses inside the channel were performed. ppTG C 1s spectra was fitted with three components, with hydroxyl/ether (C–OR) forming the major constituent (84%) at 286.6 eV relative to hydrocarbon (C–R) at 285.0 eV (14%). Carbonyl groups were found to be present (C=O at 289.2 eV) on all samples in small quantities ($2 \pm 5\%$). The ether component is known to be associated with the PEO, and has been shown previously to correlate with the non-fouling character of the coating. The result shown in Fig. S1(a)† clearly demonstrates the presence of C–OR in the unmasked channel (right). A 15 μm selected area small spot spectral analysis of the surfaces indicated that the ratio of C–O/C–R was similar in both the ppTG coated channel and fibrinogen incubated ppTG channel, *i.e.* $\sim$6 to 8. XPS images collected at the N 1s core level shown in Fig. S1(b)† illustrate that the nitrogen signal that is associated with adsorbed protein is only present in the uncoated regions of the channels. Small spot (15 μm) selected area spectral analysis confirmed that there was no detectable levels of nitrogen within the ppTG coated regions of the channels. Previous work by Wagner *et al.* has shown that XPS is sensitive to <10 ng cm^{-2} of protein, if the substrate material does not contain nitrogen, enabling us to conclude that the protein adsorption within the channels is at least less than 10 ng cm^{-2} and may well be lower. ppTG was also successfully coated onto PTFE sheets, which showed good coating adhesion with virtually identical C–O/C–R ratio and fibrinogen resistance detected by XPS. The chemistry of the coating obtained in this study correlates well with the literature.[7–9]

Coating stability

ppTG coated glass cover slips were used for the stability and water contact angle measurement studies. We studied the stability of this coating for a period of 3 months, with the amount of fibrinogen adsorbed analysed with XPS. Upon shelf storage, the ppTG coating is stable with the chemistry of the surfaces remaining unchanged. No fibrinogen adsorption was observed (0.0–0.1% N 1s). Upon storage/immersion in PBS buffer solution, we confirmed the stability of the glass surface for up to 1 week. The water contact angles of ppTG coated surfaces, determined from a static or sessile drop method, were measured using a Rame–Hart goniometer. The advanced water contact angles of ppTG coated glass cover slips, PTFE sheets and 3 months-aged surfaces were $\sim 50^\circ \pm 10^\circ$, obtained from 3 successive measurements.

Conclusions

In conclusion, plasma polymerisation was shown to be a versatile technique in modifying surfaces for microfluidic applications. Plasma polymerisation of tetraglyme monomers was shown to exhibit a reproducible and controllable coating, which is biologically non-fouling. The coating was also stable under flow conditions, shelf storage of more than 3 months, as well as with buffer and water rinses. This relatively simple and one-step coating may solve several drawbacks from current surface modification methodologies which include a limited shelf life-time, complex coating procedures, and coating instabilities under flow and non-batch processing. We believe that plasma polymerised channels show promising potential for future microfluidic biological applications, as various surface functionalities can be coated onto a wide range of substrates, regardless of the underlying bulk surface properties.

Acknowledgements

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