A novel surface modification approach for protein and cell microarrays

Mahaveer D. Kurkuri*[a], Chantelle Driever[b], Helmut Thissen[b,c], Nicolas H. Voelcker[a,b,c]

[a]School of Chemistry, Physics and Earth Sciences, Flinders University, GPO Box 2100, Bedford Park SA 5042, Australia
[b]CSIRO Molecular and Health Technologies, Bayview Avenue, Clayton VIC 3168, Australia
[c]CSIRO Food Futures Flagship, Riverside Corporate Park, 5 Julius Avenue, North Ryde NSW 2113, Australia;

ABSTRACT

Tissue engineering and stem cell technologies have led to a rapidly increasing interest in the control of the behavior of mammalian cells growing on tissue culture substrates. Multifunctional polymer coatings can assist research in this area in many ways, for example, by providing low non-specific protein adsorption properties and reactive functional groups at the surface. The latter can be used for immobilization of specific biological factors that influence cell behavior. In this study, glass slides were coated with copolymers of glycidyl methacrylate (GMA) and poly(ethylene glycol) methacrylate (PEGMA). The coatings were prepared by three different methods based on dip and spin coating as well as polymer grafting procedures. Coatings were characterized by X-ray photoelectron spectroscopy, surface sensitive infrared spectroscopy, ellipsometry and contact angle measurements. A fluorescently labelled protein was deposited onto reactive coatings using a contact microarrayer. Printing of a model protein (fluorescein labeled bovine serum albumin) was performed at different protein concentrations, pH, temperature, humidity and using different micropins. The arraying of proteins was studied with a microarray scanner. Arrays printed at a protein concentration above 50 µg/mL prepared in pH 5 phosphate buffer at 10 °C and 65 % relative humidity gave the most favourable results in terms of the homogeneity of the printed spots and the fluorescence intensity.

Keywords: Microarrays, thin films, functional polymers

*mahaveer.kurkuri@flinders.edu.au (Telephone +61 8 8201 7978) (Fax +61 8 8201 2905)

1. INTRODUCTION

The development of adequate material platforms for high-throughput assays has become a very important issue in the field of biotechnology enabling genomic[1], proteomic[2] and disease diagnosis studies. Further more, these platforms have contributed significantly to progress in underpinning stem cell technologies[3], drug discovery[4] and tissue engineering[5]. Substrate materials that are commonly used in this area of research are glass, polystyrene, stainless steel, polypropylene and gold[6-8]. In order to incorporate suitable surface chemistries on these substrates, various coating processes including dip or spin coating, surface grafting, plasma polymerization, chemical vapor deposition, self assembled monolayers and layer-by-layer deposition methods have been adopted[3,9-11]. Such functional substrate surfaces then allow the immobilization and study of specific biological factors, in particular those that can control the behaviour of mammalian cells.

Initial efforts to increase throughput in bioassays in the early 1990s has led to a mature microarray technology[12,13]. However, cell-material surface interaction studies including biocompatibility testing still typically use multi-well formats today. For these formats, relatively large amounts of support material, biomolecules, cell culture medium and cells are required. At the same time, these formats are labour- and time-intensive, especially if a large number of samples are to be tested.

Smart Materials IV, edited by Nicolas H. Voelcker, Proc. of SPIE Vol. 6413, 64130Z, (2006)
0277-786X/06/$15 · doi: 10.1117/12.715172

In this study we describe the design and in vitro testing of cell microarray substrate coatings that provide a low cell attachment background, functional groups for the covalent immobilization of biologically active signals and excellent adhesion to the microarray substrate material.

A large number of synthetic polymers have been used in cell surface interaction studies, including acrylate based polymers[13], polylactides, poly(ethylene adipate)[9], nitrocellulose[13] and polyurethanes[15]. Furthermore, Anderson et al[9,14] have used polymer microarrays to characterize a large number of human embryonic stem cell-material interactions. However for the particular purpose of the present study, we have used random copolymers of glycidyl methacrylate (GMA) and poly(ethylene glycol) methacrylate (PEGMA). The polymers were deposited onto glass slides that had been coated with an allylamine plasma polymer (ALAPP) layer. GMA provided reactive oxirane groups for both biomolecule immobilization and attachment to the underlying ALAPP layer whilst the other polymer, PEGMA, acted as the component providing low cell attachment. Different methods of attaching the copolymer to the underlying plasma polymer layer (dip coating, spin coating and grafting) were compared using surface analytical techniques. A model protein (fluorescein isothiocyanate labeled bovine serum albumin, BSA-FITC) was then printed onto the copolymer coatings using a contact microarrayer and the conditions for protein printing (temperature, pH, humidity, concentration and pin type) were optimized.

2. MATERIALS AND METHODS

2.1 Substrate Preparation

Precleaned microscope glass slides obtained from Biolab (USA) were used as the substrate material. The slides were further cleaned by 1 hour sonification in surfactant solution (2% RBS (Pierce, USA), 2% ethanol, 96% MilliQ water) and then washed with MilliQ water. They were then placed into 1M NaOH solution for 5 minutes, washed thoroughly in MilliQ water and dried using a spin dryer (Technical Video, LTD).

2.2 Plasma Polymerization

Plasma polymerization experiments were carried out in a custom-built reactor as described elsewhere[16]. Briefly, the cylindrical reactor chamber is defined by a height of 35 cm and a diameter of 17 cm. The lower and upper electrodes are both circular, with a diameter of 10.5 cm and separation of 16 cm. Samples were placed on the lower electrode. Allylamine (Aldrich, 98 % purity) plasma polymer (ALAPP) coatings were deposited using a frequency of 200 kHz, a power of 20 W, an initial monomer pressure of 0.130 mm Hg and a treatment time of 25 s. This procedure results in a pinhole-free plasma polymer with a film thickness of approximately 30 nm.

2.3 Polymer Synthesis

20% v/v monomer solutions of PEGMA (Aldrich, MW 450) and GMA (Aldrich, 97 % pure) in dioxane (BDH Chemicals, England) were chromatographed through a glass column filled with Inhibitor Remover (Aldrich). The monomer solutions were then combined in PEGMA/GMA ratios of 1:1, 2:1, and 1:2 (w/w). The thermal initiator 2,2'-azobisisobutyronitrile (AIBN) (Aldrich) was then dissolved in the PEGMA/GMA solutions at a ratio of 1 mg AIBN to 2 mL monomer solution. The solutions were purged with nitrogen for 15 minutes and then sealed and placed into a 60°C oven overnight.

2.3-1 Spin Coating

The 20% PEGMA/GMA copolymer stock solution (1:1 w/w ratio) was diluted with dioxane to a 2.5% solution before spin coating, and 0.2% 0, 0'-bis (2-aminoethyl) polyethylene glycol 3'400 (PEG diamine, Fluka) was added and dissolved to act as a crosslinker. The plasma polymerised substrates were placed on the spin coater (Laurell Technologies Corporation WS-400B-6NPP/Lite, USA) and covered with the 2.5% solution using a pipette. The substrates were then spin coated at 5000 rpm for 30 s. The resulting films were stored overnight in a vacuum chamber in order to evaporate the remaining dioxane and allow for crosslinking to occur. These slides were then removed from the vacuum chamber. Some were protein printed directly afterwards. Others were incubated in PBS solution of pH 7.4 at 37°C overnight, washed with milliQ water for 1 h and dried in the spin dryer before printing.

2.3-2 Dip Coating

The 20% PEGMA/GMA copolymer stock solutions (1:1, 2:1, and 1:2 w/w ratios) were poured over the plasma-deposited substrates and incubated at room temperature overnight. Slides were then washed for 5 hours in dioxane and dried under laminar flow. Some slides were protein printed directly afterwards. Others were incubated in PBS (pH 7.4) at 37°C overnight, washed with MilliQ water for 1 h and dried in the spin dryer before printing.

2.3-3 Grafting

Allylamine coated glass pieces were placed in to 20% PEGMA/GMA monomer solution (1:1 ratio) plus thermal initiator AIBN (1mg/2mL as described above) under nitrogen. The solution and samples were then placed into a 60°C oven overnight to allow for the monomer to polymerize. The samples were then removed from the polymerisation solution and washed for 5 hours in dioxane, followed by drying under laminar flow. Some slides were protein printed directly after drying. Others were incubated in PBS (pH 7.4) at 37°C overnight, washed with MilliQ water for 1 h and dried under laminar flow before printing.

All methods that were employed to prepare polymer coatings with different compositions are shown in Table 1.

Tab. 1. Coating methods and copolymer compositions used in this study.

Method of polymer coating	Copolymer composition PEGMA:GMA	Soaked overnight in PBS at 37 °C before printing?	Code used in this study
Spin coating	1:1	No	SC1:1
		Yes	SCB1:1
Dip coating	1:1	No	DC1:1
		Yes	DCB1:1
	2:1	No	DC2:1
		Yes	DCB2:1
	1:2	No	DC1:2
		Yes	DCB1:2
Grafting	1:1	No	G1:1
		Yes	GB1:1

2.4 XPS Analysis

X-ray photoelectron spectroscopy (XPS) analysis of surface modified samples was performed on an AXIS HSi spectrometer (Kratos Analytical Ltd, UK), equipped with a monochromatized Al Kα source. The pressure during analysis was typically 5×10^{-8} mbar. The elemental composition of samples was obtained from survey spectra, collected at a pass energy of 320 eV. Binding energies were referenced to the aliphatic carbon peak at 285.0 eV. High resolution elemental spectra were recorded at a pass energy of 40 eV.

2.5 Ellipsometry

As glass substrates are not suitable for ellipsometric analysis because of their transparency, silicon wafers were surface treated according to the same procedures described above. P-type silicon wafers with an orientation of 1-0-0 and a resistivity of 10-20 Ω-cm were obtained form Silicon Quest International (USA). Measurements were performed[17] using a SE 400 (SENTECH Instruments GmbH, Germany) on the surface of the sample at multiple angles of 40, 45, 50, 55 and 60° at room temperature, considering the refractive index of silicon and silicon dioxide as 3.85 and 1.46, respectively. The Helium/Neon laser was used with the wavelength of 632.8 nm. A minimum of ten measurements were carried out on each sample and averaged.

2.6 Profilometry

A profilometer (DEKTAK 6M Stylus Profilometer, Veeco) was used to measure the thickness of the polymer layer on the glass slides. A scratch was made on the surface of a coated slide using a needle. The slide was placed under the profilometer and a section analysis of the scratch was performed resulting in a value for the height difference between the glass and the overall polymer layer.

2.7 Contact Angle Measurements

Contact angle measurements were conducted in a custom made set-up[18] which consists of a sample stage placed in between a light source and a CCD camera. A drop of MilliQ water (2 μL) was placed on the surface of the sample at room temperature. The sample stage and camera positions were adjusted so the image of the drop was clearly obtained on the computer monitor. With the help of Scion Image 4.0.2 software, the contact angles at both sides of the drop were measured. A minimum of five drops were measured for each sample surface and averaged.

2.8 Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared measurements for surface analyses were carried out using a Nicolet Avatar 370 MCT and a DRIFT accessory supplied with the instrument. A total of 60 scans were averaged with a spectral resolution of 4 cm^{-1}. Nitrogen gas was purged throughout the measurements.

2.9 Microarray Printing

A BioOdyssey Calligrapher was used for the contact printing of bovine serum albumin (BSA) labeled with fluorescein isothiocyanate (FITC). The following printing conditions were used: pin approach speed to the source plate and glass slide was 15 and 5 mm/s, respectively; dwell time in source plate and on glass slide were 1000 and 35 ms, respectively. Three different pins were used: a round solid pin (ArrayIt SSP015) with a tip diameter of 375 μm, a square solid pin (ArrayIt SNS10) with a tip diameter of 335 μm and a quill pin (ArrayIt SMP10) with a tip diameter of 335μm. Printing was performed by varying different parameters such as pH, temperature, humidity, protein concentration and type of pins. Glass slides were stored in refrigerator for 10 hours after printing and subsequently washed and soaked in 37°C PBS overnight, then washed with MilliQ water and dried by purging with dry nitrogen gas. The analysis of printed glass substrates was carried out using an AXON GenePix 4000A microarray scanner at a resolution of 10 μm.

3. RESULTS AND DISCUSSION

In this report, we aimed to generate surface chemistries for microarray slides with excellent substrate adhesion that enable covalent immobilization of biomolecules on the printed regions, but at the same time provide a low protein fouling background that does not support cell attachment. Here, we have used a random copolymer of GMA and PEGMA, where the GMA component with its oxirane groups on the side chain is reactive towards amines (a functional group present in a large number of biologically active compounds). The PEGMA component on the other hand is known to substantially reduce cell attachment. Figure 1 describes schematically the different routes that were used in this study. Figure 1A represents a coating where a thin allylamine plasma polymer (ALAPP) film has been deposited on the glass substrate. The highly crosslinked film provides excellent substrate adhesion and at the same time amine functional groups on the surface of the coating. Subsequent incubation with PEGMA/GMA copolymer ("dip coating") leads to immobilization of PEGMA/GMA polymer chains on the plasma polymer surface via one or more covalent attachment points shown as black circles per chain. The polymerization of PEGMA/GMA copolymers in solution while having the freshly deposited plasma polymer coated glass substrate present ("grafting") leads to the same scenario shown in figure A. Figure 1B in comparison shows the coating structure achieved using the "spin coating" approach. Here, copolymer chains are covalently linked to the plasma polymer substrate, but in addition covalent inter- and intra-molecular bonds are formed due to the presence of the PEG diamine crosslinker. The coating thickness in this case is predominantly controlled by the spin coating conditions.

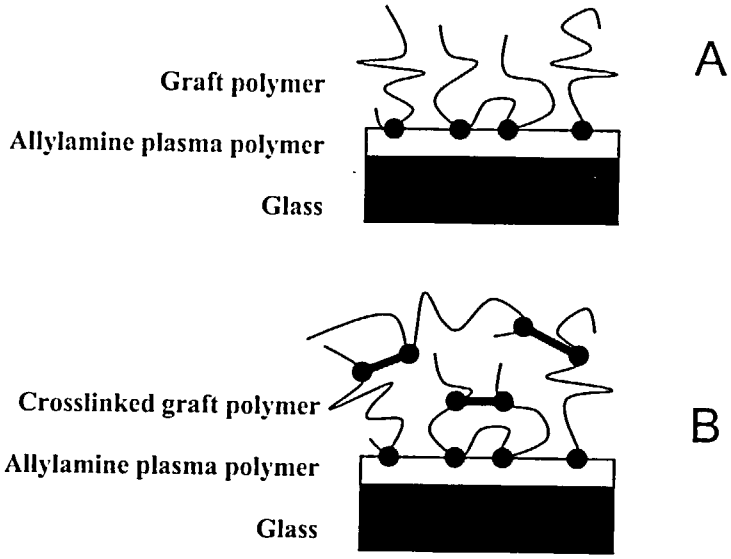

Fig. 1. Schematic representation of the coatings produced in this study.

3.1 Characterization of Polymer Coatings

To confirm that the surface modifications using the PEGMA/GMA copolymer had been successful, analysis of the surface chemistry was performed using XPS. Table 2 shows the elemental composition obtained from survey spectra. The complete attenuation of the Si signal from the underlying glass substrate for all samples confirms that the deposition of the allylamine plasma polymer (ALAPP) film created a stable, pinhole-free coating with a thickness of at least 10 nm (the approximate information depth of the XPS method). The ALAPP layer provided amino functional groups on the glass surface as indicated by the significant increase in the nitrogen content. None of the XPS spectra of PEGMA/GMA coatings showed significant amounts of nitrogen indicating that a thickness of at least 10 nm for each the spin and dip coated and grafted coatings was achieved. In addition, all of the PEGMA/GMA coatings show an elemental composition similar to the theoretical elemental composition of the PEGMA/GMA copolymer shown in Table 2.

Tab. 2. XPS elemental composition of surface modified glass substrates obtained from survey spectra (atomic concentration in %).

Sample	C	O	N	O/C	N/C
Glass-ALAPP	79	8.6	12.4	0.109	0.000
SC1:1	69.0	31.0	0.0	0.449	0.000
DC1:1	66.4	33.6	0.0	0.506	0.000
G1:1	67.5	32.2	0.3	0.477	0.000
PEGMA/GMA polymer (theory)	68.9	31.1	0.0	0.451	0.000

Figure 2 shows XPS C1s high resolution spectra recorded on the Glass-ALAPP surface, on the spin and dip coated as well as on grafted coatings. Table 3 shows the quantification of the components C1-C4 fitted to these spectra. The ALAPP coating shows a broad peak distribution typical for plasma polymers while all the PEGMA/GMA coatings show a composition close to the theoretical composition (as shown in Table 3) with a dominant C2 component. All PEGMA/GMA copolymer coatings were also incubated in PBS at 37°C overnight. XPS results (not shown here) indicate that the copolymer coatings were stable under these conditions.

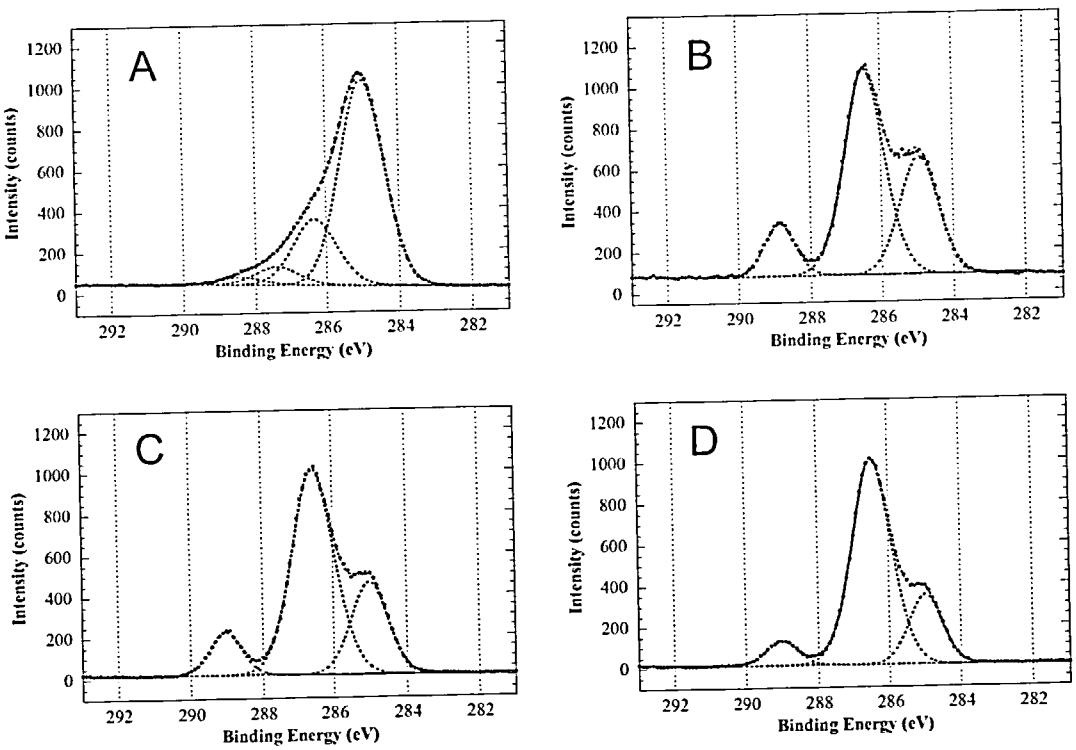

Fig. 2. XPS high resolution C1s spectra collected on pristine and surface modified glass substrates. The spectra represent Glass-ALAPP (A), DC1:1, (B), G1:1 (C) and SC1:1 (D) surfaces.

Tab. 3. Quantification of components C1-C4 fitted to XPS C1s high resolution spectra obtained on modified glass substrates (atomic concentration in %).

Sample	C1	C2	C3	C4
Glass-ALAPP	68.8	22.5	6.2	2.5
SC1:1	20.6	72.2	0.3	6.9
DC1:1	31.4	57.4	0.0	11.2
G1:1	24.8	64.7	0.3	10.2
PEGMA/GMA polymer (theory)	29.2	61.1	0.0	9.7

Ellipsometry was used to measure the thickness of the polymer coatings since XPS results showed that for all polymer coatings the thickness exceeded the information depth of the XPS. Here, polymers were deposited on silicon wafers to enable ellipsometric analysis. An average thickness of 91 ± 7.2 nm was obtained for DC1:1, whereas a thickness of 102 ± 0.8 nm and 77 ± 11.4 nm was determined for SC1:1 and G1:1, respectively. These values were in good agreement with coarse profilometry measurements performed on glass which gave an approximate thickness of 108 nm for a spin coated polymer layer. A more uniform thickness was observed for spin coated silicon wafers than for those samples that were dip coated or grafted, which indicates that spin coating allows the deposition of a polymer coating of uniform thickness. After soaking the samples overnight in PBS at 37 °C (typical cell culture conditions), they were once again subjected to thickness analysis in order to exclude delamination under cell culture conditions. They were then washed with milliQ water and dried with N_2 gas before measuring thickness with the ellipsometer. Interestingly, dip (DCB1:1) and spin coated (SCB1:1) samples had almost same thickness of 73 ± 7.1 and 72 ± 2.4 nm respectively, suggesting that a significant portion of the PEGMA/GMA coating had dissolved. In case of grafted sample GB1:1 there is not much difference in thickness between PBS soaked and non soaked samples. Both show a thickness of 62 ± 10.0 nm which indicates that the grafting procedure leads to a stable layer which does not change upon extended incubation in buffer. It

is worth noting that the measured thickness is the sum of ALAPP and PEGMA/GMA copolymer thickness. The thickness of the ALAPP layer was around 38 nm by profilometry and 42 ± 0.6 nm by ellipsometry.

Contact angles were measured and compared amongst spin coated, dip coated and grafted samples before and after soaking in PBS. The surface of a spin coated silicon wafer before soaking in PBS (SC1:1) was slightly more hydrophobic (54 ± 2.4°) than that of grafted (G1:1; 44 ± 1.1°) and dip coated (DC1:1; 41 ± 2.0°) samples. After soaking the samples in PBS overnight the surfaces of all three polymer coatings showed a similar wettability with 59, 61 and 59° for DCB1:1, SPB1:1 and G1:1 samples, respectively. Interestingly, the surfaces became more hydrophobic upon incubation in buffer which is suggestive of chain reorientation processes or removal of low molecular weight polymer species of different wettability.

Diffuse reflectance infrared spectra were obtained for the spin and dip coated silicon wafer samples and are presented in Fig. 3. For the spin coated sample, (Fig. 3 a), the peak at 1220 cm^{-1} is due to the symmetrical expansion and contraction vibrations of the oxirane ring and the peak at 1020 cm^{-1} is due to the asymmetrical expansion and contraction vibrations of the oxirane ring[19]. The characteristic peaks of carbonyl groups appear at 1720 cm^{-1} [20] and are attributed to the methacrylate esters. The two peaks at 2920 cm^{-1} and 2850 cm^{-1} correspond to aliphatic C-H stretching vibrations and the peak at 1410 cm^{-1} is due to the C-O stretching vibration of the ether. The dip coated sample showed similar peaks although the two aliphatic C-H peaks were not resolved and a series of peaks in the region of C-O stretching vibrations were observed. These differences might be due to different coating morphology or density.

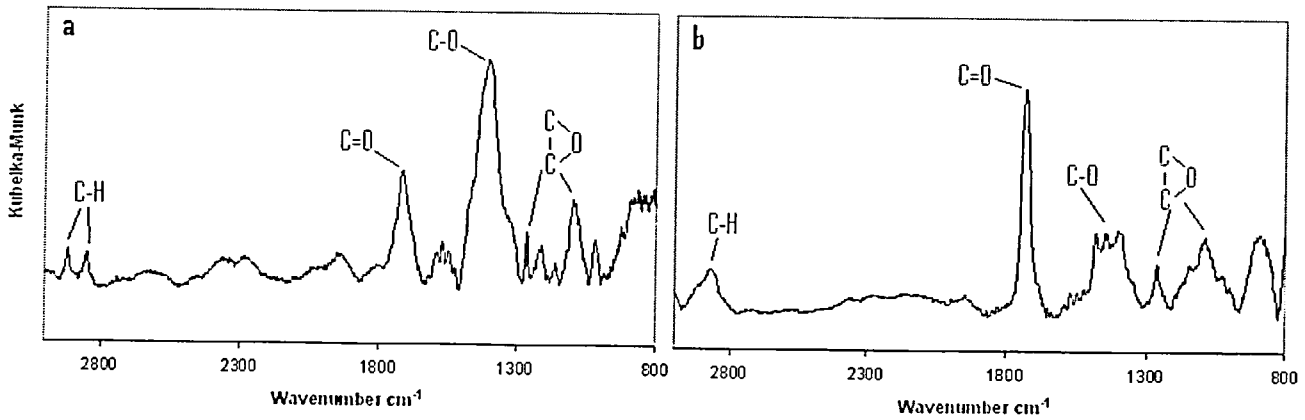

Fig. 3. FTIR spectra of (a) spin coated and (b) dip coated samples.

In summary, all surface characterization methods confirmed that the three coatings methods were appropriate for the preparation of stable polymer coatings on glass slides.

3.2 Microarraying

Spin and dip coated samples were also characterized in terms of their performance as microarray substrate surfaces. Robotic protein printing was done using fluorescein labeled bovine serum albumin (BSA-FITC) as a model protein. Upon printing, proteins would be immobilized on the polymer surface due to the reaction of amino groups on the protein with the oxirane ring of GMA. The first experiment investigated the effect of PBS soaking overnight at 37 °C on the fluorescence intensity and homogeneity of the printed protein spots on DC2:1 and DCB2:1. The images are presented in Fig. 4. It was observed that spots were more circular and the protein distribution more uniform in the case of DCB2:1 when compared to DC2:1. This indicates that the incubation in buffer leads to a surface that is more favorable for protein adhesion in the microarray format.

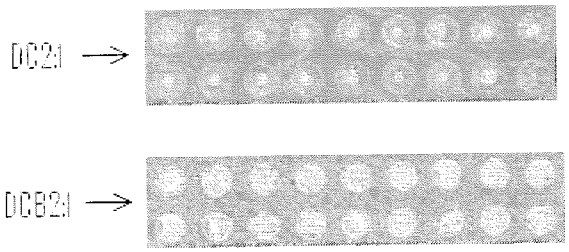

Fig. 4. Fluorescence scans of BSA-FITC spots printed onto DC2:1 and DCB2:1.

In the next step, protein spots were printed onto DCB1:1, DCB2:1 and DC1:2 to determine the suitable monomer molar ratio required on the glass substrate for the proper attachment of the protein molecules. The microarray images are presented in Fig. 5. It is observed that protein molecules detach in the case of DCB2:1 and even more so in the case of DC1:2 (see Fig 5). This is presumably the result of poor covalent attachments of the proteins to the polymer layer. Better attachment of protein molecules is observed in case of DCB1:1 (Fig 5), even after soaking the slides overnight in PBS after protein printing. This indicates that a 1:1 molar ratio between PEGMA/GMA is suitable for protein immobilization.

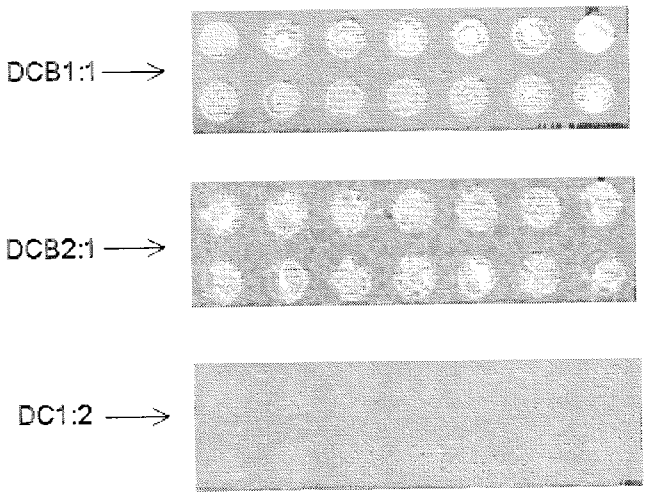

Fig. 5. Fluorescence scans of BSA-FITC arrays on DCB1:1, DCB2:1 and DC1:2.

In order to determine the optimal protein concentration and pH at which uniform circular spots can be reproducibly obtained, four protein concentrations (200, 100, 75 and 50 μg/mL) at four different pH's (5, 7, 9 and 10) were printed on SCB1:1 slides at 10°C and 65% relative humidity. The images of these slides obtained after soaking in PBS are represented in Fig. 6. Array printing was performed with a solid round pin of diameter 375 μm. All protein concentrations produced spots with sufficient fluorescence intensity. When printing was performed at pH 5 and 7, spots were rather circular whilst spots printed from pH 9 and 10 had irregular outlines. The most circular spot geometry was observed on spots printed from pH 5 buffer, although the fluorescence of spots printed from pH 9 and 10 showed greater fluorescence levels.

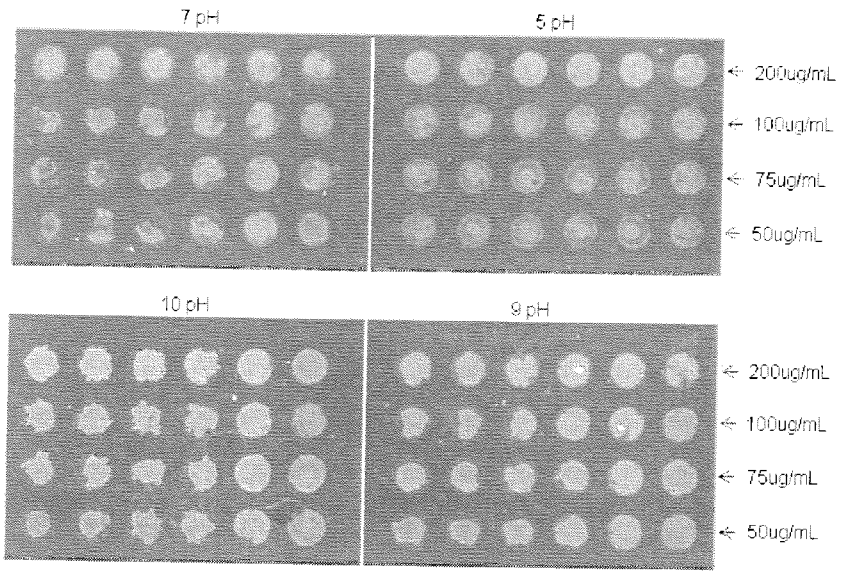

Fig. 6. Fluorescence scans of arrays of BSA-FITC printed onto SCB1:1 using 4 different concentrated proteins in 4 different buffers at 10 °C.

Temperature and humidity are known to play an important role in immobilization of proteins on microarray spots. The temperature at which the slide is held will determine the kinetics of protein immobilisation whilst the humidity will influence the rate of evaporation of the nanoliter droplets. Therefore, experiments were performed to study the effect of temperature while printing the protein molecules on a DCB1:1 slide using a protein concentration of 75 μg/mL prepared in pH 5 buffer with a relative humidity of 65%. The images of the slide printed at different temperatures are represented in Fig. 7. It is clear from the images that 10 and 15 °C are ideal for the printing of biomolecules on the reactive glass slides whereas spots are incomplete when printing was performed at 5 °C, presumably due to the poor reaction kinetics. Printings obtained at temperatures of 10 and 15 °C have shown better protein attachment to the glass substrate, however we have selected 10 °C for subsequent experiments in order to slow down the evaporation of solvent from protein solution, allowing the immobilization reaction to proceed from the liquid phase for a longer time.

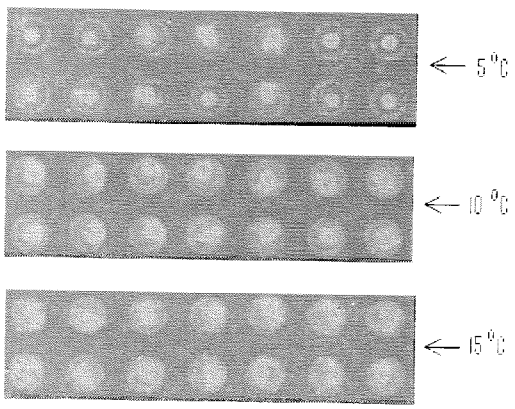

Fig. 7. Microarray images of BSA-FITC printed onto DCB1:1 with a protein concentration of 75 μg/mL at 5 pH PBS at different temperatures.

Figure 8 shows the effect of humidity on protein attachment on a DCB1:1 slide printed at 10°C. The spots are not circular and almost washed off in the case of spots printed at 35, 45 and 55% relative humidity (RH). This may be due to the fast evaporation of solvent molecules from printed protein solution spots at lower humidity conditions. The spots

printed at 65% RH remained attached even after soaking in PBS overnight, suggesting that higher humidity is better suited for printing of protein molecules onto reactive polymer coatings.

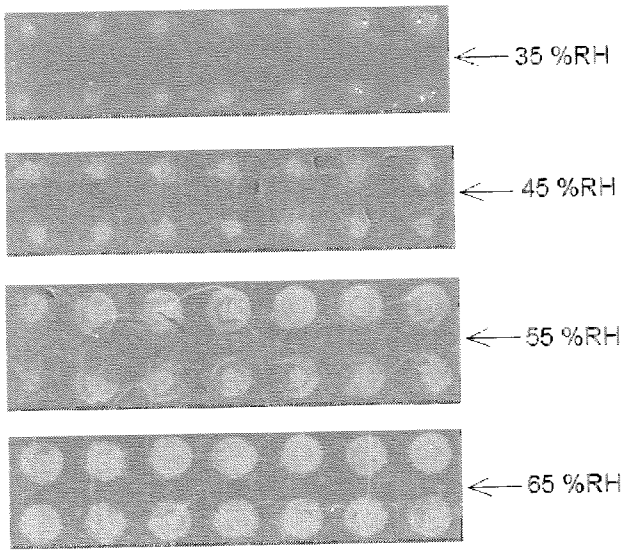

Fig. 8. Microarray images of BSA-FITC printed onto DCB1:1 with protein concentration of 75 μg/mL at 5 pH at different relative humidities.

Finally, BSA-FITC was printed using round solid, square solid and quilled pins. Scans of the resulting protein arrays are shown in Fig. 9. The figure shows that while both round and square solid pins perform reasonably well, spots generated using the quilled pin are not very homogenous in size and morphology.

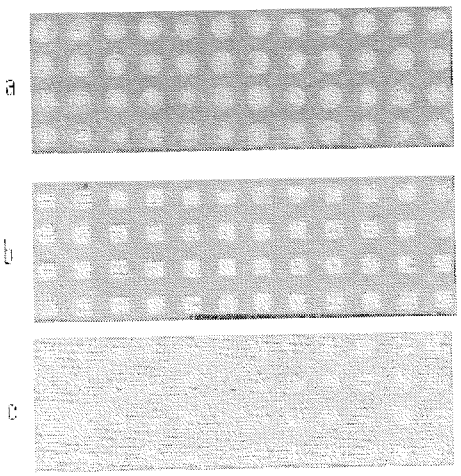

Fig. 9. Fluorescence scans of BSA-FITC printed onto SCB1:1 with (a) a solid round pin (b) a solid square pin and (c) a quilled square pin.

4. CONCLUSION

A PEGMA/GMA copolymer was attached successfully onto glass slides by three different coating methods: spin coating, dip coating and surface grafting. Surface characterization was performed by measuring the thickness by ellipsometry and profilometry, and the surface wettability by contact angle measurements. Chemical surface characterizations were carried out by XPS and diffuse reflectance IR. XPS and IR confirmed the presence of copolymer on the surfaces of samples

prepared by all three methods. Whilst the thickness of the dip and spin coated copolymer decreased upon incubation in PBS at 37 °C, the grafted copolymer thickness remained stable under these conditions. After PBS incubation all three coatings showed a similar thickness of approximately 60-70 nm. Spin coating produced the most uniform coating as indicated by the small standard deviation of the ellipsometric thickness. Microarraying of BSA-FITC was demonstrated on surface modified glass slides. Both spin and dip coated slides were used for protein printing. Optimal conditions for protein printing were identified in terms of protein concentration of the printing solution, pin geometry, temperature and humidity.

The surfaces presented in this study are currently used in cell microarray experiments and initial cell culture results confirm the ability to immobilize biologically active compounds and show the intended low background cell attachment.

ACKNOWLEDGEMENTS

Financial support from the CSIRO Food Futures Flagship, Flinders University and Bio-Rad is gratefully acknowledged.

REFERENCES

1. Drickamer, K. & Taylor, M. E. (2002) Glycan arrays for functional glycomics. *Genome Biology*, 3, Reviews 1034.1-1034.4
2. Huang, R. P. (2001). Detection of multiple proteins in an antibody-based protein microarray system. *Journal of Immunological Methods*, **255**, 1-13.
3. Yamazoe, H. & Iwata, H. (2005) Cell microarray for screening feeder cells for differentiation of embryonic stem cells. *Journal of Bioscience and Bioengineering*, **100**, 292–296.
4. Castel, D., Pitaval, A., Debily, M-A. & Gidrol, X. (2006) Cell microarrays in drug discovery. *Drug Discovery Today*, **11**, 616-622
5. Kononen, J., Bubendorf, L., Kallioniemi, A., Barlund, M., Schraml, P., Leighton, S., Torhorst, J., Mihatsch, M. J., Sauter, G. & Kallioniemi, O. P. (1998) Tissue microarrays for high-throughput molecular profiling of tumor specimens. *Nature Medicine*, **4**, 844-847.
6. Grinnell, F., Milam, M. & Srere, P. A. (1973) Attachment of normal and transformed hamster kidney cells to substrata varying in chemical composition. *Biochemical Medicine*, **7**, 87–90.
7. Bledia, Y., Domb, A. J. & Linial, M. (2000) Culturing neuronal cells on surfaces coated by a novel polyethyleneimine-based polymer. *Brain Research Protocols*, **5**, 282–289.
8. Ouyang, E. C., Wu, G. Y. & Wu, C. H. (2002) Biocompatible polymers in liver-targeted gene delivery systems. Dumitriu, S., *Polymeric Biomaterials*. 2nd Ed. New York, Marcel Dekker, 975–981.
9. Anderson, D. G., Putnam, D., Lavik, E. B., Mahmood, T. A. & Langer, R. (2005) Biomaterial microarrays: rapid, microscale screening of polymer–cell interaction. *Biomaterials*, **26**, 4892–4897.
10. Mant, A., Tourniaire, G., Diaz-Mochon, J. J., Elliott, T. J., Williams, A. P. & Bradley, M. (2006) Polymer microarrays: Identification of substrates for phagocytosis assays. *Biomaterials*, **27**, 5299–5306.
11. Ramires, P. A., Mirenghi, L., Romano, A. R., Palumbo, F. & Nicolardi, G. (2005) Plasma-treated PET surfaces improve the biocompatibility of human endothelial cells. *Journal of Biomedical Materials Research*, **51**, 535–539.
12. Wheeler, D. B., Carpenter, A. E. & Sabatini, D. M. (2005) Cell microarrays and RNA interference chip away at gene function. *Nature Genetics*, Supplement, **37**.
13. Chen, D. S. & Davis, M. M. (2006) Molecular and functional analysis using live cell microarrays. *Current Opinion in Chemical Biology*, **10**, 28–34
14. Anderson, D. G., Levenberg, S. & Langer, R. (2004) Nanoliter-scale synthesis of arrayed biomaterials and application to human embryonic stem cells. *Nature Biotechnology*, **22**, 863-866.
15. Kuschel, C., Steuer, H., Maurer, A. N., Kanzok, B., Stoop, R. & Angres, B. (2006) Cell adhesion profiling using extracellular matrix protein microarrays, *BioTechniques*, **40**, 523-531.
16. Griesser, H. J. (1989) Small scale reactor for plasma processing of moving substrate. *Vacuum*, **39**, 485-488.
17. Losic, D., Cole, M., Thissen, H. & Voelcker, N. H. (2005) Ultrathin polytyramine films by electropolymerisation on highly doped p-type silicon electrodes. *Surface Science*, **584**, 245–257.

18. Low, S. P., Williams, K. A., Canham, L. T. & Voelcker, N. H. (2006) Evaluation of mammalian cell adhesion on surface-modified porous silicon. *Biomaterials*, **27**, 4538–4546.
19. Okubo, M., Okada, M., Miya, T. & Takekoh, R. (2001) Production of micron-sized, nanodisperse composite polymer particles having epoxy groups by seeded dispersion polymerization. *Colloid Polymer Science*, **279**, 807.
20. Zhiya, M., Yueping, G. & Huizhou, L. (2005) Synthesis and characterization of micron-sized monodisperse superparamagnetic polymer particles with amino groups. *Journal of Polymer Science, Part A, Polymer Chemistry*, **43**, 3433–3439.