JOURNAL OF Materials Communication CHEMISTRY

A comparative study of cell attachment to self assembled monolayers and plasma polymers

Ros Daw,[a] Ian M. Brook,[b] A. Jane Devlin,[b] Robert D. Short,*[a] Elaine Cooper[c] and Graham J. Leggett[d]

[a] *Department of Engineering Materials, University of Sheffield, Sir Robert Hadfield Building, PO Box 600, Sheffield, UK S1 4DU. E-mail: r.short@shef.ac.uk*
[b] *Department of Oral Maxillofacial Surgery, University of Sheffield, UK S1 4DU*
[c] *Department of Materials Engineering and Materials Design, The University of Nottingham, University Park, Nottingham, UK NG7 2RD*
[d] *Department of Chemistry, University of Manchester Institute of Science and Technology, PO Box 88, Manchester, UK M60 1QD*

Received 24th August 1998, Accepted 29th September 1998

The first comparative study of cell attachment to self-assembled monolayers (SAMs) and plasma-deposited films is reported. Osteoblast-like cells attached extensively to acid-terminated alkyl thiol SAMs and to a plasma co-polymer of acrylic acid and octa-1,7-diene (acid-PCP). However, they attached poorly to methyl-terminated SAMs and a plasma polymer of octa-1,7-diene (OD-PP).

Cell attachment to synthetic substrata is strongly influenced by the surface chemical structure, both directly (in serum-free conditions) and through the adsorption of proteins from culture medium. SAMs have been used as 'model' surfaces to probe cellular responses to specific functional groups. Carboxylic acid-terminated SAMs have been shown to promote the attachment and spreading of osteoblasts,[1] fibroblasts[2] and endothelial cells,[3] whereas methyl-terminated SAMs have not promoted attachment.

A number of attachment studies have been undertaken with plasma-deposited films.[4] Plasma polymers cannot be considered 'model' surfaces for studies of this type as the technique leads to the incorporation of functional groups not present in the original monomer unit. However, plasma deposits are important in biomaterials science: a wide range of compounds can be plasma polymerised and deposition is possible onto virtually any substrate. Deposits are produced in a sterile environment free of initiator and solvents, and they can be readily used to coat medical devices and implants.

It has been shown that plasma co-polymerisation using low plasma powers may be used to control surface functional group concentration. The attachment of keratinocytes[5] and osteoblast-like cells[6] to plasma co-polymerised surfaces has been explored in previous studies. Films with oxygen-containing functional groups encouraged cell attachment,[5,6] with keratinocyte attachment correlating best with the carboxylic acid functional group.[5] Optimum attachment was found to surfaces containing 3% carboxylic acid. Pure hydrocarbon films (OD-PP) produced poor attachment.[5,6]

The purpose of this study was to compare osteoblast-like cell attachment on these plasma-polymerised materials with attachment to self-assembled monolayers of carboxylic acid and methyl-terminated adsorbates.

The reactor vessel used for the plasma polymerisations is described elsewhere.[6] The plasma was sustained by a radio-frequency (13.56 MHz) signal generator and amplifier 'inductively' coupled to the reactor vessel. The base pressure of the reactor vessel was 8×10^{-3} mbar. Acrylic acid (40%) and octa-1,7-diene (60%), from Aldrich UK, were co-polymerised at a plasma flow rate of 2 cm^3 min^{-1} (STP), and power of 2 W. Octa-1,7-diene was polymerised using the same plasma parameters. The pressure during polymerisation was typically 4×10^{-2} mbar. Films were deposited on Thermanox tissue culture plastic coverslips.

SAMs were prepared according to well-established procedures, by the immersion of gold-coated (50 nm) chromium-primed (2 nm) glass microscope slides in 1 mM ethanolic solutions of mercaptoundecanoic acid (MUA), 3-mercapto-propanoic acid (MPA), butanethiol (BT), octanethiol (OT) and dodecanethiol (DDT) for *ca.* 16 h. BT, OT and DDT were obtained from Fluka, UK, and MPA was obtained from Sigma, UK, and were used as received. MUA was synthesised following a procedure adapted from the method of Bain *et al.*[7] Following removal of the SAMs from the thiol solution, they were rinsed with ethanol and dried in a stream of nitrogen.

ROS 17/2.8 cells were donated by G.A. Rodan of Merck, Sharp and Dohme. Cells were removed from liquid nitrogen, and fast thawed. They were suspended in serum supplemented medium, seeded in tissue culture flasks and incubated for seven days. The cells were then trypsinised using 0.05% trypsin containing 0.5 mM disodium ethylenediaminetetraacetic acid (Na_2EDTA), before centrifuging at 405*g*. The cell pellet was re-suspended in fresh medium. Three samples of each film were placed into 24 well trays. Cells were seeded at a density of 1.1×10^5 cells ml^{-1} (with 1 ml per well), and allowed to attach in an incubator at 37 °C and 5% CO_2 for 90 min. Each sample was then washed three times with warmed PBS to remove unattached cells. Cells were counted using a microscope graticule and at least four fields of view for each sample. The mean cells per mm^2 and standard deviation were calculated. Total DNA content of the samples was estimated using a Hoechst stain. The technique is described elsewhere.[6] Statistical significance was calculated using the Students' t-test. Data were taken to be significant when a p value of 0.05 or less was obtained (showing a 95% confidence limit).

Fig. 1 shows the mean number of osteoblast cells per mm^2 attached to each sample. Two distinct levels of attachment were seen. Almost equal numbers of cells attached to the acid-terminated SAMs and the acid plasma co-polymer, with the possible suggestion of higher levels of attachment to the plasma co-polymer (acid-PCP). On the methyl-terminated SAMs and OD-PP, statistically similar numbers of cells were found to have attached, although slightly higher mean levels of attachment were observed on the OD-PP and BT surfaces. The numbers of cells on the acid-terminated SAMs and the acid-PCP surfaces were significantly greater than the numbers of cells attaching to the methyl-terminated SAMs and the OD-PP surfaces.

On the hydrocarbon surfaces, cells were only weakly attached, clumped together and had a rounded morphology. The cells more strongly adhered to the acid surfaces; they were better spread and less clumped.

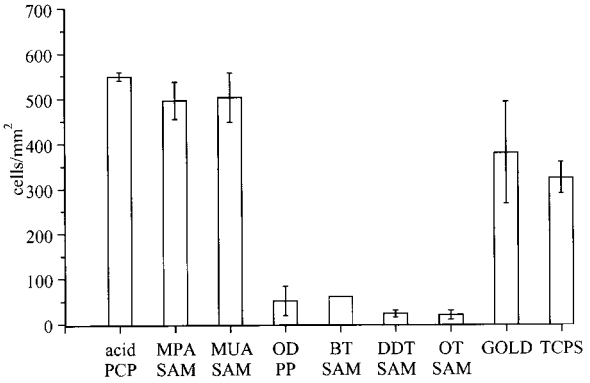

Fig. 1 Cell attachment to SAMs, plasma-deposited surfaces, TCPS and gold. Values are the average of 12 measurements (three samples $\times$ four fields of view) except for BT.† The statistical significance (p) between the acid surfaces and methyl surfaces was $\leqslant 0.002$; between the OD-PP and methyl-terminated SAMs, $p = 0.1$.

Fig. 2 shows the measured DNA concentrations per mm^2 on these samples. The amounts of DNA measured on the acid-PCP and the acid-terminated SAMs were almost equal. There appeared significantly more DNA on the OD-PP than both the DDT- and OT-SAMs, but a similar value was recorded for the BT-SAM. The amounts of DNA on the acid-containing surfaces were significantly higher than on the hydrocarbon surfaces.

Also included in Fig. 1 are data for cells cultured on gold and tissue culture polystyrene (TCPS). The former is the substrate on which the SAMs were prepared. It has been previously observed that cells attach well to this surface. TCPS is the usual substratum material used in tissue culture, although it may be subsequently coated with various extracellular matrix (ECM) components, prior to cell culture. Good attachment to TCPS was expected, but as described elsewhere, the surface chemistry of TCPS can be variable and it has only limited value as a control.[5]

The degree of correlation observed in the cell counts and in the DNA between the acid-terminated SAMs and the acid-PCP is perhaps surprising. Although both contain carboxylic acid, the numbers of carboxylic acid groups per 100 carbons are very different for these two types of surface. Furthermore, in the SAMs, the acid groups are known to be at the liquid interface; this has not been established for the acid-PCP. In aqueous media, the acid-PCP can hydrate, as we will demonstrate elsewhere.[8] This behaviour is not expected of the acid-terminated SAMs. Significant re-orientation of acid functional groups within the surface of the acid-PCP, to present a higher concentration of acid at the liquid interface, can be discounted as the advancing contact angle measured on the acid-PCP (80° for distilled water) reflects the essentially hydrocarbon nature of this surface.

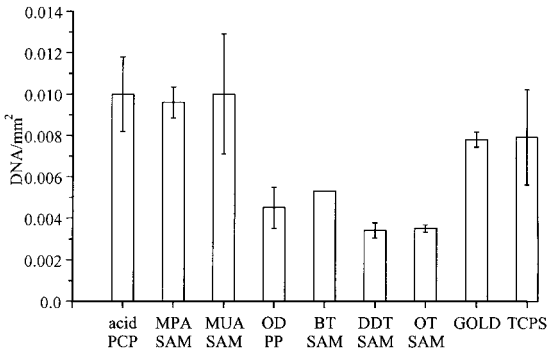

Fig. 2 Total (μg mm^{-2}) measured on the SAMs, PP, PCP, TCPS and gold. The statistical significance (p) between the acid surfaces and methyl surfaces was $\leqslant 0.04$; between the OD-PP and methyl-terminated SAMs, $p \leqslant 0.05$.

The result from the OD-PP and methyl-terminated SAMs is also interesting. Structural irregularity in the surface of the OD-PP was anticipated. The surface will present a number of different carbon–hydrogen bonding arrangements (methyl, methylene, olefinic *etc.*). Order in SAMs is thought to be influenced by adsorbate chain length. Long chain SAMs, such as those formed from DDT, exhibit ordered, crystalline structures in which the alkyl chains pack relatively rigidly. Short chain SAMs, such as those formed from BT, are thought to exist in a two dimensional liquid state, in which the alkyl chains are relatively mobile. Consequently, while the surface of a long chain SAM is composed almost exclusively of methyl groups, the short chain SAMs are much more disordered and the alkyl chain methylene groups are exposed as well as terminal methyl groups. In view of this it is not surprising that the data for OD-PP resemble those for the short-chain SAM, BT, rather than those formed from longer adsorbates (OT and DDT).

In serum-containing media, cell attachment is influenced by the adsorbed protein layer. Identification of the factors that control protein adsorption and retention of activity (conformation) once adsorbed, has been the subject of considerable research effort, with much emphasis put upon surface hydrophilicity/hydrophobicity. However, adsorption may also be controlled by factors at the molecular level, such as the availability of a specific functional group that acts at a specific binding site for the protein. In this study, no attempt was made to control the nature of the layer of adsorbed protein at the sample surfaces. Undoubtedly, the nature of the adsorbed protein layer, which forms rapidly following exposure of the samples to the culture medium, plays a critical role in determining the ultimate outcome of the cell–material interaction. Future studies must address this problem. However, the contrasting effects of the different chemistries, and the similarity in the response of the plasma co-polymer and the acid terminated SAM, are clearly demonstrated.

This study draws attention to the importance of having well-defined surfaces on which to work. It illustrates the importance of the acid functionality, but also questions whether cell attachment requires high concentrations of this functionality at the surface. The comparison of the OD-PP with the methyl-terminated SAMs illustrates how SAMs may possibly be used to probe structural disorder in PPs.

R.D.S. and R.D. thank the EPSRC for a studentship (for R.D.); G.J.L. and E.C. thank the Leverhulme Trust for the provision of a research grant and G.J.L. thanks the Nuffield Foundation for provision of a Science Research Fellowship.

Notes and references

† Cell counts comparable to those made on gold were made on two of the BT SAMs, but these values were discounted on the grounds that the BT SAM is the least stable of the SAM surfaces studied. We strongly suspected that regions of the gold substratum had become exposed. The anomaly was verified by the respective DNA values, which were also subsequently discarded.

1 C. A. Scotchford, E. Cooper, S. Downes and G. J. Leggett, *J. Biomed. Mater. Res.*, 1998, **41**, 431.
2 E. Cooper, W. Robin, D. A. Hunt, L. Parker, G. J. Leggett and T. L. Parker, *J. Mater. Chem.*, 1997, **7**, 435.
3 C. D. Tidwell, A. M. Belu, B. D. Ratner, B. Tarasevich B. S. Atre and D. L. Allara, *Transactions Soc. Biomater*, *23rd Annual Meeting*, 1997, vol. XX, 203.
4 S. I. Ertel, A. Chilkoti, T. A. Horbett and B. D. Ratner, *J. Biomater. Sci., Polym. Ed.*, 1991, **3**, 163.
5 R. M. France, R. D Short, R. A. Dawson and S. MacNeil, *J. Mater. Chem.*, 1998, **8**, 1, 37.
6 R. Daw, A. J. Beck, R. D. Short, A. J. Devlin and I. M. Brook, *Biomaterials*, 1998, in the press.
7 C. D. Bain, E. B. Troughton, Y.-T. Tao, J. Evall, G. M. Whitesides and R. G. Nuzzo, *J. Am. Chem. Soc.*, 1989, **111**, 321.
8 R. M. France, R. J. Heaton, M. C. Davies, S. J. B. Tendler, C. J. Roberts, P. Williams, R. Daw and R. D. Short, unpublished work.

Communication 8/06612D