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The effect of alkyl chain length and terminal group chemistry on the attachment and growth of murine 3T3 fibroblasts and primary human osteoblasts on self-assembled monolayers of alkanethiols on gold†

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Murine 3T3 fibroblasts and primary human osteoblasts have been cultured on self-assembled monolayers (SAMs) formed by the adsorption of alkanethiols of varying terminal group and alkyl chain length on gold. It has been found that the interactions of both cell types with SAMs are influenced by surprisingly subtle changes in adsorbate structure. Both the nature of the adsorbate terminal group and the length of the alkyl chain have a significant influence on cellular attachment and growth. For both cell types, attachment and growth on the carboxylic acid terminated SAMs was extensive and was accompanied by a high degree of cell spreading. In contrast, attachment and growth on long-chain methyl terminated SAMs were poor. Attachment and growth on short-chain methyl terminated SAMs were intermediate, with reasonable numbers of attached cells being observed and some degree of cell spreading. Sharply contrasting behaviour was observed for the two cell types when cultured on hydroxy terminated SAMs. It was found that fibroblasts attached poorly to these surfaces, while osteoblasts attached and grew nearly as successfully on short-chain hydroxy terminated SAMs as on carboxylic acid terminated SAMs. Osteoblasts attached less well to long-chain hydroxy terminated SAMs, but better than to methyl terminated SAMs.

Introduction

The interaction of biological media with surfaces is important in many aspects of biotechnology, including the development of novel biomaterials, the production of effective biosensors and the design of diagnostic testing systems. For three decades there has been intense research activity directed towards the development of an understanding of the parameters that control the interaction of cells and proteins with surfaces. There has been a substantial amount of work attempting to understand the role of the solid substratum in influencing cell adhesion, and many properties of the surface have been shown to be important, including substrate wettability,[1,2] surface chemistry[3–9] and surface topography.[10–13] There has been much interest in understanding the interactions of cells with organic polymers.[1,2,14–17] However, polymer surfaces are prone to reorientation on transfer to aqueous media, they are often heterogeneous and their chemical structures and micromorphological characteristics are difficult to analyse. Consequently progress has been slow and the formulation of general principles for the design of surfaces to elicit specific cellular responses has been difficult. The advent of self-assembled monolayers (SAMs) promises to revolutionise work in this area, providing the capability for the production of well-defined model surfaces of known structures and properties that may be carefully regulated and manipulated. There has therefore been much interest in the use of SAMs as substrates for the investigation of the influence of surface chemistry on cell attachment.[3–9,18–27]

Two different types of SAMs have been widely used to study cell attachment: alkylsiloxanes on hydroxylated surfaces[3–9] and alkanethiolates on gold.[18–27] The adsorption of alkanethiolates on gold provides an ideal model for studying the effect of surface chemistry on cell attachment, because a very ordered monolayer is formed and strict control can be exerted over terminal group functionality and surface chemistry. While alkylsilane SAMs are also valuable models, they afford less precise control over surface chemistry, particularly because of the difficulties associated with the formation of monolayers with polar terminal groups; typically, it is necessary to employ a chemical transformation of an apolar terminal group following monolayer formation and 100% effectiveness is rarely, if ever, achieved.[28]

In previous studies,[25,26,29] we have demonstrated that both fibroblast and osteoblast cells attach more extensively to carboxylic acid terminated SAMs than to methyl terminated SAMs, with both cell types spreading effectively on the former surface but not the latter. Similar results have also been observed for keratinocytes.[30] Actin filament organisation was found to be extensive in osteoblasts cultured on acid-terminated SAMs, but only low levels of organisation were observed on methyl-terminated SAMs. Spatial control of SAM terminal group chemistry provided an effective means of controlling the location of attachment and the spreading behaviour of both fibroblasts and osteoblasts.

These studies provided good indications of the depth of information available through studies of cellular interactions with SAMs. However, a limited number of SAM systems were employed. In the present study we have sought to address two key questions through the utilisation of a broader range of surfaces. First, we have reported that fibroblast and osteoblast-like cell attachment to carboxylic acid terminated

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SAMs is extensive. Is this because the cells exhibit a preference for high energy surfaces, or is there a specific chemical effect? Tidwell *et al.* reported that bovine aortic endothelial cells attached poorly to hydroxy terminated SAMs,[27] but in contrast, DiMilla *et al.* reported that osteosarcoma cells exhibited a preference for attachment to hydroxy rather than methyl terminated SAMs.[20] We have compared the behaviour of fibroblasts and osteoblast cells on hydroxy, carboxylic acid and methyl terminated SAMs. Second, it has been reported that the alkyl chain length of a SAM exerts a significant influence on its behaviour. For example, the wettability varies with chain length,[31,32] and the rates of photo-oxidation of SAMs on gold and silver are strongly influenced by the length of the adsorbate alkyl chains.[33–35] This has been largely attributed to changes in adsorbate ordering as a result of changing numbers of interchain van der Waals interactions (although Miller and Abbott have suggested that changes in wettability with alkyl chain length may instead reflect variations in the van der Waals force exerted by the metal substrate[36]). We wished to determine whether the length of the adsorbate alkyl chain had any influence on cell attachment to SAMs.

In order to address both of these questions, hydroxy, carboxylic acid and methyl terminated SAMs with short and long alkyl chains have been prepared. Murine 3T3 fibroblast cells and primary human osteoblast cells have been cultured on each surface and the number of cells counted after 24 hours. The cell morphologies have been compared. It has been found that both the nature of the terminal functional group and the adsorbate alkyl chain have an influence on the interactions of cells with SAMs, but that this effect is different for each cell type.

Experimental

Preparation of monolayers

The self-assembled monolayers were prepared on evaporated gold films supported on chromium ($\sim$10 Å thick) primed glass coverslip substrates. The gold was deposited at a rate of *ca.* 0.5 Å s^{-1} to a thickness of 20–40 nm. Immediately after gold deposition the substrates were immersed in 1mM solutions of the thiols in degassed ethanol for 12–18 hours. Following removal of the samples from the adsorbate solution, they were rinsed with degassed ethanol and dried under a stream of nitrogen. Octanethiol, OT (97%), 3-mercaptopropanoic acid, MPA (99%), propanethiol, PT (97%), and dodecanethiol, DDT (97%) were obtained from Fluka and used as received. 3-Mercaptopropan-1-ol, MPL (95%) was obtained from Aldrich and also used as received. 11-Mercaptoundecanol, MUL, and 11-mercaptoundecanoic acid, MUA, were both synthesised using a method adapted from that of Bain and co-workers.[32]

Contact angle measurements

Advancing water contact angles were measured using the sessile drop method of Bain *et al.* on a Rame-Hart 100-00 contact angle goniometer. A 2 µl droplet of water was suspended from the tip of a microlitre syringe supported above the sample stage. The syringe tip was advanced towards the sample until the drop made contact with the sample surface. The syringe was then retracted, leaving the droplet on the sample. A sample was said to be wetting if the contact angle was less than 10° and the drop perimeter was observed to be deformed.

Fibroblast culture

An anchorage-dependent murine fibroblast cell line (3T3-L1) was maintained in Dulbecco's Minimum Essential Medium supplemented with 10% newborn bovine serum (Gibco), 7.5% $NaHCO_3$ (Gibco), 1 mM sodium pyruvate (Gibco), 2 mM L-glutamine and 100 IU/100 mg penicillin/streptomycin and routinely subcultured by trypsination (0.25% trypsin in EDTA in Ca^{2+}/Mg^{2+}-free phosphate buffered saline). Cells were passaged every 2–3 days and were discarded after 50 passages. Cells were seeded at a concentration of 1×10^4 cells per mL in 3 mL of complete medium, and cultured in humidified air with 5% CO_2 at 37 °C and pH 7.2. For cell attachment assays, coverslips were dipped briefly in Earle's balanced salt solution (EBSS) (pH 7.2–7.4), fixed after 24 hours *in situ* with 5% acetic acid in ethanol and counted by means of systematic random sampling using a computer-based image analysis system connected to a video microscope.

Osteoblast culture

Primary human osteoblasts (HOS) derived from femoral head trabecular bone were maintained in culture in Dulbecco's Modified Eagles Medium (DMEM; Gibco BRL, Paisley, UK) supplemented with 10% (v/v) foetal calf serum, 0.02 M HEPES buffer, 2 mM glutamine, 50 µg/mL ascorbate and 1% (v/v) non essential amino acids at 37 °C, 5% CO_2 and pH 7.2. The osteoblastic phenotype of these cells has been previously confirmed. The SAM samples were sterilised in 70% ethanol then washed in sterile PBS prior to incubation with cells. Fresh confluent flasks of HOS cells were incubated with trypsin/HEPES (0.02% trypsin, 10 mM HEPES in Ca^{2+}/Mg^{2+}-free PBS) for 10 minutes then resuspended in culture medium. Osteoblasts were seeded on to the surfaces at a density of 4×10^4 cells cm^{-2} and maintained in culture. For each experiment, samples of each SAM were prepared in quadruplicate, and assayed post incubation in triplicate, with the remaining sample fixed for microscopic examination. Cell activity was measured using the alamar Blue assay (Serotec, Oxford, UK). After 24 hours incubation with cells, culture medium was removed from the monolayers which were then washed with Earle's Balanced Salt Solution (37 °C, Gibco BRL, Paisley), to remove non adherent cells. Samples were transferred to another 24 well plate containing 2 mL alamar Blue solution (1:9 dilution in Hank's Balanced Salt Solution, Gibco BRL, Paisley). Samples were then incubated for 90 minutes at 37 °C. After this period, 100 µL aliquots of the incubate were excited at 530 nm and read at 590 nm using a Perceptive Biosystems Cytofluor fluorimeter.

Quantification of numbers of attached cells

Clean gold substrates were employed as a control surface for both cell types. The numbers of attached cells quoted in the text are normalised to the data for the gold control surface for both cell types, enabling comparison between them, even though different methods of cell counting were employed. The data represent averages of either seven sets of data (fibroblasts) or three sets of data (osteoblasts). One-way analysis of variance was used to determine the significance of differences in cell attachment and growth on the range of SAMs, with significance determined at the 95% level.

Results

All monolayers were characterised using contact angle goniometry prior to cell culture, in order to ensure repeatable standards of sample preparation. The values measured (MPA $< 15°$, MUA $< 10°$, MPL $< 10°$, MUL $14 \pm 3°$, PT $100 \pm 3°$, OT $110 \pm 3°$ and DDT $115 \pm 2°$) were in good agreement with the literature. Representative samples were characterised using X-ray photoelectron spectroscopy (XPS) and static secondary ion mass spectrometry (SSIMS) to confirm their purity, as part of an extensive study of the structures and oxidative stability of

these materials, the results of which have been published elsewhere.[33,35,37] The results confirmed that a high standard of sample integrity was maintained throughout the study.

Fibroblast attachment

Fig. 1 displays the relative numbers of fibroblasts attached to each of the seven monolayers, compared to the gold control surface, after 24 hours culture. A large variation in the number of attached cells was observed, and it is clear from the graph that surface chemistry is an important parameter in controlling fibroblast attachment. Of the seven SAMs studied, the largest number of cells was found to attach to the acid terminated surfaces. Approximately equal numbers of cells attached to MPA and MUA surfaces, and this figure was approximately twice as large as the number of cells that were found to attach to the next most favourable surface, PT. All four of the other surfaces exhibited low numbers of attached cells—less than 10% of the figure for the gold control. The numbers of cells attached to the two alcohol terminated SAMs, MPL and MUL, were approximately equal within experimental error, and only DDT exhibited a lower number of attached cells.

The similarity of the data for MUA and MPA and the data for MUL and MPL suggests that for these molecules, the length of the adsorbate alkyl chain has no significant effect on the number of attached cells. However, for the three methyl terminated SAMs, PT, OT and DDT, there was a clear trend: the number of attached cells decreased significantly with increasing alkyl chain length.

Micrographs showing the cell morphologies provide further evidence for the influence of surface chemical structure on cell-material interactions. Fig. 2 shows light micrographs of cells grown on the different surfaces. The results may be divided roughly into three groups. First, on the gold control surfaces and for the MPA and MUA surfaces, the cells were observed to be uniformly and densely distributed at the surface. There was evidence for significant process formation and the cells exhibited a high degree of spreading, indicative of a very favourable interaction with the surface. These surfaces clearly supported cell growth. Second, on PT and OT surfaces, the density of attached cells was much lower. However, there was no evidence of clustering of cells, and those cells that did attach exhibited a significant degree of spreading and some process formation. Third, on the remaining surfaces (MUL, MPL and DDT), there were few cells and those that did attach to the surface were rounded, with no evidence of spreading, and exhibited a tendency to cluster together, indicating a more favourable interaction with each other than the surface. Clearly the cells interacted unfavourably with these surfaces.

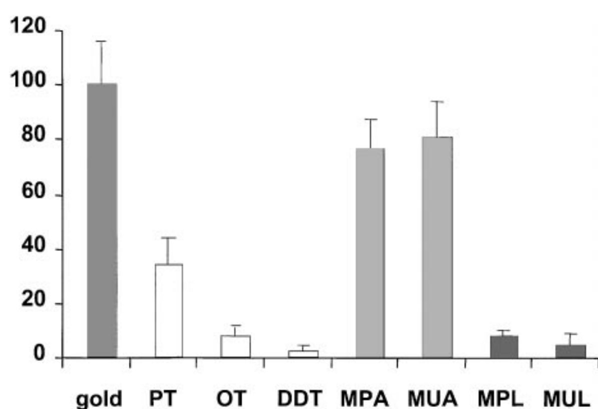

Fig. 1 Fibroblast growth on SAMs after 24 hours culture. The data are means and the error bars are the standard errors of the means. All values are normalised to gold (=100%).

Osteoblast attachment

Fig. 3 shows the numbers of osteoblast cells attached to the seven different SAMs and the gold control surface after 24 hours. As for the fibroblasts, the values quoted have been normalised to the number of cells attached to the gold control surface. It is again clear that the surface chemical structure has a strong influence on cell attachment. However, there are some important differences from the results for fibroblasts. The most prominent difference is for the results of cell culture on the hydroxy terminated SAMs. In contrast to the fibroblast behaviour, there was no significant difference in cell growth between MUA, MPL and MPA surfaces. Cell growth on MUL surfaces, though still large, was significantly less than on MPA and MPL. Cell growth on all three methyl terminated surfaces was significantly lower than on hydroxy and carboxylic acid terminated surfaces, with the exception that there was no significant difference between MUL and PT SAMs. Cell growth on PT surfaces was significantly greater than that measured on DDT and OT surfaces.

The alkyl chain length appears to have a significant effect on cell attachment to surfaces with methyl and hydroxy terminal group chemistry, but for MPA and MUA, the difference is not significant. The number of cells attached to PT SAMs was significantly greater than that attached to either of the other methyl-terminated surfaces. The figures for OT and DDT were not significantly different. This appears to represent a difference from the behaviour exhibited by the fibroblasts, for which the number of cells attached to DDT SAMs was smaller than that attached to OT SAMs. However, when the magnitude of the experimental uncertainty is properly taken into account, the discrepancy between these figures is not significant.

The trends in the numbers of attached cells were reflected in the cell morphologies observed by light microscopy (Fig. 4). For the surfaces exhibiting the highest levels of attachment, MPA, MUA, MPL and MUL, the cells were observed to be evenly distributed at the surface and exhibited a high degree of spreading. On PT, there were fewer attached cells. Some cells exhibited a high degree of spreading; however, others were rounded and there was some clustering together of cells. On the OT and DDT surfaces, cells were observed to be tightly rounded and there was some evidence of clustering together.

Discussion

The results of the present study provide strong evidence that cellular attachment to surfaces is influenced by subtle changes in substratum chemical composition and molecular structure. They demonstrate that different cell types exhibit different responses to specific surface chemistries when cultured under similar conditions and show that the length of the adsorbate alkyl chain exerts a significant influence over the outcome of cellular interactions with SAMs.

Effect of terminal group chemistry

The effect of surface chemistry on cell adhesion has been widely studied, with much of the published work utilising polymer surfaces. Curtis *et al.* studied the adhesion of BHK fibroblast cells to a variety of polymer surfaces with varying densities of hydroxy and carboxy groups.[16] They utilised simple treatments to modify polymer surfaces, undoubtedly leading to complex, polyfunctional structures and a large reaction volume (*i.e.* a poor surface specificity). However, important findings were nevertheless made. Using oxidative treatments, they introduced carboxy groups at the surfaces of four polymers and attempted to measure the density of hydroxyl groups using radiolabelled diazomethane. They concluded that carboxy groups did not

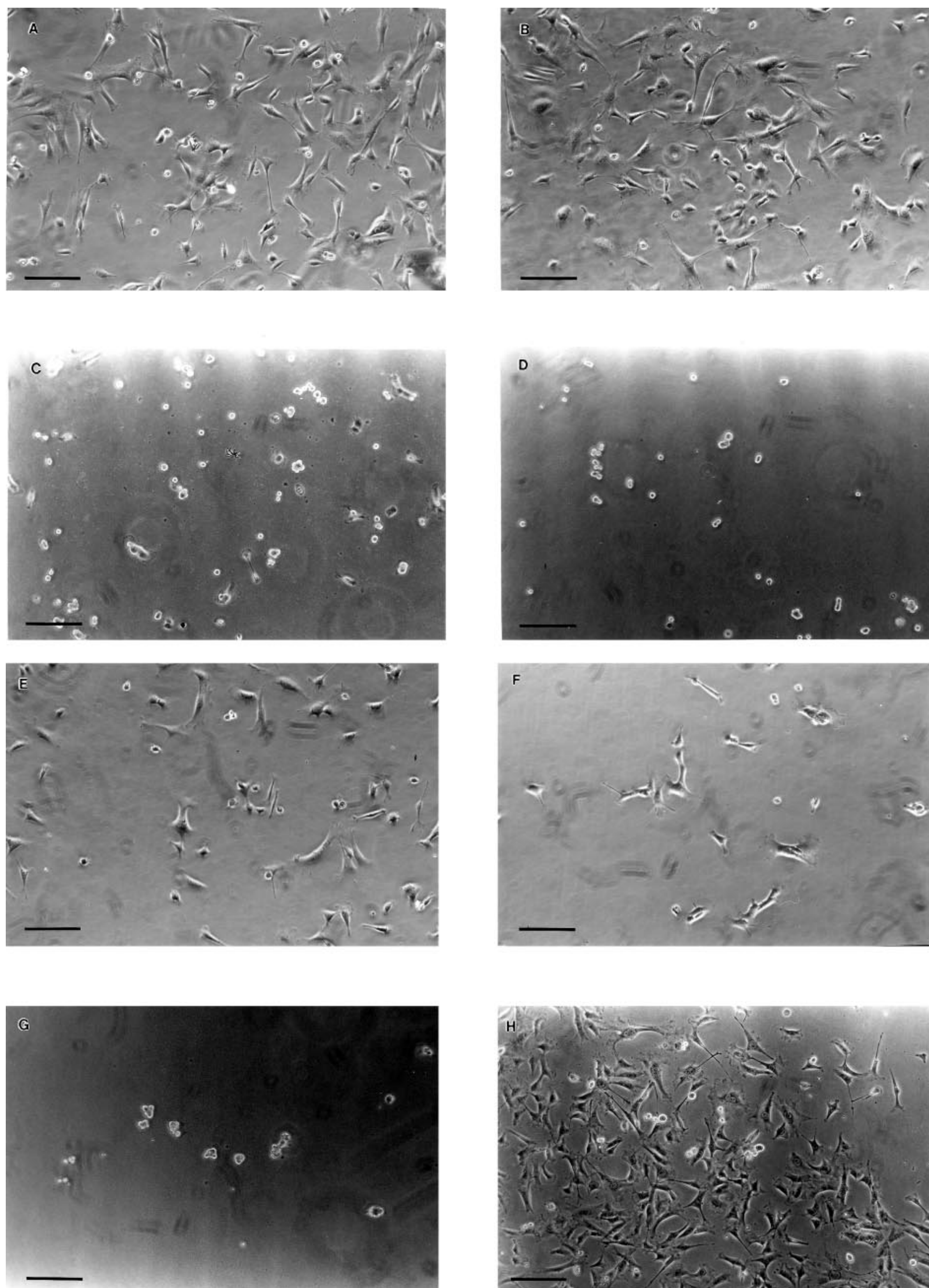

Fig. 2 Optical micrographs showing fibroblasts cultured on (a) MPA; (b) MUA; (c) MPL; (d) MUL; (e) PT; (f) OT; (g) DDT; (h) bare gold. The scale bar represents 50 μm in each case.

increase cell adhesion. However, we have observed that for both osteoblasts and fibroblasts, the carboxylic acid terminated SAM appeared to be the most favourable surface with comparable numbers of cells attaching to these surfaces as were observed to attach to the gold control surfaces, and with significant spreading being observed.

Our findings are consistent with those of Tidwell *et al.*, who studied the attachment of endothelial cells to SAMs formed by the adsorption of long chain thiols $HS(CH_2)_{15}X$ on Au, where X is COOH, CH_3, CO_2CH_3 or CH_2OH.[27] In agreement with the present study, they observed maximum attachment to carboxylic acid terminated SAMs. A recent study of keratino-

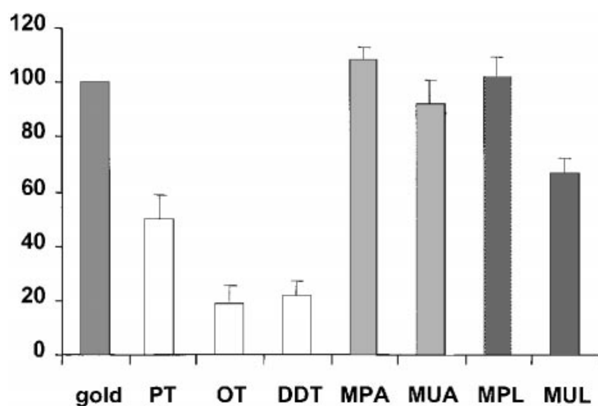

Fig. 3 Osteoblast growth on SAMs after 24 hours culture. The data are means and the error bars are the standard errors of the means. All values are normalised to gold (=100%).

cyte attachment to SAMs also reported high levels of attachment to acid terminated surfaces.[30]

In their study, Curtis *et al.* concluded that other workers who had reported increased attachment to oxidised polymer surfaces may have overlooked the importance of hydroxy groups created in the same process. In the present study, there can be no doubt about the homogeneity of the surface, illustrating a key advantage of SAMs as substrates for fundamental studies of cell-material interactions. However, there is evidence that complex chemical structures may facilitate synergistic interactions leading to high levels of cellular attachment. Significantly, tissue culture polystyrene, which was employed as a control by Tidwell *et al.*,[27] was a much better promoter of attachment than any of the four SAM surfaces they studied. They concluded from this observation that a number of different chemical groups are required to be present at a surface for the promotion of cell growth. A similar finding was reported by France *et al.*, who studied keratinocyte attachment to plasma co-polymers of acrylic acid and octa-1,7-diene.[38] They found that surfaces containing low amounts of carboxylic acid groups (2–3%) promoted cell attachment. Future studies of cellular attachment to SAMs must address this issue if they are to facilitate further progress towards understanding the relationship between surface chemical structure and cell–material interactions. The utilisation of mixed SAMs containing two or more thiols with contrasting terminal functional groups is likely to be an important strategy in such work.

Curtis *et al.* concluded that hydroxy groups were required for cell adhesion, although very high densities of hydroxy groups resulted in diminished attachment. Support for this finding has been provided by a study of fibroblast adhesion to hydroxyethyl methacrylate (HEMA) and ethyl methacrylate (EMA) copolymers.[15] Very low spreading was observed on copolymers enriched in HEMA, but maximal cell spreading was observed on a 50% EMA copolymer, suggesting that a purely hydroxylated surface is less adhesive than a mixed surface. In the present study, osteoblast attachment to hydroxy terminated SAMs was extensive, indicating a highly favourable interaction with this chemistry. In contrast, however, fibroblasts were found to attach poorly to hydroxy terminated SAMs, exhibiting poor spreading. While the osteoblast data appear to be consistent with the findings of Curtis *et al.*, the fibroblast data characterise a quite different response to hydroxy terminal groups, possibly indicative of differences in the mechanism of attachment. Tidwell *et al.* also reported a highly unfavourable response to hydroxylated SAMs by endothelial cells.[27] While these data do seem to refute the finding of Curtis *et al.* that hydroxy groups encourage cell attachment, it should be borne in mind that the density of hydroxy groups at the SAM surface is very high indeed. Thus the hydroxy terminated SAMs may correspond to the very highly hydroxylated surfaces used by Curtis *et al.*, and may not necessarily be expected to yield high levels of attachment. Again, studies involving mixed SAM systems may assist in clarifying this point.

The observations reported in the present study clearly demonstrate that different cell types exhibit different responses to given surfaces. Given that both fibroblasts and osteoblasts attached extensively to the hydrophilic carboxylic acid terminated SAMs, this result also suggests that the nature of the surface chemical structure is more important than the surface energy in determining the outcome of the cell–material interaction. High surface energy is not synonymous with high levels of cell attachment and spreading.

Effect of adsorbate alkyl chain length

Perhaps the most significant finding of the present study was that the alkyl chain length of the adsorbate molecule can have a significant effect on the number of cells attaching to a surface of given terminal group chemistry. This indicates that cellular attachment is a process that is highly sensitive to the molecular structure of the surface onto which cells adhere. It seems remarkable that entities as large as cells should be influenced in their behaviour by such small-scale changes in structure; however, the evidence presented here is clear. The influence of such subtle structural effects may realistically be investigated for cells cultured on a SAM surface; such a level of detail would be inaccessible for polymer surfaces, illustrating the importance of studies of cell–SAM interactions for the development of a thorough understanding of the behaviour of cells at interfaces.

The effect of alkyl chain length on the structures of SAMs has been studied by many techniques,[39–41] including scanning tunnelling microscopy (STM).[39] Most interest has centred on the degree of disorder within the monolayer, and it has generally been found that long-chain thiols $HS(CH_2)_{n-1}CH_3$ where $n \geqslant 8$, exhibit long-range order, reaching a quasi-crystalline state. In contrast, short chain thiols with $n \leqslant 4$ are liquid-like in character, with hexanethiol ($n = 6$) exhibiting intermediate behaviour.

Changes in the contact angles of alkanethiols with chain length have also been observed, and these have been attributed to differences in the degree of ordering.[31,32] The advancing water contact angle, θ_a, increases with n from 100° for PT to 115° for DDT. It has been argued that the greater disorder in the short chain monolayers results in exposure of methylene groups from the adsorbate alkyl chain at the surface, along with the terminal methyl groups. These have a slightly higher surface energy, and the value of θ_a is thus reduced. An alternative explanation has been put forward by Miller and Abbott, however.[36] They have argued that the change in the value of θ_a is due to changes in the van der Waals force exerted on the water droplet by the underlying gold substrate. They calculated the values expected for the advancing contact angles of hexadecane and perfluorononane on SAMs with $3 \leqslant n \leqslant 18$ using Lifshitz theory, and found good agreement between the theoretical and experimental results.

A variety of SAM interfacial interactions have been found to be influenced by adsorbate alkyl chain length. Sun and Crooks have shown using IR spectroscopy and ellipsometry that pK_a values of carboxylic acid terminated SAMs—and therefore their likelihood of participating in proton-transfer reactions—are influenced by the degree of ordering within the monolayer.[42] Generally, the pK_a values of carboxylic acid terminated SAMs are significantly higher than those of the corresponding solution-phase molecules, as a result of highly effective hydrogen bonding between adjacent carboxylic acid groups and the resulting reduction in the extent of dissociation in aqueous media. Studies of SAM photo-oxidation have revealed that the alkyl chain length has a significant effect on rates of

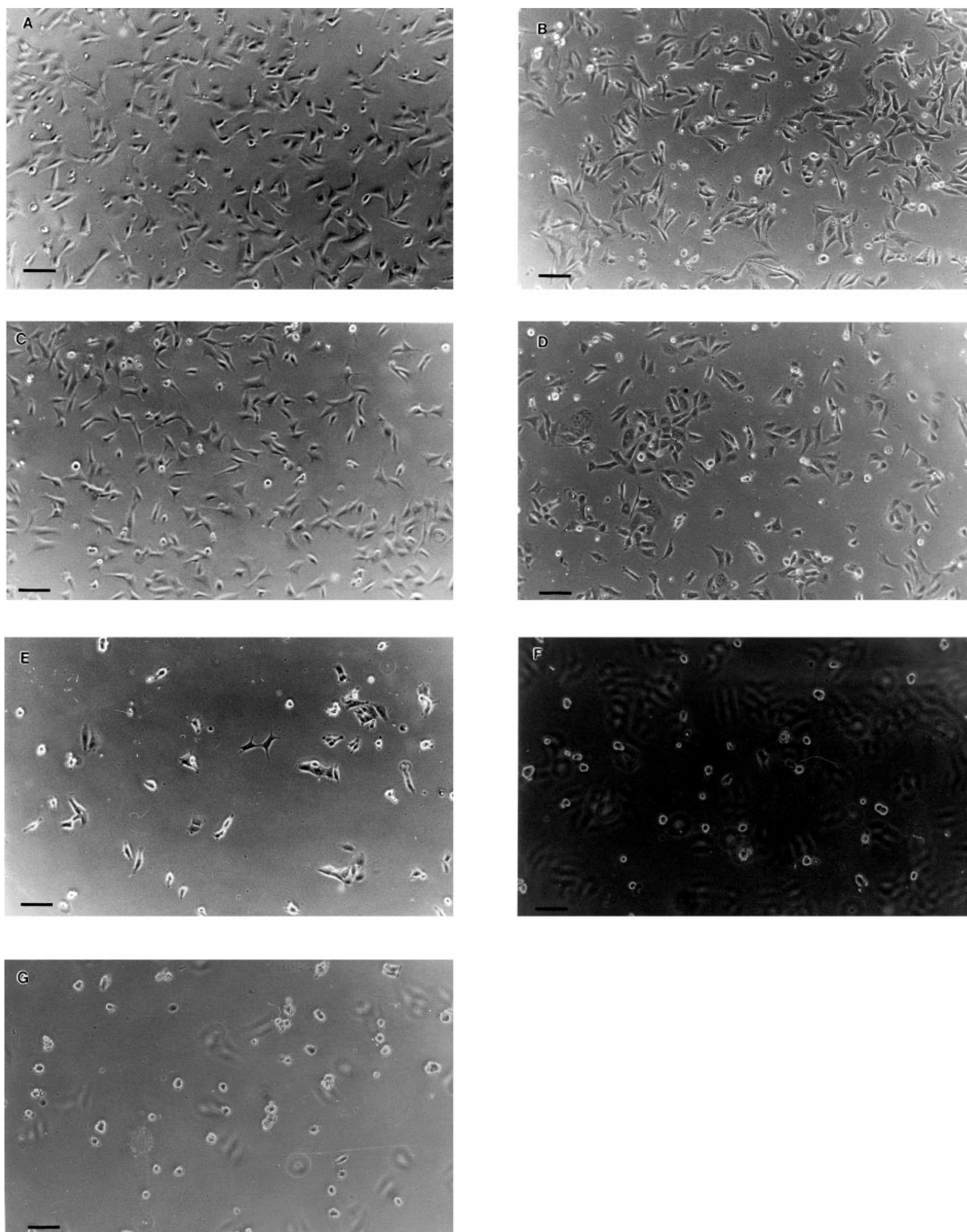

Fig. 4 Optical micrographs showing osteoblasts cultured on (a) MPA; (b) MUA; (c) MPL; (d) MUL; (e) PT; (f) OT; (g) DDT. The scale bar represents 100 μm in each case.

reaction, with long chain thiols oxidising slowly and short-chain thiols oxidising increasingly rapidly with decreasing alkyl chain length.[33–35] A recent static SIMS study concluded that there was a fourfold reduction in the rate of photo-oxidation for an increase in the alkyl chain of eight methylene groups.[35]

The reduction in numbers of attached cells with increasing alkyl chain length for fibroblasts cultured on methyl terminated SAMs is dramatic. The reduction in the numbers of attached osteoblasts on going from PT to OT is similarly dramatic, although there is little change on going from OT to DDT. However, for both cell types, the numbers of cells attaching to MPA and MUA surfaces appear to be independent of alkyl chain length; here the nature of the terminal group is the dominant influence. We suggest that the decline in numbers of cells attached to methyl terminated SAMs with increasing alkyl chain length reflects the increase in order in the SAMs. Most likely, the exposure of methylene groups at the surface together with terminal methyl groups leads to the formation of a more adhesive surface for the short chain SAMs. The hypothesis of Miller and Abbott, relating to van der Waals interactions with the substrate,[36] does not appear to explain the cell attachment data because of the similarity of the data for the acid

terminated SAMs with different chain lengths and, for fibroblasts, the similarity in data for short and long chain hydroxy terminated SAMs. Although the trend observed for the methyl terminated SAMs could fit their model, it would be expected that, given the magnitude of the difference between the PT and OT data for both cell types, a significant difference would also be observed between the numbers of cells attached to the short and long chain acid and alcohol terminated SAMs. This was not the case.

Protein adsorption

The principal criticism of the present study is that its findings are largely phenomenological; we are unable at present to provide a mechanistic explanation for our findings. Against this, it needs to be borne in mind that even the establishment of reliable phenomenological correlations between specific surface chemical structures and cell responses has been very difficult for polymeric materials. Moreover, with SAMs it is possible to reliably examine the influence of subtle structural effects (such as variations in order and orientation arising from different alkyl chain lengths) that would be lost in a polymeric system. However, at a mechanistic level, the explanation of the phenomena we have observed will surely lie in an understanding of the interactions of specific proteins with SAMs. Future studies must address this problem, and its relationship to cellular behaviour on SAM surfaces.

Conclusions

The attachment of murine 3T3 fibroblast cells and primary human osteoblast cells to self-assembled monolayers is strongly influenced by both the nature of the adsorbate terminal group and the length of the alkyl chain. For both cell types studied here, the highest levels of attachment and growth were observed on carboxylic acid terminated SAMs, and the lowest levels on the long chain methyl terminated SAM. Short chain methyl terminated SAMs exhibited intermediate levels of attachment and growth. A sharp contrast was observed in the behaviour observed on hydroxy terminated SAMs, however. For these surfaces, very low levels of fibroblast attachment were observed. Osteoblast attachment to short chain hydroxy terminated SAMs was comparable to levels of attachment to carboxylic acid terminated SAMs, and was greater on long chain hydroxy terminated SAMs than was observed on any of the methyl terminated SAMs. The number of attached cells was observed to decrease with increasing chain length for the methyl terminated SAMs for both cell types. This behaviour is attributed to an increase in order in the SAMs with increasing adsorbate alkyl chain length.

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