 DOI: 10.1002/masy.201050537

# Cell Adhesion to Polymethacrylate Networks Prepared by Photopolymerization and Functionalized with GRGDS Peptide or Fibrinogen

*C. Johnson,*[1] *L. Perlin,*[1,2] *P. Wyman,*[2] *B. Zhao,*[3] *N. J. Fullwood,*[3] *S. MacNeil,*[2] *S. Rimmer**[1]

**Summary**: Polymethacrylate hydrogels are useful scaffolds for tissue engineering and cell-based therapies provided they can be modified to give good cell adhesion characteristics. They have advantages over non-swollen materials, such as good diffusion properties, tunable moduli and porosities. However, conventional non-charged hydrogels are essentially non-adhesive for cells and they are poor substrates for cell culture. In a previous volume in this series of symposia from the Polymer Networks Group, we described how both amphiphilic conetworks and alkyl-aminated hydrogels were excellent substrates for cell culture.[1] In this article we report our most recent work on understanding how to modify hydrogels for cell attachment. We report how the choice of photoinitiator affects the viability of human dermal fibroblasts (HDFs). Next, we provide further results involving the use of an enzyme deprotection strategy that can be carried out after copolymerization of a GRGDS methacrylate monomer. We show that the peptide enhanced the culture of HDFs in serum when the peptide was attached to poly(1,2-propane diol methacrylate-*co*-ethane diol dimethacrylate). On the other hand, poly(*n*-butyl methacrylate-*co*-ethane diol dimethacrylate) networks adsorb larger amounts of protein non-specifically, and they are reasonable substrates for adhesion of HDFs without peptide modification. Attachment of GRGDS to an example of these networks did not increase the attachment and proliferation of HDFs in serum containing media. We also describe the attachment of fibrinogen/fibrin coatings by the transglutaminase mediated reaction of alkyl amines attached to a poly(1,2-propane diol methacrylate-*co*-ethane diol dimethacrylate-*co*-dodecyl methacrylate) network. This work illustrates the reactivity of alkyl-aminated hydrogels in transglutaminase catalyzed reactions, although the addition of the fibrin coating did not improve the performance of the material for cell culture which was already good on these hydrogels.

**Keywords:** amine; cell culture; hydrogel; methacrylate; peptide; photoinitiator; tissue engineering

## Introduction

The adhesion of human cells to synthetic substrates is central to the technologies that underpin regenerative medicine and cell therapy. Recently, the underlying principles that control these properties are emerging but there is still much to be determined in the detail of how synthetic materials affect cell attachment, proliferation and differentiation.

[1] Department of Engineering Materials, University of Sheffield, The Kroto Research Institute, North Campus, Broad Lane, Sheffield, S3 7HQ, United Kingdom
[2] The Polymer and Biomaterials Chemistry Laboratories, Department of Chemistry, The Polymer Centre and CTEB, University of Sheffield, Sheffield, South Yorkshire, S3 7HF, United Kingdom
[3] Department of Biomedical Sciences, Lancaster University, Lancaster, Lancashire, LA 1 4YQ, United Kingdom

In our recent work, we have shown that control of polymer network architecture and synthesis conditions, which ultimately control bulk and surface morphology, can have profound effects on cell behavior.[1–3] We have also shown that the addition of specific alkyl amine groups can convert previously non-adhesive hydrogel substrates into excellent supports for cell culture.[1,4] While the mechanisms underlying these observations on alkyl amine-functional hydrogels are not clear, it is likely that, as the alkyl group is key to the performance in cell culture, the processes involved are enzymatic. In contrast to approaches focused on control of polymer morphology/physical structure and addition of alkyl amine functionality, the often-used alternative approach of adding cell-adhesive peptides is designed to mimic the complex interactions of extracellular cell adhesion molecules with cell surface receptors. For example, Arg-Gly-Asp (RGD) peptides can bind to integrins resident on cell surfaces and these interactions often result in promotion of cell adhesion.[5] The RGD-containing peptides can be formed into monomers suitable for copolymerization,[6–9] or they can be attached directly to polymer substrates.[5] However, synthesis of the peptides requires protection and deprotection of the guanidine group on R, and we considered that a better strategy would be to carry out the deprotection after copolymerization. Unfortunately, the conventional deprotection strategies (typically the use of concentrated trifluoracetic acid) degrade many polymers of use as scaffolds. Thus, with this in mind, we recently introduced a mild enzymatic deprotection that is compatible with the use of a wide range of polymers.[10]

Hydrogels are typically highly non-cell adhesive substrates, but any of the three strategies described above (control of network architecture, addition of alkyl amines or addition of biomimetic cell adhesion peptides) can produce materials that perform well for cell culture. Hydrogels provide other advantages in tissue engineering, such as controlled delivery of growth factors and other biomolecules, and free diffusion of nutrients and metabolic waste products.

The following describes some of our recent results in this area. In particular, we describe new data on the culture of cells in serum containing media on GRGDS-functionalized networks and the enzymatic attachment of a model protein to an alkyl amine functionalized hydrogel catalyzed by transglutaminase.

## Experimental Part

### Amine Functional Polymers

Amine functional polymers were produced as described in reference 5, using dodecyl methacrylate (DMA, 1.26 g), 1,2-propane diol methacrylate (1-glyceryl methacrylate, GMA, 6.75 g) ethane diol dimethacrylate (ethylene glycol dimethacrylate, EGDMA, 0.54 g) and glycidyl methacrylate (GME, 0.45 g) dissolved in isopropyl alcohol (IPA, 4 mL) along with 2-hydroxy-2-methylpropiophenone (HMPP, 90 mg).

### Preparation of Coated Poly(ethylene terephthalate) Sheets (GRGDS Polymers and Polymers for the Photointiator Study)

Solutions of the monofunctional monomers (GMA, n-butyl methacrylate (BuMA) or the peptide monomer) plus 2.5 wt % EGDMA and 1 wt % initiator 2-methyl-4'-(methylthio)-2-morpholinopropiophenone (MMP), 2,2-dimethoxy-2-phenylacetophenone (DPP), 2-chlorothioxanthen-9-one (CTX), HMPP or a 50:50 blend of diphenyl(2,4,6-trimethylbenzoyl) phosphine oxide (DPO) and HMPP) were dissolved in methanol and purged with nitrogen for 20 minutes in a covered vessel. The solutions were syringed onto a sheet of material and drawn down the sheet using a 60 micron K-Bar. The sheet was then placed in a box with a quartz window. The box was evacuated and then flushed with nitrogen repeatedly. After a minimum of 3 cycles, the box was placed in a Dymax bond box where it was irradiated with ultraviolet light for 5 minutes.

### Preparation of Polymers for Cell Culture

Polymer sheets were washed five times in phosphate buffered saline under aseptic

 

conditions to remove the ethanol and were then cut into 13 mm discs with a no. 9 cork borer and stored in a 0.1% $NaN_3$ solution. Before the addition of cells, polymer discs were washed three times in PBS, including one overnight wash.

### Cell Culture

Human dermal fibroblast (HDF) or bovine keratocyte (BK) cells were routinely cultured in DMEM (Sigma-Aldrich, UK) supplemented with 10% (HDF) or 20% (BK) fetal bovine serum (Biosera, UK), 2 mM L-glutamine, penicillin (100 units per mL), streptomycin (100 μg per mL) and amphotericin B solution (0.625 μg per mL). The human corneal epithelial cell (HCEC) line was obtained from LGC Promochem UK (the European distributor for the American Tissue Culture Collection (ATCC)) and cultured in Epilife® medium with Human Cornea Growth Supplement (HCGS) (Cascade Biologics, UK). Each cell type was grown in a humidified atmosphere of 5% $CO_2$/95% air at 37 °C until nearly confluent. Fibroblasts were obtained from human skin obtained from patients undergoing elective surgery who gave informed consent for skin not required for their treatment to be used for research. The isolation of the cells was described in reference 10.

### Culture of Cells on Polymer Discs-Amine/Fibrin Samples

Polymer discs were transferred to a 24-well tissue culture plate and incubated overnight in 6 mg $mL^{-1}$ fibrinogen (in Epilife® supplemented with 2.5 mM $CaCl_2$) and 2U $mL^{-1}$ transglutaminase. Polymers were washed once in Epilife® before seeding cells. Polymers without fibrin were incubated overnight in either serum-free DMEM for the culture of BKs, or Epilife® medium with antibiotics for the culture of HCEC and coculture of HCEC and BK. For single culture, $2 \times 10^4$ cells were seeded in their cell specific fresh medium onto each of the polymers (in triplicate). For coculture, $1 \times 10^4$ of each cell type was seeded using serum-free conditions in Epilife® media appropriate for the culture of HCEC. All cultures, single culture and coculture, were cultured for 6 days and photographs were taken at 1, 2, 3 and 6 days. Cell viability of the cultures was assessed using MTT on day 6.

### MTT Assay

Cells were washed with PBS and then 1 mL of MTT solution was added (0.5 mg MTT / mL PBS) to each well. The cells were incubated at 37 °C and 5% $CO_2$ for 40 minutes. The MTT solution was removed and 300 μL of ethoxy ethanol was added to each well to solubilize the formazan color formed by the assay. 150 μL of ethoxy ethanol was removed from each well and placed in a 96 well plate. The absorbance at 540 nm was read using a Dynatech MR5000 plate reader with a reference wavelength of 630 nm.

### Immunofluorescence of Polymers with Fibrin

Polymers incubated overnight in 6 mg $mL^{-1}$ fibrinogen (in Epilife® supplemented with 2.5 mM $CaCl_2$) and 2U $mL^{-1}$ transglutaminase were washed three times in PBS and blocked in PBS-TM (PBS with 0.05% Tween20 and 5% skimmed milk powder) for 4 h at RT. Sheep anti-human fibrinogen-IgG (1:2000 in PBS-TM) was incubated overnight at 4 °C, followed by a donkey anti-sheep IgG conjugated to AlexaFluor® 546 (1:1000 in PBS-TM) (three PBS washed were performed before and after each step). Images of the polymers were taken at 620 nm using an automated cellular and imaging analysis system, ImageXpress™.

## Results

### Cell Adhesion to Non-Functionalized Methacrylate Networks

UV free-radical initiation has been extensively used for many years in coating applications. However, it is only in recent years that it has been used for biomaterials produced by radical polymerization.[11–15]

 

Therefore, we first set out to examine any cytotoxic effects that were inherent in HMPP, which has been approved by the US FDA and the UK MHRA for use in biomaterials and so appears to be a good candidate for the photoinitiation of these materials. In this work, its cytocompatibility was compared to some other common photoinitiators. Figure 1 shows the structures of the four photoinitiators examined. MMP, DPP and HMPP were used alone and DPO was used in combination with HMPP. They were used to polymerize mixtures of GMA with EGDMA or BuMA with EGDMA.

Generally, photoinitiators that produce radicals by the Norrish I homolytic cleavage route are highly susceptible to oxygen inhibition, which, in these systems, was minimized by carrying out the polymerizations in a nitrogen atmosphere. Cell culture was performed on films produced by UV irradiation of coatings (thickness = 60 μm) on PET sheets. In our previous work, we showed that GMA hydrogels were poor substrates for HDFs,[1–3] HCECs,[4] BKs[4] or A549 epithelial cells (article submitted), unless features such as alkyl amines, cell adhesive peptides or other hydrophobic segments were included. On the other hand copolymer networks of BuMA and EGDMA proved to be excellent substrates for HDF culture.[1–3] Figure 2 illustrates cell viability data, which clearly show the substantial difference between the performance of the poly(GMA-*co*-EGDMA) and poly(BuMA-*co*-EGDMA) copolymers. The data confirm the results of our earlier work described above. However, there were also significant differences between materials prepared with the different photoinitiators. The polymers synthesized with the DPO/HMPP mixture were significantly better at supporting cell adhesion and viability than the polymers with the same monomer synthesized with any of the other initiators. However, HMPP does have FDA and MHRA approval for use in biomaterials and its performance did appear to be satisfactory for our further studies. Further work would be required before it will be possible to deduce the mechanism of the

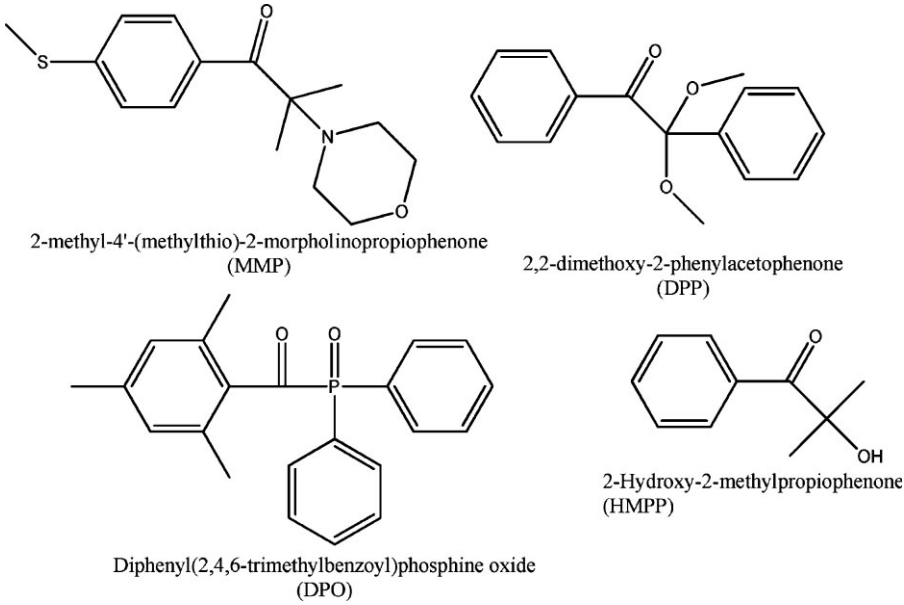


***Figure 1.***
Structures of the photoinitiators 2-methyl-4'-(methylthio)-2-morpholinopropio-phenone (MMP), 2,2-dimethoxy-2-phenylacetophenone (DPP), diphenyl(2,4,6-trimethylbenzoyl) phosphine oxide (DPO), and 2-hydroxy-2-methylpropiophenone (HMPP).

 

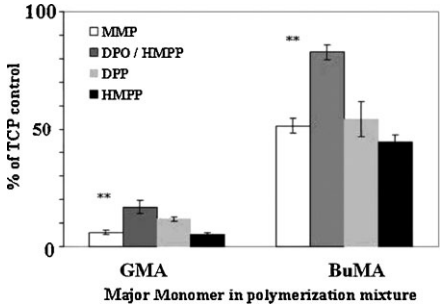


***Figure 2.***
Viability (MTT assay) of HDFs cultured for 3 days on poly(GMA-*co*-EGDMA or poly(BuMA-*co*-EGDMA) containing 2.5 wt % EGDMA and 1 wt % of the relevant initiator. All results are expressed relevant to cell viability on TCP. Error bars are standard errors of triplicate cultures ($n = 3$). Student's non-paired T-test was performed on the data, all polymers were significantly different to culture of cells on TCP ($p < 0.01$) and $^{**}$ indicates that $p < 0.01$ compared to the other polymers of the same type synthesized with different initiators.

enhancement in cell viability observed when DPO is used in combination with HMPP. However, it is likely that this effect is associated with a change in the degree of polymerization of the coating.

Figure 3 shows the morphology of the cells on the substrates poly(GMA-*co*-EGDMA), poly(BuMA-*co*-EGDMA) and TCP after 4 and 24 hours prepared with HMPP as photoinitiator. On each material, after 4 hours the cells had started to attach to the surfaces but had not yet fully spread and obtained their final morphologies. On the poly(GMA-*co*-EGDMA) network the cells adhered poorly at 4 and 24 hours. By 24 hours, a few cells had spread out but the majority remained rounded and some had aggregated into large cellular bundles. Cells on the poly(BuMA-*co*-EGDMA) surfaces adhered well after 4 hours and

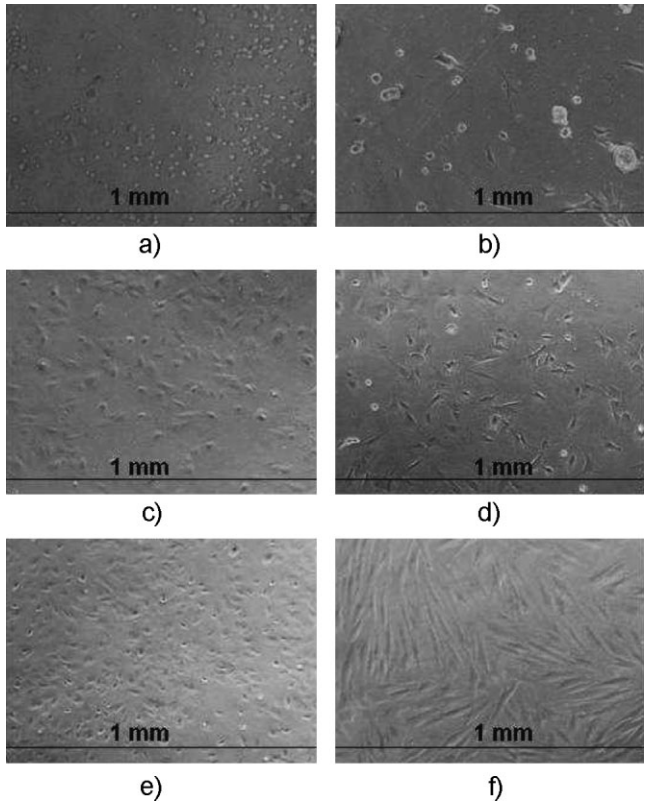


***Figure 3.***
HDFs cultured in media containing 10% FCS on: poly(GMA-*co*-EGDMA) (a) 4 h and (b) 24 h; poly(BuMA-*co*-EGDMA) (c) and 4 h (d) 24 h; TCP (e) 4 h and (f) 24 h.

 

the cells were well-spread after 24 hours. On tissue culture plastic, a larger fraction of the cells adhered than on poly(BuMA-*co*-EGDMA) but they had a similar rounded shape. However, at 24 hours the cells on TCP were clearly more elongated. Thus, the polymers induced a range of cell behavior, which was a function of the substrate and probably reflected the difference in extent and character of non-specific protein interactions to these materials.

### Functionalization of Poly(GMA-co-EGDMA) with GRGDS Peptides

Previously we reported that, in common with most non-charged hydrogels, poly(GMA-*co*-EGDMA)-based hydrogels were non-cell adhesive because they did not sorb appreciable amounts of protein.[16,17] Following this work and the observations reported in the previous section, we were interested to compare the effect of adding the GRGDS peptide to either a network that is relatively non-protein sorbing, poly(GMA-*co*-EGDMA), or to a network that could sorb significant amounts of protein from serum, poly(BuMA-*co*-EGDMA). The networks were functionalized with the GRGDS peptide by copolymerization with a GRGDS-methacrylate monomer in which the arginine unit was protected as the 4-bromosulphonamide. Following copolymerization by UV irradiation of comonomer films, the protecting group was removed by glutathione-S-transferase (GST) in combination with the cosubstrate, reduced glutathione (GSH). The two peptide monomers are shown in Figure 4 and their synthesis and their subsequent post-polymerization deprotection is described in reference 10.

### Poly(GMA-*co*-EGDMA) Networks Modified with Attached GRGDS

In Perlin et al,[10] we described how poly(GMA-*co*-EGDMA) functionalized with these peptides was an excellent substrate for the culture of HDFs in

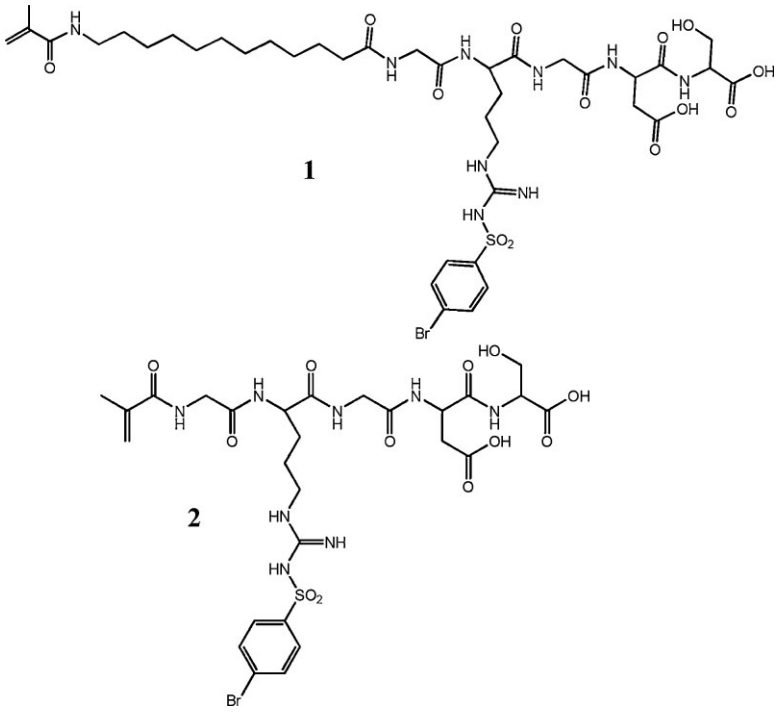


***Figure 4.***
GRGDS methacrylate monomers with 4-bromosulphonamide-protected arginine.

 

serum-free media, and here we show the results of experiments carried out in media containing fetal calf serum. Figure 5 shows micrographs taken after 4 hours culture of fibroblasts on poly(GMA-*co*-EGDMA-*co*-**1**) using various loadings of peptide **1** and after deprotection with GST/GSH.

Clearly, after 4 hours there are already large differences between the way cells behave on polymers containing different concentrations of RGD. At the lowest RGD concentration the cells appeared very similar to those on the non-peptide containing hydrogel (Figure 4a), whereas at the higher concentrations the cells appeared more like those on tissue culture plastic at the same time point.

Figure 6 shows micrographs of cells cultured for 24 hours on poly(GMA-*co*-EGDMA) functionalized with **1** or **2**. After 24 hours, the cells on each material had spread to a larger degree, but at 0.01 μmol $g^{-1}$ there was little difference compared to the cells on the non-functionalized material. There was a continual improvement in cell morphology and cell density as the amount of peptide in the monomer feed increased, and the materials with the highest concentrations appeared indistinguishable from the positive control, TCP. There appeared to be little difference between the materials functionalized with **1** or **2**.

### Poly(BuMA-*co*-EGDMA) Networks Modified with the GRGDS Peptide

BuMA polymers, in common with other hydrophobic and non-swollen polymers, generally adsorb large amounts of proteins from serum and consequently the effects of the GRGDS peptide may be masked by the presence of the protein layers. Figure 7 illustrates that there were no differences in the cell morphologies after 24 hours between the polymers containing the long peptide (poly(BuMA-*co*-EGDMA-*co*-**1**) sequence with the spacer arm and morphologies observed with the peptide without the spacer arm at the same concentration (poly(BuMA-*co*-EGDMA-*co*-**2**). There is some indication that cells are better spread on the polymers containing larger amounts of peptides but the effects are much less pronounced than in the poly(GMA-*co*-EGDMA) systems.

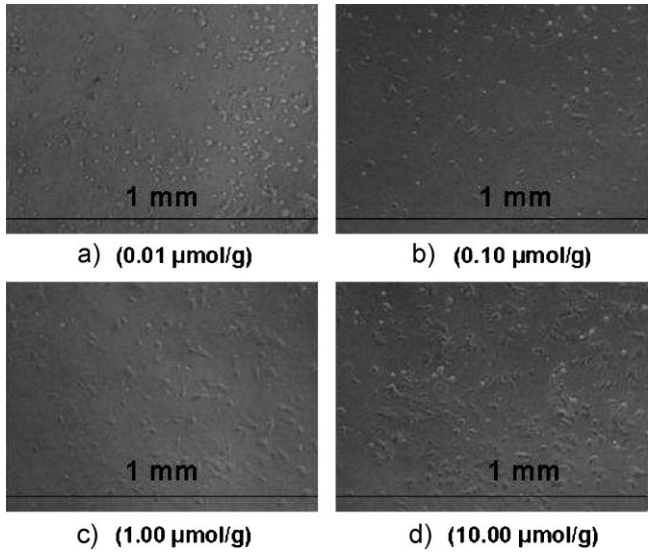


***Figure 5.***
HDFs cultured in media containing 10% FCS for 4 hours on poly(GMA-*co*-EGDMA-*co* -**1**) pre-incubated with GST/GSH for 1 day. Values in parentheses are amounts of peptide monomer in the feed based on total mass of monomer.

 

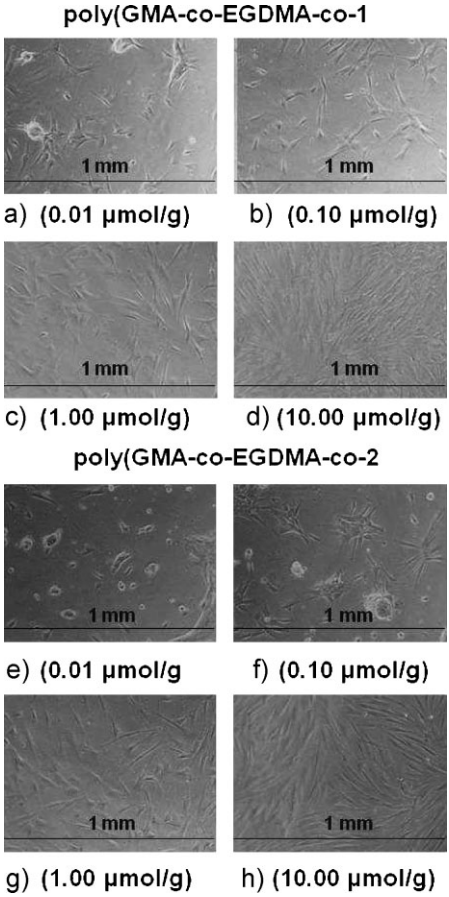


*Figure 6.*
HDFs cultured in media containing 10% FCS for 24 hours on poly(GMA-*co*-EGDMA-*co*-**1** (a-d) or poly(GMA-*co*-EGDMA-*co*-**2**) (e-h) pre-incubated with GST and GSH for 1 day. Values in parentheses are amounts of peptide monomer in the feed based on total mass of monomer.

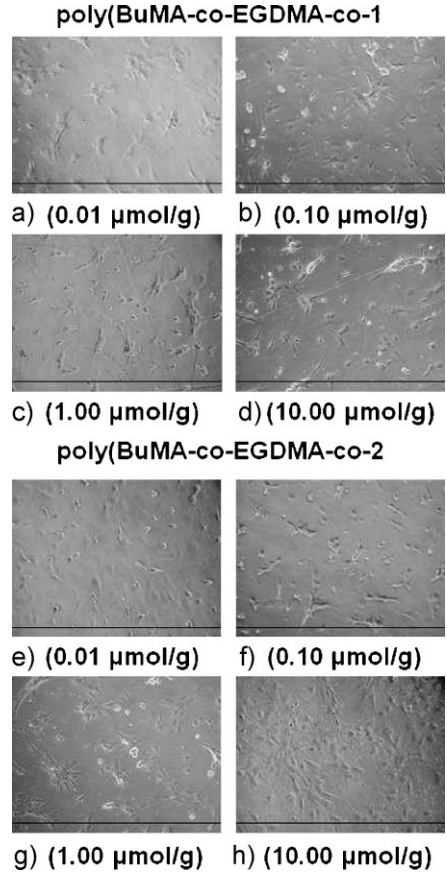


*Figure 7.*
Primary human dermal fibroblasts cultured in media containing 10% FCS for 24 hours on poly(BuMA-*co*-EGDMA-*co*-**1**) (a-d) or poly(BuMA-*co*-EGDMA-*co*-**2**) (e-h) pre-incubated with 0.05 mg $mL^{-1}$ GST and 500 µmol $dm^{-3}$ GSH for 1 day. Values in parentheses are amounts of peptide monomer in the feed based on total mass of monomer.

**Protein Modification of Poly(1,2-propane diol methacrylate-*co*-ethylene Glycol dimethacrylate-*co*-dodecyl methacrylate) (Poly(GMA-*co*-EGDMA-*co*-DMA)) Functionalized with Amine-Groups Using Transglutaminase**

Transglutaminases are extracellular enzymes that form protein cross-links by catalyzing the reaction of lysine with glutamate residues. In our previous work, we showed that the alkyl amination of hydrogels formed from copolymers of GMA containing GME, as shown in Figure 8, produced materials that were excellent substrates for the adhesion of various cells. Although amination had previously been shown to improve the adhesion of cells, the highly significant effect of the alkyl group had not been reported prior to our work. Our work showed that alkyl groups of 3, 4 or 6 carbons were required to convert poorly cell adhesive hydrogels into substrates that were suitable for culture. On the other hand, no enhancement of cell adhesion was observed by modification with ammonia or alkyl amines containing alkyl groups with 2 carbons.

These observations and the structural similarity of the alkyl-aminated units,

 

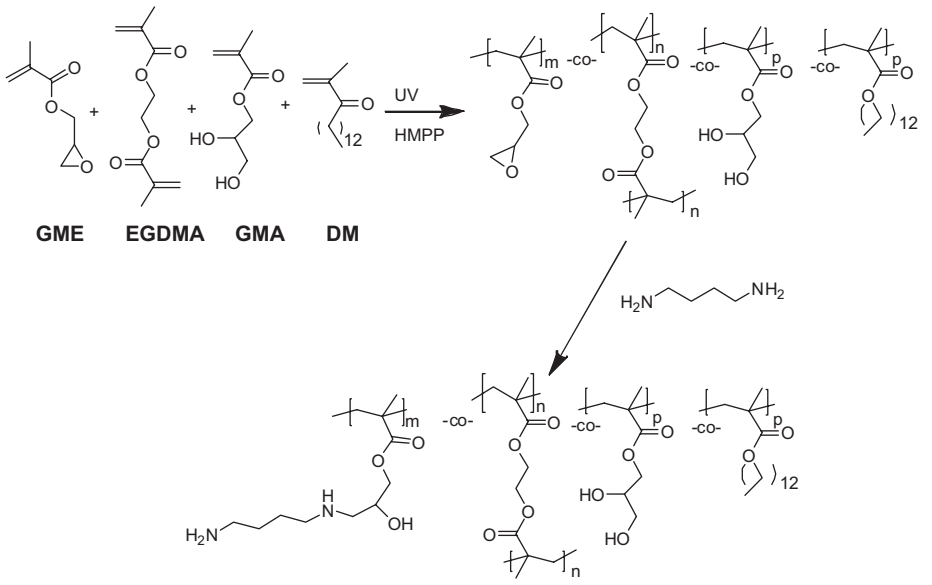


***Figure 8.***
Preparation of alkyl-aminated hydrogels illustrated by the use of 1,4-butane diamine (alkyl group containing 4 carbons).

which provide useful cell adhesive functionality, to lysine have led us to the hypothesis that the source of the enhancement in cell adhesion is enzymatic modification of the hydrogel by extracellular enzymes. In our first attempts to test this hypothesis we studied the feasibility of attaching a model protein, fibrin, with known activity in the transglutaminase mediated lysine/glutamate reaction.

Figure 9a shows a SEM image of the surface of poly(GMA-*co*-EGDMA-*co*-DMA) modified by reaction with 1,4-butane diamine. The micrograph shows a relatively uniform porous surface with pores in the 100 nm range. As expected, exposure to a solution of fibrinogen resulted in adsorption of fibrinogen to the hydrogel surface. Figure 9b shows that these surfaces were irregular and uneven and were different in appearance to surfaces produced by exposing this hydrogel to a solution of fibrinogen and transglutaminase. Figure 9c then presents an example of the images of the surface after exposure to this enzyme. The surface is clearly modified by a fibrillar network structure. At higher magnification (Figure 9d), one can discern that the fibrils have diameters in the range 200–500 nm. As a direct comparison, we also prepared a similar hydrogel that was functionalized by reaction of the epoxy groups on the GME units with ammonia. Similar materials were previously shown by us to be poor substrates for cell culture. This material was also treated with a solution of fibrinogen and transglutaminase. Figure 9e shows an example of a typical electron micrograph of this material and clearly shows that treatment of this surface also results in the deposition of fibrin. However, the degree of modification was much lower and the fibrin appears to be deposited in aggregates rather than in the fibrils observed in Figure 9c and 9d.

The presence of the fibrin coating was further verified by immunofluorescence of the material modified with 1,4-butane diamine. Sheep anti-human fibrinogen-IgG was first bound to the fibrinogen surface, then anti-sheep IgG conjugated to Alexa-Fluor® was added. Thus functionalization with fibrin/fibrinogen could be detected as a

 

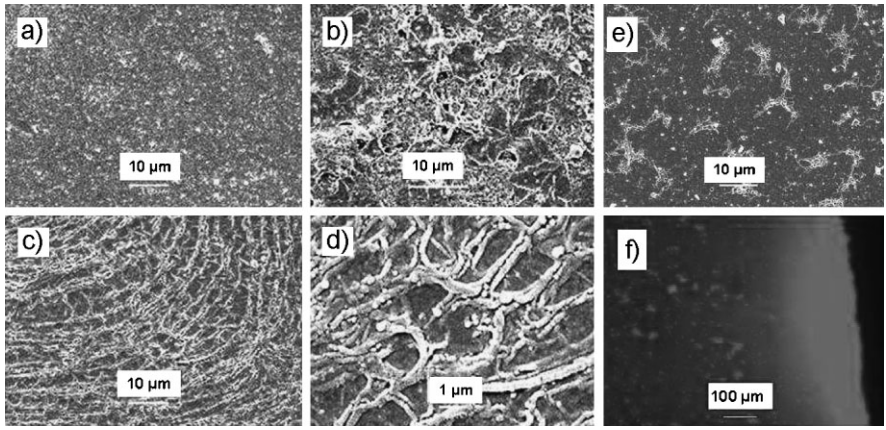


***Figure 9.***
Scanning electron micrographs of poly(GMA-*co*-EGDMA-*co*-DMA) functionalized with 1,4-butane diamine. (a) The surface of the 1,4-butane diamine polymer after amination; (b) after adsorption of fibrinogen; (c) and (d) after treatment with transglutaminase and fibrinogen; (e) the surface of a hydrogel functionalized with ammonia followed by treatment with fibrinogen and transglutaminase, and, (f) a fluorescence micrograph (×10) of a polymer functionalized with 1,4-butane diamine, fibrinogen/transglutaminase and visualized by binding of sheep anti-human fibrinogen-IgG/Alexafluor® anti sheep IgG.

fluorescent coating. Figure 9f shows a fluorescence micrograph of the hydrogel after reaction with fibrin and transglutaminase and then labeling with the antibody fluorophore system. The micrograph shows clear evidence for the presence of a fibrinogen layer after treatment with fibrin and transglutaminase.

Culture of BKs, HCECs and co-culture of these cells on these fibrinogen functional hydrogels was more dependent on the nature of the amine functionality than the presence of fibrinogen coatings. For example Figure 10 shows MTT cell viability data obtained from the culture of cells for 6 days on these hydrogels. Each plot compares the cell viability of control samples with a fibrinogen functional hydrogel and the precursor, amine functional hydrogel. Three experiments were performed for each material with the cell systems stated above. Clearly the MTT assay indicated a larger number of cells were present in a viable state ($p < 0.05$ in all comparisons) on each of the materials functionalized with 1,4-butane diamine. Also, there appears to be little difference between materials with or without fibrin. As we previously reported, modification with ammonia produced materials that were not useful as surfaces for cell adhesion and the addition of fibrin did not enhance the performance of these materials.

## Discussion

The adhesion and proliferation of cells to synthetic materials is affected by several materials variables as well as by culture conditions. Several of the variables are interconnected so that producing structure-performance predictions and models has been difficult. However, we are now in a position to address these complex systems by using carefully designed experiments that exploit three separate hydrogel systems that can support cell culture: optimized amphiphilic conetworks; alkyl amine functional hydrogels and hydrogels functionalized with cell adhesive peptides.

It seems reasonable to assume that the cells are using different mechanisms to adhere and proliferate in each system. In the conetwork system, it is most likely that non-specific protein adsorption is the key factor.[3] On the other hand, cell adhesion to alkyl-amine functionalized random

 

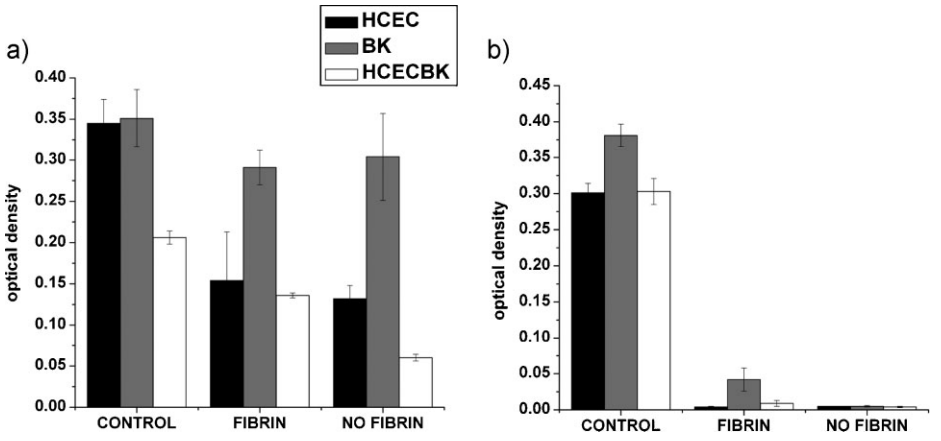


***Figure 10.***
Cell viability data (MTT assay) of cells cultured on poly(GMA-*co*-EGDMA-*co*-DMA) functionalized with either 1,4-butane diamine (a) or ammonia (b) followed by further modification with fibrin/transglutaminase. Data were obtained after 6 days culture with bovine keratocytes (BKs), human corneal epithelial cells (HCEC) and BK and HCEC in co-culture. Results shown are means +SEM of triplicate experiments.

copolymer hydrogels is strongly influenced by the structure of the alkyl group with alkyl groups that resemble the side chain of lysine being the most effective.[4] In this work, we show that addition of a transglutaminase enzyme can mediate the attachment of an extracellular protein, fibrin, in a manner that mimics the reaction of lysine with glutamate in proteins of the extracellular matrix. Interestingly, transglutaminase appears to be inactive in catalyzing a reaction of the amine functional hydrogel that does not contain an alkyl spacer between the primary amine and the polymer chain. This difference in enzymatic activity is reflected in the cell culture data reported in the previous volume of this series,[1,4] and it is likely that the enhancement in cell culture performance is due to enzymatic modification of the alkyl amine functional interface during cell adhesion and proliferation. Further work is underway in our laboratories to investigate this hypothesis and will be reported in due course.

Finally, in a third mode of cell adhesion, cell adhesive peptides are thought to bind to cell surface integrins. In previous work, we showed that, in serum free media, non-cell-adhesive hydrogels can be converted to excellent substrates for cell culture and that the cells can be removed by competitive binding of soluble GRGDS, which blocks the integrin receptor sites on the cells.[11] In this work, we showed that hydrogels that do not sorb large amounts of protein can also be enhanced for cell adhesion in this way, even in serum-containing media. In future experiments, the hypothesis that cell adhesion to each of the three systems operates by different mechanisms will allow us to investigate the details of these cellular processes by carefully designing parallel experiments in which the cells are cultured on different materials from each class.

## Conclusion

We have shown that attachment of GRGDS to a hydrogel that does not sorb appreciable amounts of protein produces a large enhancement in the ability of the material to support cell adhesion and proliferation in serum containing media. However, the attachment of the peptide had a much smaller effect in serum containing media in culture on a network that sorbs larger amounts of protein non-selectively. Also, we have shown that transglutaminase can catalyze the attachment of fibrin fibers to a hydrogel functionalized with an alkyl amine.

 

Fibrinogen was also deposited on a hydrogel functionalized with a primary amine without an alkyl spacer but the material did not have the fibrillar structure observed with the alkyl amine functionalized material.

[1] Y. Sun, J. Maughan, R. Haigh, S. A. Hopkins, P. Wyman, C. Johnson, N. J. Fullwood, J. Ebdon, S. MacNeil, S. Rimmer, *Macromol. Symp.* **2007**, *256*, 137.
[2] S. Rimmer, M. J. German, J. Maughan, Y. Sun, N. Fullwood, J. Ebdon, S. MacNeil, *Biomaterials* **2005**, *26*, 2219.
[3] Y. Sun, J. Collett, N. J. Fullwood, S. Mac Neil, S. Rimmer, *Biomaterials* **2007**, *28*, 661.
[4] S. Rimmer, C. Johnson, B. Zhao, J. Collier, L. Gilmore, S. Sabnis, P. Wyman, C. Sammon, N. J. Fullwood, S. MacNeil, *Biomaterials* **2007**, *28*, 5319.
[5] L. Perlin, S. MacNeil, S. Rimmer, *Soft Matter* **2008**, *4*, 2331.
[6] L. M. Weber, K. N. Hayd, K. Haskins, K. S. Anseth, *Biomaterials* **2007**, *28*, 3004.
[7] C. R. Nuttelman, M. C. Tripod, K. S. Anseth, *Matrix Biology* **2005**, *24*, 208.
[8] D. L. Hern, J. A. Hubbell, *J. Biomed. Mater. Res.* **1998**, *39*, 266.
[9] C. E. Kang, E. J. Gemeinhart, R. A. Gemeinhart, *J. Biomed. Mater. Res.* **2004**, *71A*, 403.
[10] L. Perlin, S. MacNeil, S. Rimmer, *Chem. Commun.* **2008**, 5951.
[11] A. S. Hoffman, *Adv. Drug Deliv. Rev.* **2002**, *43*, 3.
[12] S. Halstenberg, A. Panitch, S. Rizzi, H. Hall, J. A. Hubbell, *Biomacromolecules* **2002**, *3*, 710.
[13] S. J. Bryant, K. S. Anseth, *J. Biomed. Mater. Res.* **2003**, *64A*, 70.
[14] B. K. Mann, J. L. West, *Anat. Rec.* **2001**, *263*, 367.
[15] J. A. Burdick, K. S. Anseth, *Biomaterials* **2002**, *23*, 4315.
[16] R. Haigh, N. Fullwood, S. Rimmer, *Biomaterials* **2000**, *21*, 735.
[17] R. Haigh, N. Fullwood, S. Rimmer, *Biomaterials* **2002**, *23*, 3509.

 