Polymethacrylate Networks as Substrates for Cell Culture

Y. Sun,[1] *J. Maughan,*[1] *R. Haigh,*[3] *S. A. Hopkins,*[1] *P. Wyman,*[1] *C. Johnson,*[2] *N. J. Fullwood,*[3] *J. Ebdon,*[1] *S. MacNeil,*[2] *S. Rimmer**[1]

Summary: methacrylate networks have a long history of applications in medical technology and much is known of their non-fouling properties. However, in recent times it has become clear that the swollen nature of these materials may provide some advantages if they are used as scaffolds in tissue engineering. In general however these hydrogels are resistant to protein adsorption and human cells do not easily adhere. In this work we provide an overview of several strategies that are designed to improve the cell-adhesive properties of hydrogels while maintaining their useful properties, mainly ease of diffusion of nutrients and growth factors. We describe our early attempts at modifying hydrogels based on 2,3-propandiol -1-methacrylate, with either hydrophobic units or acid groups. Modification with lauryl methacrylate produced an improvement but acid modification failed to provide surfaces that were conducive to cell culture. Much better scaffolds were prepared by amination of epoxy functional 2,3-propandiol-1-methacrylate networks. Optimized materials in this class were shown to be good substrates for the co-culture of bovine keratocytes with human corneal epithelial cells. We also describe the synthesis and biological properties of methacrylate conetworks, which phase separate during synthesis to give porous amphiphilic materials. Optimization of these materials produces materials that perform as well as tissue culture plastic so that confluent sheets of human dermal fibroblasts can be produced using standard culture techniques.

Keywords: amine; cell culture; conetworks; hydrogel; porous; methacrmethylate; tissue engineering

Introduction

Amphiphilic networks often have properties that make them suitable for medical applications such as contact lenses, drug delivery, tissue engineering, etc.[1–13] They are composed of either random repeat units of hydrophobic and hydrophilic monomers or block copolymer sequences (conetworks). Both classes are generally cross-linked by incorporation of difunctional agents. Random copolymer networks have been known for many years and they form the basis of several technologies. On the other hand amphiphilic conetworks are a more recent innovation. One of the earliest studied amphiphilic conetworks were those based on the copolymerization of oligoisobutylene macromonomers.[2,3] These materials can have a range of morphologies including co-continuous structures as well as phase separated structures on the nano or micron length scales. These features can enable rapid surface reorganization of hydrophobic and hydrophilic domains and diffusion of biomoleulecules. The utility of

[1] The Polymer and Biomaterials Chemistry Laboratories, Department of Chemistry (The Polymer Centre and CTEB), University of Sheffield, Sheffield, South Yorkshire, S3 7HF, UK
E-mail: S.Rimmer@sheffield.ac.uk
[2] Department of Engineering Materials and Division of Biomedical Sciences and Medicine University of Sheffield, The Kroto Research Institute, North Campus, Broad Lane, Sheffield, S3 7HQ, UK
[3] Department of Biological Sciences, Lancaster University, Lancaster, Lancs, UK LA 1 4YQ

these materials is related to the presence of large amounts of water. In this way they resemble living tissue and it is this feature that makes them generally acceptable in clinical and surgical applications. However, unlike physicochemical-structure property relationships, the complex nature of the biological milieu and the scarcity of data on well-characterized materials means that biological-structure relationships are less well understood. With a view to elucidating these relationships Kohn et al. have introduced the concepts of parallel synthesis/experimental design to Biomaterials Science.[14] The concept involves producing libraries of materials that contain regular changes in materials structural variables. These libraries are then examined by both physical and biological techniques. Since it is possible to examine the libraries with many different techniques, the power of this methodology lies in the fact that it is not necessary to formulate a hypothesis to determine structure-property relationships. Most tissue engineering and some drug delivery applications require materials that are porous. Porosity can be induced in hydrogel/amphiphilic materials in several ways including leaching of salts and other porogens;[15] phase separation during polymerization[16] and by mechanical means.[17]

In this paper we will provide an overview of our work along with previously unpublished results on two series of amphiphilic networks. The first of these is a series of hydrogels composed of random repeat units derived from 2,3,-propandiol-1-methacrylate (GMMA), dodecyl methacrylate (DM) and ethandiol dimethacrylate (EDMA)[18,19] and functionalized derivatives of these networks. The second set of materials is a series of amphiphilic networks derived from the terpolymerization of oligo(butyl methacrylate) macromonomers, EDMA and GMMA.[20–23]

Experimental Part

Preparation of Oligo(butyl methacrylate) Macromonomers

Oligo(butyl methacrylate)s with carboxylic acid end groups (OBMA + 2COOH) were prepared by ozonolysis of poly(butyl methacrylate-co-butadiene) latexes.[22] The resultant materials were then further functionalized by reaction with glycidyl methacrylate to yield OBMA-dimethacrylate.[20,21]

Hydrogel Preparation

Preparation of the networks, except those containing vinyl sulphonic acid (VSA), has already been described in detail previously.[18,20,21] Briefly, the required monomers were dissolved in 2-propanol and were polymerized by radical polymerization in a glass mould, lined with PET sheets. The networks were then purified by extraction with 2-propanol and ethanol. The synthesis of aminated networks is described in reference 24. Poly(GMMA-co-EDMA-co-VSA) networks were prepared in water (50 wt%) using azobis (cyanopentanoic acid) (1 wt%) as the initiator at 60 °C. The equilibrium water contents (EWC) were obtained by hydration in ultrapure water prepared by filtration (Milli-Q Systems) and are calculated according to Equation (1).

$$\mathrm{EWC}\ (\%\ \mathrm{wt}) = ((\text{wet weight} - \text{dry weight})/\text{wet weight}) \times 100 \quad (1)$$

Deprotection of Acetal-Functional Monomers Using Iodine in Methanol

Hydrogels composed of methacrylic acid, the acetal of GMMA (GMAc) and EDMA were deprotected in 1% I_2 in Methanol as follows: Disks of the networks were cut with a N° 10 cork borer (total 250 mg). The disks were soaked in methanol (HPLC grade, 15 ml) containing Iodine (10 mg ml^{-1}) at room temperature for 16 hrs. The networks turned orange over this time period and the colouration was removed by washing with ethanol (20 ml × 5 over 3 days).

Cell Growth Studies

Cell Culture Carried out on poly(GMMA-co-DM-co-EDMA) Networks[19]

1BR3-transformed human skin fibroblasts were maintained in minimum essential medium containing bovine foetal calf

serum in a humidified atmosphere with elevated CO_2 at 37 °C until confluent. The fibroblasts were then harvested and seeded onto hydrated samples of hydrogels in 96 or 24-well cell culture plates. The culture plates were then incubated for 24 hours.

Cell Culture Carried out on poly(GMMA-co-MA), poly(GMMA-co-VSA) and Block Copolymer Networks[20,21]

Assessment of the extent of cell attachment and proliferation on the networks was examined using human dermal fibroblasts. DMEM tissue culture media supplemented with 10% fetal calf serum was used and the cells were examined after 1,4 and 6 days. Fibroblast adhesion was assessed by reacting cells with a vital dye (MTT ESTA assay), which reacts to intercellular dehydrogenase activity with a change in optical density.[24]

Co-Culture on Amine-Functional Polymers[25]

Hydrogel discs were transferred to a 24-well tissue culture plate and incubated overnight in Epilife® medium with antibiotics. 1×10^4 of each cell type were seeded using serum-free conditions in Epilife media appropriate for the culture of HCEC. Cultures were incubated for 8 days and photographs were taken at 1, 2, 3 and 8 days. Cell viability was assessed using the MTT ESTA assay.

Results and Discussion

Several series of statistical terpolymer amphiphilic networks and similar variants with block conetwork architecture were prepared. The variables associated with these materials are: hydrophilicity (ratio of hydrophilic to hydrophobic monomer); cross-link density (in this case amount of ethandiol dimethacrylate (EDMA) in the monomer feed content) and equilibrium water content (EWC). Statistical terpolymers prepared by radical polymerization of GMMA, DM and EDMA did not show evidence of phase separation and even when swollen in water they were optically clear at all compositions.[18,19] The EWCs (degree of swelling) of these materials increased with decreasing EDMA content and increased with GMMA content; the former produces an increase in cross-link density and the latter an increase in $\chi_{1,2}$.

Examination of the effect of network composition on the EWC has also allowed us to identify sets of materials that have constant EWC (*iso*-EWC materials) and these materials have allowed us to design experiments that allow us to investigate the effect of the water structure on cell adhesion and proliferation. In a DSC study of materials with equal gross EWC but different repeat unit compositions, these were found to contain different fractions of swelling water that could be frozen.[19] The fraction of freezing water can be associated with water that is mobile and not hydrogen bonded to the polymer chain and this type of water was at the lowest level in hydrogels that contained a hydrophobic repeat unit (DM) as well as the hydrophilic monomer (GMMA). The explanation for this behaviour lies in a consideration of the overall dielectric constant of these homogeneous materials. Thus, the dielectric constant of poly(GMMA-co-EDMA) swollen with water is likely to be higher than poly(GMMA-co-DM-co-EDMA) swollen with the same amount of water. The extent of hydrogen bonding will then be greater in the poly(GMMA-co-DM-co-EDMA) materials and smaller fractions of water are available to freeze because of hydrogen bonding interactions with the polymer chain. These changes in the structure of water could affect the use of hydrogels as substrata for cell culture. Those materials containing large fractions of freezing water tended to be poor substrates for cell culture; few cells adhered and those that did had rounded morphologies. On the other hand substantial improvements in the morphology and numbers of cells were observed by using the poly(GMMA-co-DM-co-EDMA) substrates. Figure 1 illustrates these phenomena using scanning electron micrographs of human dermal fibroblasts grown on two materials of equal EWC. Material (a) is composed of residues of GMMA, EDMA

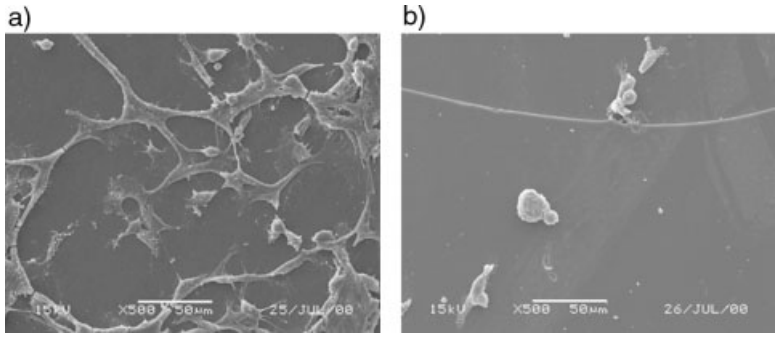


Figure 1.
Human dermal fibroblasts cultured for 24 hours on materials of equal EWC but containing differing fractions of freezing water (a) GMMA:DM:EDMA 75:25:4.8, EWC 38% (b) GMMA:EDMA 100:11.5EWC 38%.

and DM and the cells spread and adhered whereas (b) is composed of GMMA and EDMA and the cells showed rounded morphologies in order to minimize contact with the surface of the hydrogel.

Whilst improvements in cell adhesion and spreading were obtained by inclusion of hydrophobic monomers we were unable to form confluent sheets of fibroblasts in this manner. With this in mind we considered that modification of the polymers by addition of the acid monomer vinyl sulphonic acid could be a useful strategy for adding charge to the hydrogels, which is occasionally claimed to enhance cell adhesion. A series of terpolymers of vinyl sulphonic acid (VSA), GMMA and EDMA were produced (EWC = 64–75 wt%). However, assessment of cell viability (Figure 2), using the MTT assay, showed that modification with VSA did not provide materials that were useful as substrates for cell culture.

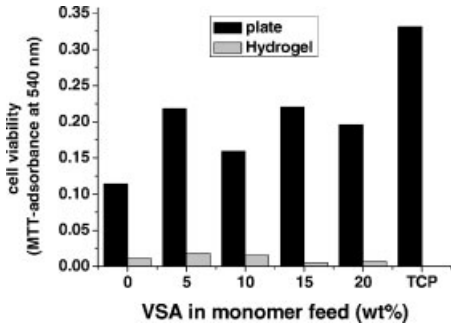
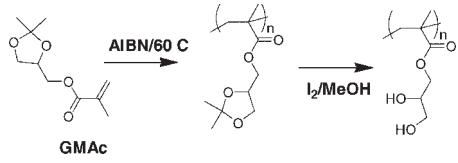


Figure 2.
Cell viability data obtained from HDFs cultured on a series of Poly(GMMA-co-EDMA-co-VSA) networks. The data show that the cells tend to migrate away from the hydrogel surfaces on to the tissue culture plate (TCP) so that the majority of the viable cells are observed on the TCP.

In experiments (Figure 2) in which HDFs were initially seeded onto the hydrogels and then allowed to migrate from these onto the tissue culture plastic (TCP) wells over 2 days, there was no significant difference between any of the VSA-modified compositions and the non-cell adhesive control poly(GMMA-co-EDMA). Figure 2 shows that the majority of the viable cells had migrated from the samples onto the underlying plate.

As an alternative to modification with VSA we also produced hydrogels containing methacrylic acid (MA) residues, which modify the materials by adding carboxylic acid (COOH) groups. Hydrogels composed of GMMA, DM and EDMA (in the weight ratio 10.1:2.7:1 respectively) modified with various amounts of MA (0.5, 1 and 2.1 wgt.%) were used as substrata for the culture of bovine keratocytes (BK). However, in this set of experiments we examined an alternative route to preparation of



Scheme 1.
Polymerization of the acetal derivative of GMMA (GMAc) and removal of the acetal group with iodine.

polymers containing the GMMA repeat unit. Since GMMA is relatively difficult to purify by distillation we prepared a polymerizable acetal precursor, GMAc, which was polymerized and then deprotected by reaction with iodine in methanol, as shown in Scheme 1.

Figure 3 shows optical micrographs of attempts to culture HDFs on examples of the MA modified polymers. Successful

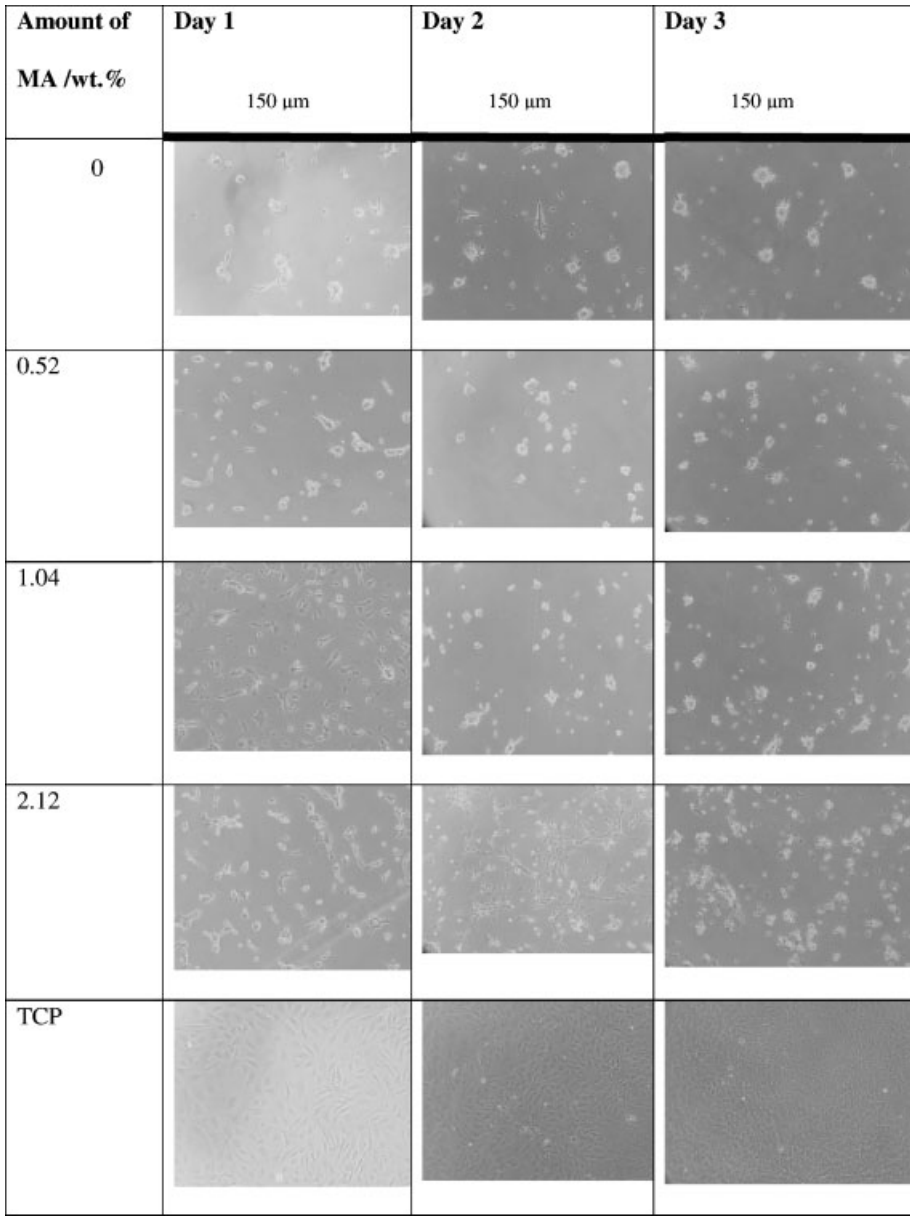
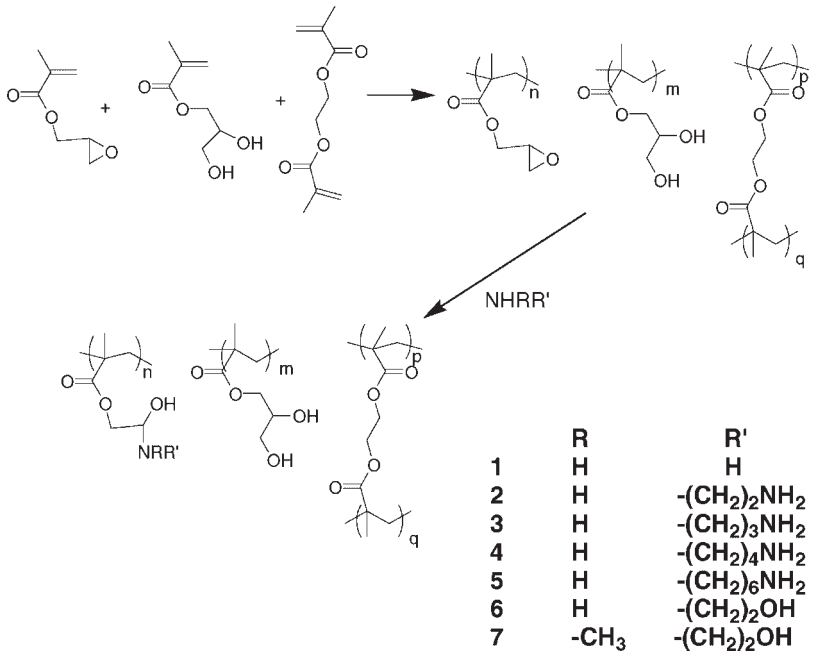


Figure 3.
Optical micrographs of HDFs cultured on hydrogels containing various amounts of MA compared to control cultures on tissue culture plastic. The data show that although these materials are not toxic (established in separate experiments in which cells were exposed to hydrogels in co-culture conditions in the same media but physically separated from the cells) the cells do not spread well and are poorly attached to all of the hydrogels.

cultures of HDFs produces elongated cells that are spread continuously over the substrate as shown in the micrographs of cells grown on TCP. HDFs grown on the poly(GMMA-co-DM-co-EDMA-co-MA) networks are clumped and rounded and similar to cells grown on the non-modified poly(GMMA-co-DM-co-EDMA) network. As can be seen from these micrographs and the MTT data no improvements were achieved using either of these acid-modification strategies.

Following these results we considered that functionalization with basic groups such as amines might provide the desired improvements in cell adhesion and proliferation.[24] The synthesis of this set of materials required a two-stage technique since the polymerization of amine functional methacrylates can result in Michael addition of the amine to the methacrylate unit during either monomer or polymer synthesis. The problem was easily circumvented by synthesis of the polymers containing the epoxy derivative of GMMA (glycidyl methacrylate (GME)).Various amine compounds were then reacted with these networks, as shown in Scheme 2. This route also provided a convenient way to examine the effects of changing the alkyl spacer between the hydrogel main-chain and the amine moiety.

The culture of bovine keratocytes (BK) (a stromal-like cell in the eye) and human corneal epithelial cells, both in mono-culture and in co-culture with each other showed a remarkable dependence on the structure of R'. Both BK mono-cultures and BK/HCEC co-cultures achieved essentially full coverage of the materials to give confluent sheets of cells provided alkyl amines with at least 3 carbons were employed (structures **3**, **4** and **5**). Modifications with **3**, **4** or **5** produced materials that were in general, as effective for cell culture as TCP. It was possible to use networks **1**, **2**, **6** or **7** for the mono-culture of BKs but cell viability was much reduced compared to the control (culture on TCP). Examples of the results from a cell viability assay (MTT) of a co-culture experiment are illustrated in Figure 4.



	R	R'
1	H	H
2	H	$-(CH_2)_2NH_2$
3	H	$-(CH_2)_3NH_2$
4	H	$-(CH_2)_4NH_2$
5	H	$-(CH_2)_6NH_2$
6	H	$-(CH_2)_2OH$
7	$-CH_3$	$-(CH_2)_2OH$

Scheme 2.
Synthesis of amine modified hydrogels.

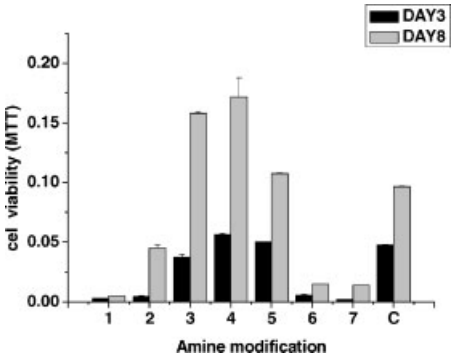


Figure 4.
Example of the viability of HCEC/BKs co-cultured on amine functional hydrogels studied by MTT assay (MTT measured in units of optical density at 410 nm). The control, C, is TCP coated with collagen and fibronectin.

The effect of amine structure was seen most clearly in the co-culture experiments. In these experiments amines **3, 4** and **5** produced materials that performed as well as the control but amines **1**, **2**, **6** or **7** supported only negligible numbers of cells. Calculation of pKas for model compounds using the pKa plugin available in Marvin (Chemaxon Kft) allowed us to compute the charge state distribution of these amine functionalities at pH 7.2; the approximate pH of culture medium. Figure 5 shows the relationship between alkyl chain length and the distribution of the possible protonated charge states from modifications with compounds **2**–**5**. Each of these amines presents a positively charged surface to the cells. However, amine **2** is not fully ionized although each repeat unit is predicted to carry positive charge. Modifications with compounds **1**, **6** and **7** produced essentially fully ionized materials at pH 7.2 (the predictions are 99.52, 99.02 and 98.67 mol% of repeat units in the protonated form respectively).

Thus, these three modifications present similarly charged surfaces. Modification with **2** produced an increase in charge density but none of these modifications provided enhanced substrates for cell culture. Modifications with **3**, **4** and **5** provided a further increase in charge density because in these systems both amine groups are fully ionized. However, the preference for alkyl spacers of 3 to 6 carbons may imply a role for extracellular enzymatic processes and further work is required to fully elucidate the mechanisms underlying these observations. As described above amphiphilic statistical copolymers can support cell adhesion and proliferation. However, cell cultures could only be progressed to confluency after further functionalization with specific amines,

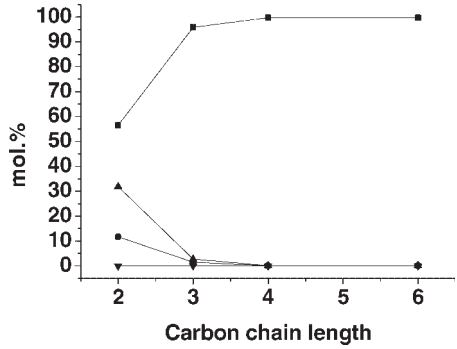
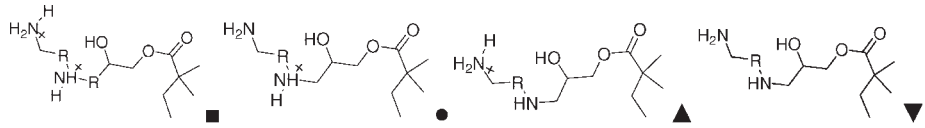
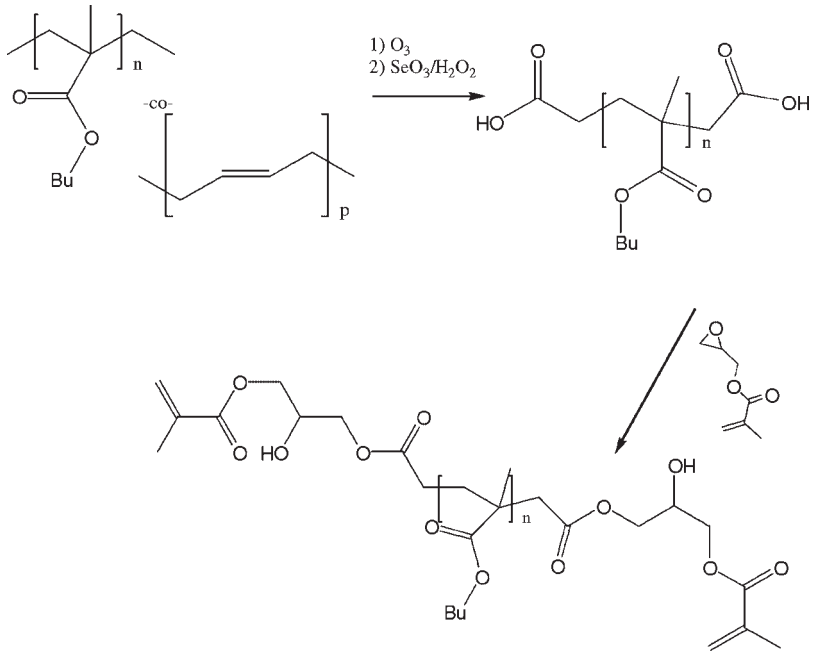


Figure 5.
Distributions of charge states for modifications with diamines 2–5 calculated from predicted pKas of model compounds with the general structures:





Scheme 3.
Synthesis of oligo(butyl methacrylate) macromonomers.

accordingly we chose to next investigate the use of block conetworks.

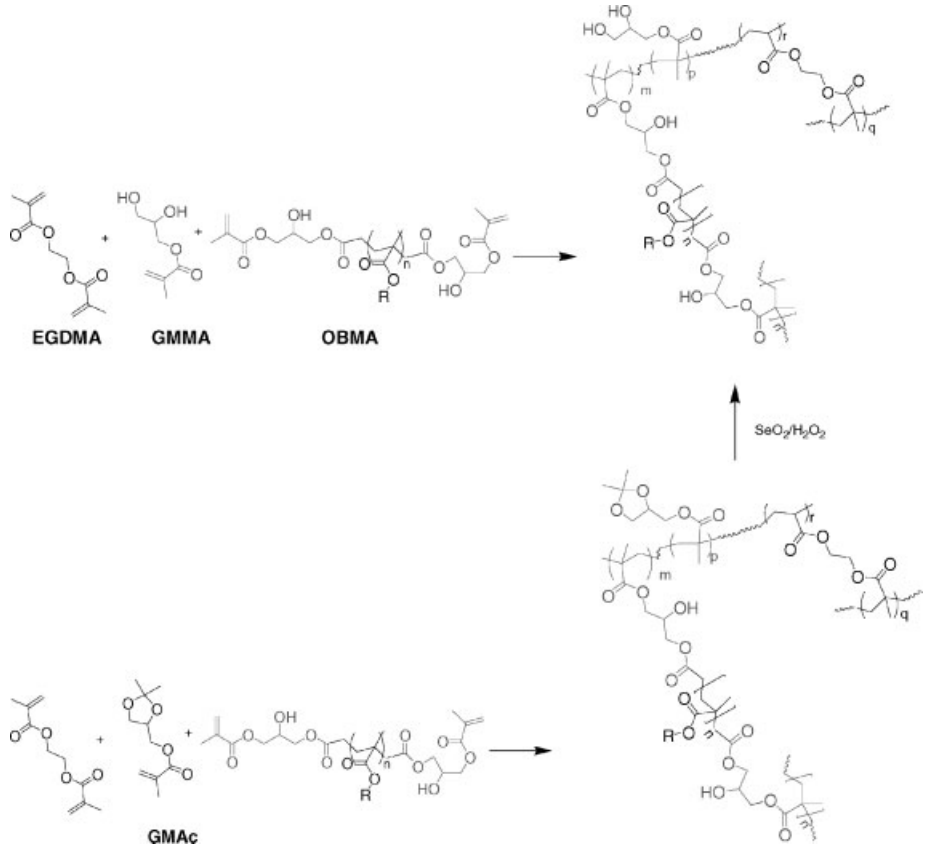
Block copolymer amphiphilic networks were prepared as shown in Scheme 3.[20,21] That is we first prepared oligo(butyl methacrylate)s with dicarboxylic end groups by the ozonolysis and oxidative work up of latexes of poly(butyl methacrylate-co-butadiene).[22,23] The oligomer latex produced from this procedure was then reacted with GME to yield a flocculated mass of oligo(butyl methacrylate)s with di-methacrylate chain ends (OBMA).

Copolymerization of these with GMMA yielded amphiphilic block conetworks, which were similar to the random poly-(GMMA-co-EDMA-co-DM) terpolymers described above, as shown in Scheme 4.

The EWCs of these networks are presented in Figure 6(a). As expected the EWCs increased, in each series, as the weight fraction of GMMA increases. Also, as the molecular weight of the oligomer increased the effective crosslink density decreased and this has the expected effect of increasing the EWC. However, these parameters had a much smaller effect than in the random copolymer series. Thus the EWCs of materials prepared in the absence of EDMA were higher than would be expected from comparison to similar random copolymers (EWC = 89–97 wgt.%) and this result was rationalized by considering that, in these highly phase separated and porous materials, a substantial fraction of water is held by capillary forces within the pores rather than by solvation of the polymer chain.

Further control of the EWC can be achieved by the addition of a low molecular weight difunctional monomer, such as EDMA.

Figure 6(b) shows EWC data derived from two types of network prepared as shown in Scheme 4. The first set of networks (series-**GMMA**) was similar to those examined in Figure 6(a) but EDMA was added to the monomer feed and the second set (series-**GMAc**) were derived from similar feed compositions but GMMA was replaced with GMAc. The acetal was then removed from the GMAc units by reaction with SeO_2/H_2O_2. The data show that the EWCs were reduced from those obtained in



Scheme 4.
Synthesis of conetworks by coplymerization of macronomers with either GMMA or GMAc.

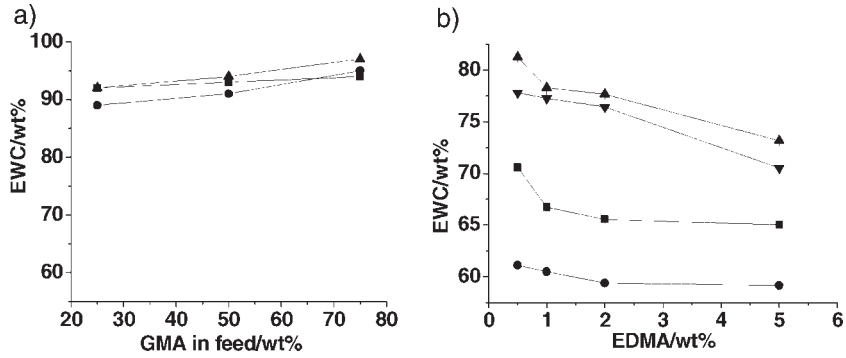


Figure 6.
(a) Variation of EWC in conetworks with weight fraction of GMMA (a) poly(BMA-block-GMMA)): ■ $M_n = 1{,}350$ $gmol^{-1}$; ● $M_n = 2{,}300$ $gmol^{-1}$; ◆ $M_n = 2{,}950$ $gmol^{-1}$ (b)variation of EWC with EDMA content in feed of poly(BMA-block-GMMA-co-EDMA) derived from monomer feeds as follows: ▲50:50 GMMA:OBMA, ▼25:75 GMMA:OBMA, ■ 50:50 GMAc:OBMA, 25:75 GMAc:OBMA.

the absence of EDMA and there was a small decrease in EWC as the amount of EDMA increases.

It is clear from these SEM data that there are substantial increases in porosity as the amount of EDMA in the monomer feed increases and that the changes due to this variable are greater than changes associated with the synthesis procedure. However, at each EDMA concentration the series GMMA materials appear to be more porous.

Each of these conetworks phase separate from the reaction medium during polymerization and this process produces porous amphiphilic materials. Scanning electron microscopy (SEM) of freeze fractured surfaces shows the influence that the formulation variables have on the pore structure of these materials. Figure 7 shows micrographs of fracture surfaces from series-**GMMA** networks and Figure 8 shows similar micrographs from the series-**GMAc** materials. The use of these materials as substrata for cell culture was affected by the synthesis route. Thus, one of our objectives in using **GMAc**, followed by removal of the acetal, was to promote mixing between the hydrophilic and hydrophobic blocks of the network. This increase in interfacial mixing was manifested as increased toughness in the series-**GMAc**.

However, the morphological changes, which presumably underpin this increase in toughness, had a detrimental effect on cell adhesion.[21] For example, the culture of HDFs, on series-**GMMA** networks gave confluent sheets of cells, which gave MTT cell viabilities that were far in excess of those obtained when the same cells were cultured on equivalent series-**GMAc** networks. Examples of these data shown in Figure 9, which also shows that in some cases the MTT data indicate that the substrates are comparable to the positive control, TCP.

Scanning electron microscopy of the HDFs cultured on these polymers shows

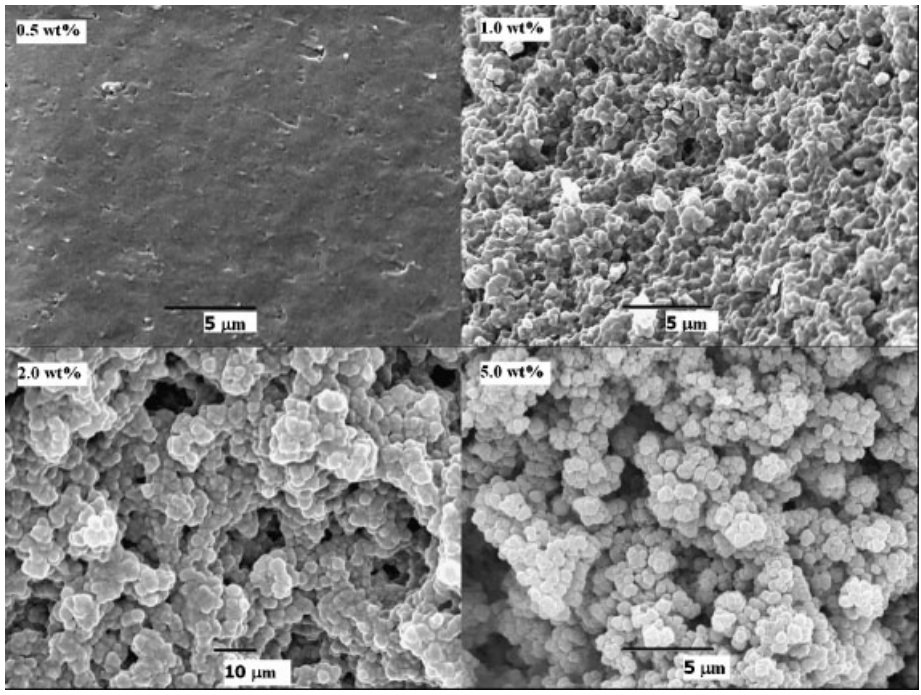


Figure 7.
SEMs of fracture surfaces of series-GMMA-conetworks. The numbers are amounts of EDMA in the monomer feed.

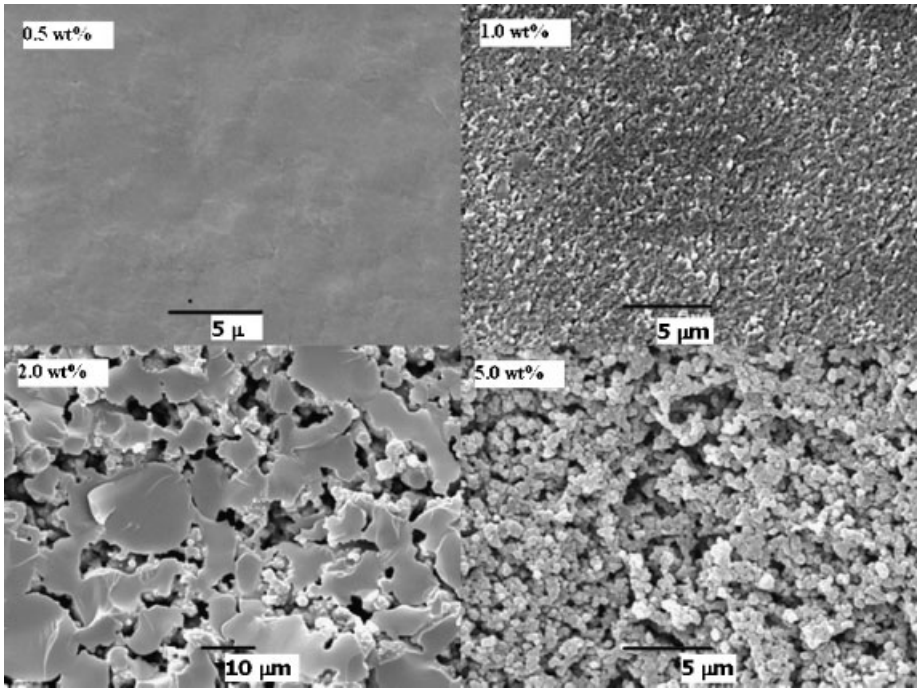


Figure 8.
SEMs of fracture surfaces of series-GMAc-conetworks. The numbers are amounts of EDMA in the monomer feed.

that the cells form confluent sheets and that in some cases there are indications that the cells can penetrate into the pores of these materials. If we consider isolated materials of constant EDMA content; i.e. constant overall polymer composition after deprotection it is tempting to conclude that the differences in cell viability and observed numbers of proliferating cells are due to differences in porosity since in each case, at constant EDMA content, The series-**GMMA** appear to have higher porosity than the series-**GMAc** materials.

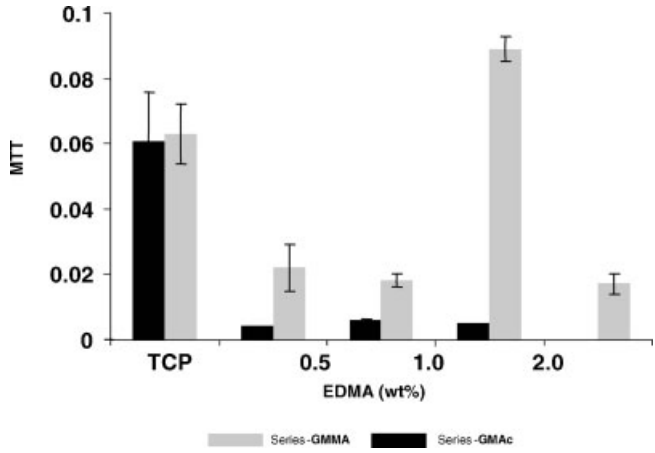


Figure 9.
Results from MTT assay of dermal fibroblasts cultured on series-GMMA and series-GMAc materials.

However, reference to Figure 7 and 8 clearly shows that it is possible to produce series-**GMAc** materials that are more porous than particular series-**GMMA** materials. Cross examination of these data shows that series-**GMAc** with 1.0 wt% EDMA is more porous than the series-**GMMA** with 0.5 wt% EDMA and the series-**GMAc** with 5.0 wt% EDMA is more porous than the series-**GMMA** with 1.0 wt% EDMA but the series-**GMMA** materials are in every case better substrates for cell culture than any of the series-**GMAc** materials. Therefore, although porosity has an important role to play in the optimization of these materials, as in many aspects of tissue engineering, there are also other important compositional variables to be considered simultaneously within the system.

Conclusions

In vivo cells form tissues within a natural hydrogel known as the extracellular matrix. This is a complex and high water content polymer network that can be mimicked by synthetic materials. However, synthetic hydrogels are generally non-cell adhesive materials and if they are to be used as scaffolds in tissue engineering they must be modified to promote cell adhesion and proliferation. In our work on the modification of GMMA based hydrogels we have investigated the use of various strategies for promoting cell adhesion and proliferation. The addition of acidic groups failed to promote the adhesion of dermal fibroblasts but similar materials that were modified with alkyl amines proved to be excellent substrates for both mono-culture of bovine keratocytes and co-culture of these cells with human epithelial corneal cells. The relationship between the detailed chemical structure of the amine modification and the performance of these materials suggests an explanation that must involve mechanisms other than just changes in charge on these hydrogels. Similar improvements in the ability of the hydrogels to support cell attachment can also be achieved by adopting block conetwork architecture. However, there are several interdependent variables that have an effect on the performance of these materials. Careful attention to the detailed chemical and morphological structure of these materials will be essential if the full biological potential of these materials for use in tissue engineering is to be realized.

[1] C. S. Patrickios, T. K. Georgiou, *Curr. Opin. Coll Inter. Sci.* **2003**, *8*, 76.
[2] J. P. Kennedy, *Trends Polym. Sci.* **1993**, *1*, 381.
[3] J. P. Kennedy, G. Fenyvesi, R. P. Levy, K. S. Rosenthal, *Macromol. Symp.* **2001**, *172*, 56.
[4] G. Erdodi, J. P. Kennedy, *Prog Polym. Sci.* **2006**, *31*, 1.
[5] I. Barakat, Ph. Dubois, Ch. Grandfils, R. Jerome, *J. Polym. Sci., Part A: Polym. Chem.* **1999**, *37*, 2401.
[6] Y. Guan, Y. Peng, *Huaxue Jinzhan* **1999**, *11*, 86.
[7] B. Ivan, J. Feldthusen, A. H. E. Mueller, *Macromol. Symp.* **1996**, *102*, 81.
[8] R. Blezer, T. Lindhout, B. Keszler, J. P. Kennedy, *Polym. Bull. (Berlin)* **1995**, *34*, 101.
[9] B. Keszler, J. P. Kennedy, *J. Polym. Sci., Part A: Polym. Chem.* **1994**, *32*, 3153.
[10] J. P. Kennedy, *J. Macromol. Sci., Pure Appl. Chem.* **1994**, *A31*, 1771.
[11] B. Keszler, J. P. Kennedy, P. W. Mackey, *J. Contr. Rel.* **1993**, *25*, 115.
[12] H. Yu, D. W. Grainger, *J. Contr. Rel.* **1995**, *34*, 117.
[13] D. Chen, J. P. Kennedy, M. M. Kory, D. L. Ely, *J. Biomed. Mater. Res.* **1989**, *23*, 1327.
[14] S. Brocchini, K. James, V. Tangpasuthadol, J. Kohn, *J. Biomed. Mater. Res.* **1998**, *42*, 66.
[15] J. J. Yoon, T. G. Park, *J. Biomed. Mater. Res.* **2001**, *55*, 401.
[16] S. V. Madihally, H. W. T. Matthew, *Biomaterials* **1999**, *20*, 1133.
[17] Y. Nakayama, S. Nishi, H. Ishibashi-Ueda, H. T. Matsuda, *J. Biomed. Mater. Res.* **2000**, *51*, 520.
[18] R. Haigh, N. J. Fullwood, S. Rimmer, *Biomaterials* **2000**, *21*, 735.
[19] R. Haigh, N. Fullwood, S. Rimmer, *Biomaterials* **2002**, *23*, 3509.
[20] S. Rimmer, M. J. German, J. Maughan, Y. Sun, N. Fullwood, J. Ebdon, S. MacNeil, *Biomaterials* **2005**, *26*, 2219.
[21] Y. Sun, J. Collett, N. J. Fullwood, S. Mac Neil, S. Rimmer, *Biomaterials* **2007**, *28*, 661.
[22] J. R. Ebdon, S. Rimmer, *J. Polym. Sci. A Polym. Chem. Ed.* **1997**, *35*, 3255.
[23] S. Rimmer, P. Tattersall, J. R. Ebdon, N. Fullwood, *React. Funct. Polym.* **1999**, *41*, 177.
[24] R. A. Dawson, N. J. Goberdhan, F. Freedlander, S. Mac Neil, *Burns* **1996**, *22*, 93.
[25] S. Rimmer, P. Wyman, C. Johnson, B. Zhao, N. J. Fullwood, S. MacNeil, *Biomaterials* submitted.

