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Formation of porous natural-synthetic polymer composites using emulsion templating and supercritical fluid assisted impregnation

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Summary

Porous natural-synthetic polymer composites were prepared using an alginate emulsion templating step followed by supercritical carbon dioxide (sc-CO_2) assisted impregnation (and subsequent polymerisation) of synthetic monomer mixtures. In the impregnation step, an initiator and either 2-hydroxyethylmethacrylate (HEMA), butylmethacrylate (BMA), ethyleneglycoldimethacrylate (EGDMA) or trimethylolpropanetrimethacrylate (TRIM) monomers, respectively, were used. After impregnation into the porous alginate foam, the synthetic monomer(s) were polymerised *in situ*, forming porous composites with increased stiffness. A number of methods were used to assess the effects of the impregnation/polymerisation process including uniaxial compression testing, scanning electron microscopy (SEM), mercury intrusion porosimetry (MIP), helium pycnometry and Fourier transform infra-red (FTIR) spectroscopy. Our results suggest that alginate foams impregnated with HEMA show higher weight gains and are stiffer than those impregnated with BMA. Such stiffer porous composites are potentially better suited than the unmodified materials in applications such as tissue engineering (cell-seeded) scaffolds, where mechanical conditioning is desired to stimulate cells for development of neo tissue growth.

Introduction

Porous natural polymers are of interest as degradable or partially-degradable tissue engineering (TE) scaffolds, which create and maintain a space for neo tissue formation [1-3]. They provide a site for cell attachment, differentiation and proliferation [4], as well as mechanical stability to support the growing neo tissue [5] until it has regenerated and gained strength [6,7]. Controlling the mechanical properties of hydrogels precisely is one of the prerequisites for their successful utilisation in TE applications (particularly for cell culture within a bioreactor). Despite the excellent biological properties (*e.g.* biocompatibility) of many natural hydrogels, their poor mechanical properties [8] and the difficulties associated with tailoring their degradation rates, makes the use of synthetic hydrogels more attractive. Therefore,

combining synthetic and natural polymers allows a suitable balance of biological, mechanical and degradation properties to be achieved.

There are several examples in the biomaterials literature, where researchers have combined natural and synthetic polymers for soft tissue applications. Coombes *et al.* [9] prepared biocomposite films from a non crosslinked natural polymer collagen and the synthetic polymer poly(caprolactone) (PCL). Films were prepared by impregnating freeze-dried collagen films with a solution of PCL in solvent (dichloromethane) followed by evaporation of the solvent. The biocomposite films were washed extensively before being subjected to cell culture studies using human osteoblasts. Such methods that use organic solvents may leave residues in the materials that can cause cytotoxic responses [10]. Furthermore, such methods often give uneven mixing of the two polymer phases during the drying stage (due to capillary effects) or impregnation steps (due to surface tension and poor diffusivity of the solvent into smaller pores). Impregnating a second phase into a polymer has also been applied more simply by Zhou *et al.* [11] who swelled crosslinked poly(stearylmethacrylate-co-divinylbenzene) (PSMA-co-DVB) in a monomer solution of DVB and 4-*t*-butylstyrene (t-BS) to make an interpenetrating polymer network (IPN) with particularly good oil absorption capabilities, and also by Budd *et al.* [12] who coated the porous structure of crosslinked poly(styrene-co-divinylbenzene) (PS-co-DVB) by immersing in a solution of poly(vinyl alcohol) and letting it dry resulting in a novel medium for thin-layer electrophoresis. These methods are however quite time consuming, taking long time periods for the immersing solution to diffuse into the crosslinked polymer morphology and could also contribute to a lesser ability to penetrate fine pore structure. Thus, there has been much interest in the use of so called "clean" solvents such as supercritical fluids, which can overcome diffusion limitations posed by more conventional solvents, and do not leave substantial residues.

Supercritical fluids (SCFs) are compressed, single-phase fluids that can display both the properties of liquids and gases, *e.g.* they can act as solvents but have higher diffusivities compared to conventional liquids. In particular, supercritical carbon dioxide (sc-CO_2) has a relatively low critical pressure and temperature of 73.8 Bar and 31.1°C, respectively (above which it exists as a single phase compressible fluid in the pure form). Sc-CO_2 has been widely used for many applications, including the manufacture of porous biomaterials and drug delivery formulations [13-15]. In many of these reports, sc-CO_2 has been used as a low temperature plasticising agent, as a solvent [16,17], as a porogen [18,19], as a reagent [20,21] and as an antisolvent [22,23]. Watkins and McCarthy [24,25] developed a polymer modification route using sc-CO_2 to swell the substrate as well as aid infusion of a monomer and initiator into it. The impregnated monomer was subsequently polymerised *in situ* by a thermally initiated free radical reaction. This was followed by a depressurisation step to remove the SCF and possibly any unreacted monomer. They first used this methodology to modify poly(chlorotrifluroethylene) (PCTFE) and high density polyethylene (HDPE) substrates with styrene to yield poly(styrene)-substrate polymer blends. Muth *et al.* [26] used a similar approach to modify poly(vinylchloride) (PVC) and poly(methylmethacrylate) (PMMA) substrates, respectively, with the monomers styrene, methylmethacrylate or methacrylic acid. The polymers were impregnated with a monomer and a free radical initiator, followed by polymerisation inside the SCF swollen matrix to generate a composite "polymer within a polymer". This route has enabled the synthesis of novel polymer blends with unique properties; *e.g.* the PMAA-PVC polymer system in which a hydrophilic monomer was incorporated

inside a hydrophobic matrix. Zhang *et al.* [27] investigated the morphological structure of ultra high molecular weight poly(ethylene) (UHMWPE) impregnated with methylmethacrylate (MMA). Kung *et al.* [28] investigated the synthesis of polystyrene (PS)/high-density polyethylene (HDPE) blends in SCFs. The polystyrene reinforced the HDPE, which displayed increasing stiffness and strength as the polystyrene content increased.

Herein, we describe our approach towards the manufacture of natural-synthetic porous hydrogel composites, (for TE applications) by using a synthetic polymer to reinforce a low modulus natural porous polymer. In this work, porous alginate foams were impregnated with the synthetic monomers [(2-hydroxyethylmethacrylate (HEMA) and butylmethacrylate (BMA) respectively], with or without crosslinkers [ethyleneglycoldimethacrylate (EGDMA) and trimethylolpropanetrimethacrylate (TRIM), respectively], using sc-CO_2 assisted impregnation, followed by a subsequent polymerisation step. By changing the type and amount of synthetic monomer, the mechanical properties and porosity of the alginate hydrogels were modulated.

Experimental

Materials

All chemicals were purchased from Aldrich Chemical Company (Dorset, UK) unless otherwise specified. Adipic acid (high purity, 99%), sodium citrate dihydrate (99+%), isooctane (99.7+%), *bis* 2-ethylhexylsulfosuccinate sodium salt, (AOT) (min 99%), butylmethacrylate (>99.0%), 2-hydroxyethylmethacrylate (98%), 2,2'azo*bis*(2-methylpropnitrile) (AIBN) (98%), trimethylolpropanetrimethacrylate (98%), ethyleneglycoldimethacrylate (98%), Protanal LF 10/60 sodium alginate, (M_w of 120,000 – 180,000, guluronic content 65 - 75%), (pharmaceutical grade, Honeywill & Stein Ltd. UK), calcium carbonate, (min 99.0% AnalaR grade, BDH, UK), liquid carbon dioxide (99.8 %, BOC, UK).

Synthesis of porous alginate foams

The porous alginate foams were prepared according to a method recently developed in our laboratories and initially reported elsewhere [18]. Briefly, calcium carbonate and trisodium citrate were dispersed in 100 ml distilled water (to form a 0.1 M calcium mixture) by stirring on a heated magnetic stirrer for 15 min at 50°C. 8.0 g sodium alginate was added very slowly to the mixture to give an 8 % w/v dispersion (mixed with an overhead stirrer). Once a homogeneous dispersion was obtained, 60 ml of a 0.1 M solution of AOT in isooctane was added very slowly to the alginate mixture under stirring. Finally, adipic acid solution (1.48 g adipic acid dissolved in 20 ml distilled water at 70°C) was added under stirring. The solution was left to stir until it became too viscous, where upon it was left to stand for 24 hrs. The hydrogel was soaked for a further 24 hrs in a 10 % ethanol/water mixture and then rinsed with distilled water before being freeze dried for 18 hrs at $12x10^{-3}$ Torr.

Impregnation of alginate foams

Reactions were conducted in a 400 ml Parr 4562 stainless steel autoclave using porous alginates made using the method described above. In a typical experiment,

the autoclave was charged with 2-hydroxyethylmethacrylate (HEMA) or butylmethacrylate (BMA) monomer, 2,2'azo *bis*(2-methylpropnitrile) (1 wt % with respect to monomer). Freeze dried porous alginate foams (*ca.* 25 mm^3) were placed in a special raised wire "tray" inside the autoclave to prevent them from contacting the stirrer blades at the bottom. Using this method, the foams were not directly immersed in the monomer liquids. The autoclave was then sealed. The monomer concentrations used varied from 2.5 v/v % (10 ml) to 10 v/v % (40 ml). [See Table 1]. The desired starting pressure and temperature was achieved within 20 mins. In all experiments, a soaking temperature of 50°C and polymerisation temperature of 70°C were used. Altogether, two methods were used; in method 1, the soaking and polymerisation times were maintained for 1 hr each. In method 2, the soaking time and polymerisation time were increased to 4 and 2 hrs, respectively. After the total reaction time period (soaking and polymerisation time), the CO_2 was vented from the vessel over a 20 minute period, and the autoclave was cooled to room temperature before the samples were recovered. The samples were then left overnight to allow the CO_2 to desorb and achieve a constant weight, prior to calculation of percentage weight gain (W_G) according to the following equation;

$$W_G = \frac{W_{blend} - W_{original}}{W_{original}} x100 \qquad (\mathbf{1})$$

Where W_G is percentage weight gain (%), $W_{original}$ is the weight of the polymer (alginate foam) before it was modified, and W_{blend} is the total weight of the polymer after modification (impregnated alginate foam).

In reactions where a crosslinker (EGDMA or TRIM) was used, the autoclave was charged with 5 v/v % (20 ml) of HEMA monomer, with 1-5 wt % (0.2 – 1.0 ml, with respect to monomer weight) of either TRIM or EGDMA. Thereafter, the reaction was conducted in a similar method to others. (See Table 1 for precise experimental conditions).

Characterisation and sample preparation

Prior to scanning electron microscopy (SEM) analysis, the samples were immersed in liquid nitrogen for five minutes, fractured into smaller pieces with a sharp razor blade, and then mounted on aluminium stubs with carbon black paste. Samples were then coated with gold (Agar Auto Sputter Coater 103A) before analysis using the JEOL 6300F SEM.

Uniaxial compression tests were performed on an Instron 5564 mechanical tester (Instron Corporation, USA) with a 100N load cell. Prior to mechanical testing, gel specimens were swollen for a minimum of 24 hrs in a 1.5 mM calcium chloride solution. Cylindrical specimens were made using a borer with a internal diameter of 12 mm, and specimens were then cut with a sharp razor blade to 6 mm thicknesses; it was important to obtain flat, parallel surfaces to ensure an even distribution of the load during compression testing. The compressive strain was set to a maximum of 50 % and testing was carried out at a crosshead speed of 3.6 mm/min (strain rate of 0.01 s^{-1}). Extension (mm) and load (N) were recorded using Merlin™ materials testing software (Instron Corporation). Four specimens were tested for each sample type. A stress-strain curve was constructed by converting the force-displacement data obtained from the compression tests using the following equations;

$$\sigma = \frac{F}{A} \quad (2)$$

$$\varepsilon = \frac{\Delta l}{l_o} \quad (3)$$

Where σ is the stress (Pa), F is the force (N), A is the area of the specimen (mm^2), ε is the strain, Δl is the change in length or extension (mm) and l_0 is the original length (mm) of the specimen. The compressive elastic moduli of the gels were calculated from the stress-strain curves limited to the first 10 % of strain [29-31].

Fourier transform infra red (FTIR) spectra were recorded on a Nicolet 800 spectrometer in conjunction with a diffuse reflectance infra-red Fourier transform sampling (DRIFTS) cell. Spectra were collected in the range 4000 and 400 cm^{-1} for 32 scans and a spectral resolution of 4 cm^{-1}. Samples were ground into a fine powder and diluted with approximately 20 % potassium bromide.

Mercury intrusion porosimetry (MIP) was performed using the PoreMaster™ 33 porosimeter (Quantachrome). Low-pressure intrusion porosimetry (0-28 psi) was employed on porous monoliths to obtain interconnected macropore diameter distributions in the range of 1-250 μm.

The skeletal density of porous samples was determined by automated helium displacement pycnometry using the Ultrapycnometer™ 1000 (Quantachrome).

Results and Discussion

Porous alginates were made using a previously described emulsion templating–freeze drying method developed by our group [18]. Since it has been shown by previous papers [13-15,24,25] (and our own unpublished results) that both hydrophilic and hydrophobic polymer matrices can be swollen under pressure by sc-CO_2, we felt that the supercritical fluid was a clean and suitable solvent that should allow infusion of low molecular weight methacrylates into the alginate matrix. After each experiment, the mass gain of the original porous alginate served as an important measure of the amount of impregnated synthetic polymer in the composite [26]. An increase in weight indicated that the monomer had successfully impregnated the alginate foam and that polymerisation had occurred (Table 1). Monomers used included B = butylmethacrylate, H = 2-hydroxyethylmethacrylate, E = ethyleneglycoldimethacrylate and T = trimethylolpropanetrimethacrylate.

Samples impregnated with 2-hydroxyethylmethacrylate (HEMA) exhibited higher mass gains than those samples impregnated with butylmethacrylate (BMA). For example, under the same conditions, Sample 6 increased in weight by 140 %, whereas sample 3 only increased by 4 %. Doubling the concentration of BMA in the autoclave only resulted in a 3 % further weight gain (Sample 3 *vs.* Sample 4), whereas doubling the concentration of HEMA in the autoclave, resulted in a 50 % further weight gain (Sample 6 *vs.* Sample 7). The amount of monomer that can be impregnated into the alginate substrate is highly dependent upon the solubility of the former in SCF [32]. HEMA [33] and BMA [34] are both known to dissolve in sc-CO_2, thus, higher HEMA uptake was possibly due to its higher solubility. Solubility of molecules in sc-CO_2 is highly sensitive to factors such as molecular structure, molecular weight, polarity, and the presence of interacting functional groups. Kazarian *et al.* [35] demonstrated that

molecules *e.g.* polymers possessing certain electron donating functional groups (*e.g.* C=O) exhibit substantial interactions with sc-CO_2 molecules. Molecular simulation data obtained by Wick *et al.* [36] is consistent with these experimental observations. The authors used molecular simulations to show that polycarbonate poly(ethyleneoxide) copolymers (CARB-PEO) are more soluble than PEO homopolymers in CO_2 because of the strong interactions of the C=O group interaction with CO_2. This is because of the larger accessible surface area of the exposed carbonyl oxygen in CARB-PEO, which leads to an enhanced local CO_2 density around this functional group and, therefore, leads to higher solubility compare to the sterically hindered ether oxygen of PEO. Additionally, other factors such as flexibility of the molecule also influences solubility; the stiffer the molecule, generally the more difficult it is to dissolve in sc-CO_2 [34]. Therefore, under the same experimental conditions, BMA may be less soluble than HEMA because of the more rigid structure, and because of the steric effects of the "bulky" butyl group making the C=O group more inaccessible to sc-CO_2 molecules.
Generally, the impregnation of crosslinkers did not significantly enhance the modulus of the hydrogel after polymerisation (Sample 6 *vs.* Samples 12-14). This may imply that at the relatively low pressures and temperatures used, the crosslinkers were not soluble enough in sc-CO_2 due to higher molecular weights and for the same reasons mentioned above; poor solubility due to the steric effects from bulky side groups. An exception was Sample 11 which was made from 5 % v/v HEMA and 5 % v/v EGDMA, respectively, which was the stiffest composite at 39.9 ± 4.2 kPa.

Table 1. The percentage weight gain and average compressive moduli of alginate foams modified with 2-hydroxyethylmethacrylate (HEMA = H) and butylmethacrylate (BMA= B), as well as with the crosslinkers ethyleneglycoldimethacrylate (EGDMA = E) and trimethylolpropanetrimethacrylate (TRIM = T).

	Sample	IP [Bar]	IT [h]	PT [h]	WG [%]	CM [kPa ±SD]
1	Wet	-	-	-	0	24.3 ± 5.1
2	Unmodified[a]	-	-	-	0	9.8 ± 3.7
3	B-5	115	1	1	4	10.5 ± 7.9
4	B-10	115	1	1	7	13.4 ± 3.4
5	B-10	125	4	2	12	12.6 ± 3.4
6	H-5	115	1	1	140	30.1 ± 5.0
7	H-2.5	115	1	1	73	18.0 ± 2.6
8	H-2.5	110	4	2	40	18.0 ± 3.0
9	H-2.5	115	4	2	51	15.8 ± 3.1
10	H-2.5	130	4	2	63	15.8 ± 3.6
11	H-5 E-5	115	1	1	140	39.9 ± 4.2
12	H-5 E-1	115	1	1	142	32.1 ± 2.3
13	H-5 T-5	115	1	1	170	26.9 ± 4.9
14	H-5 T-1	115	1	1	136	28.7 ± 5.4

In column 2, the number refers to the concentration of the monomer based on the volume of the autoclave (% v/v). IP = impregnation pressure; IT = impregnation time; PT = polymerisation time; WG = weight gain; CM = compressive modulus. [a] Freeze dried and then re-hydrated.

Uniaxial compression testing was performed on re-hydrated samples. Polymer networks impregnated with BMA showed no significant increase in the modulus of the

hydrogels due to low mass uptakes (Sample 2 *vs.* Samples 3-5). In contrast, when HEMA was used, there was a dramatic difference in the stiffness; sample 6 (H-5) has a modulus of 30.1 ± 5.0 kPa *vs.* 9.8 ± 3.7 kPa for Sample 2 (unmodified). The compressive modulus can be related to the total weight gain of the composite foam; higher percentage mass gains generally lead to a stiffer polymeric network (see Figure 1).

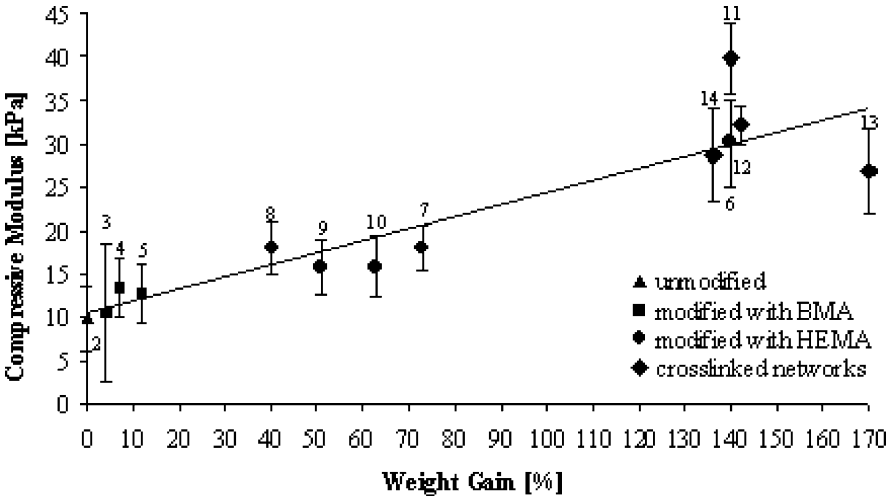


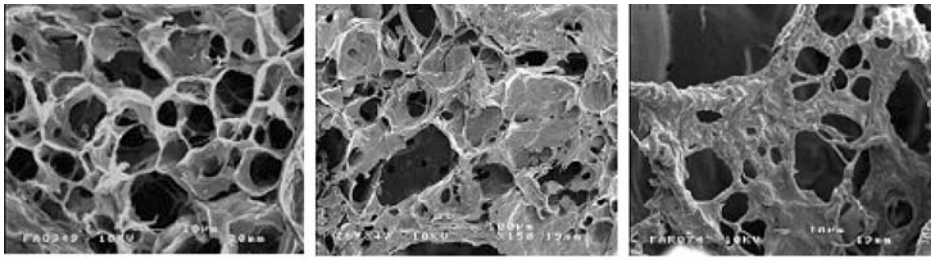
Figure 1. Relationship between the compressive modulus and weight gain for alginate foams modified with 2-hydroxyethylmethacrylate (HEMA) and butylmethacrylate (BMA), as well as with the crosslinkers ethyleneglycoldimethacrylate (EGDMA) and trimethylolpropanetrimethacrylate (TRIM).

The compressive modulus of the wet unmodified hydrogel Sample 1 was measured prior to freeze drying (*i.e.* post synthesis), and was determined to be 24.3 ± 5.1 kPa. When such hydrogels were freeze dried and then rehydrated, the modulus dropped to 9.8 ± 3.7 kPa (Sample 2). The large loss in stiffness was attributed to some cracking of the porous structure during the freeze-drying process, as has been observed elsewhere [37].

Factors that do not have a significant effect on the either the mass gain or compressive modulus are impregnation/polymerisation times and to a limited extent, the pressure. When the impregnation and polymerisation times were increased, or the pressure was varied (over 110-130 Bar) there was little or no change in modulus (Samples 7 - 10) indicating that the maximum level of impregnation was achieved in the first two hours or less. This finding was rather unexpected and will require further investigation to understand fully. As pressure is known to affect solubility, it is assumed that the solubilities of the monomers would be increased if a pressure of 200 Bar or so was used; such experiments will form part of a future study.

The pore microstructure was investigated using scanning electron microscopy (SEM), mercury intrusion porosimetry (MIP) and helium pycnometry. SEM pictures show that when high concentrations of HEMA (5 v/v %) were used the pore structure is substantially altered; the surfaces became smoother where the synthetic polymer appeared to coat walls of the pores, and fill some of the larger pores (Figure 2b). In

comparison, at lower concentrations (2.5 v/v %), the polymer largely coated the walls of the pores only (Figure 2c).



(a) (b) (c)

Figure 2. Scanning electron microscopy (SEM) pictures of (a) unmodified porous alginate foam (Sample 2), and alginate foams impregnated with 2-hydroxyethylmethacrylate, (HEMA=H) for (b) Sample 6 (H-5) and (c) Sample 8 (H-2.5).

MIP of the unmodified sample (Figure 3a) shows that there is a broad range of pore sizes, with some pores larger than 250 μm (detection limit of the machine), as shown by the continually upward rising curve of the MIP plots. MIP plots of the impregnated foams showed a downward trend with increasing pore diameter, implying that there are fewer larger pores (especially those approaching 250 μm and above). This suggests that some of the larger pores may have been partially filled by the synthetic polymer, particularly for Samples 6 (H-5) and 11 (H-5 E-5), where a high concentration of HEMA was used. The decrease in pore volume for samples 6, 11 and 13 (H-5 T-5) also indicates that the larger pores/voids may have been filled, *e.g.,* for Sample 13 the pore volume decreased from 5.9 to 2.0 cm^3/g. For Sample 7 (H-2.5), where lower concentrations of HEMA (2.5 v/v %) were used, MIP data suggests that the polymer largely coats the pore walls (Figure 3d); the pore volume is very similar to that of the unmodified foams (Table 2) suggesting that there was little or no impregnation into the smaller pores, as is also the case for Sample 4.

Table 2. Mercury intrusion porosimetry (MIP) data for unmodified and impregnated samples. The table is similar to Table 1; a few representative samples were analysed.

	Sample	Weight Gain [%]	Compressive Modulus [kPa ±SD]	Mean Pore Diameter [μm]	Total Pore Volume [cm^3/g]	Porosity [%]
2	Unmodified[a]	0	9.8 ± 3.7	102	5.9	91
4	B-10	7	13.4 ± 3.4	85	6.2	94
6	H-5	140	30.1 ± 5.0	21	2.1	96
7	H-2.5	73	18.0 ± 2.6	67	6.4	95
11	H-5 E-5	140	39.9 ± 4.2	104	2.8	95
13	H-5 T-5	170	26.9 ± 4.9	105	2.0	94

Key; B = butylmethacrylate, H = 2-hydroxyethylmethacrylate,
E = ethyleneglycoldimethacrylate, T = trimethylolpropanetrimethacrylate. The number refers to the concentration of the monomer based on the volume of the autoclave (% v/v). [a] freeze dried and then rehydrated. In all cases, an impregnation pressure of 115Bar was used except for Sample 2, which was unmodified.

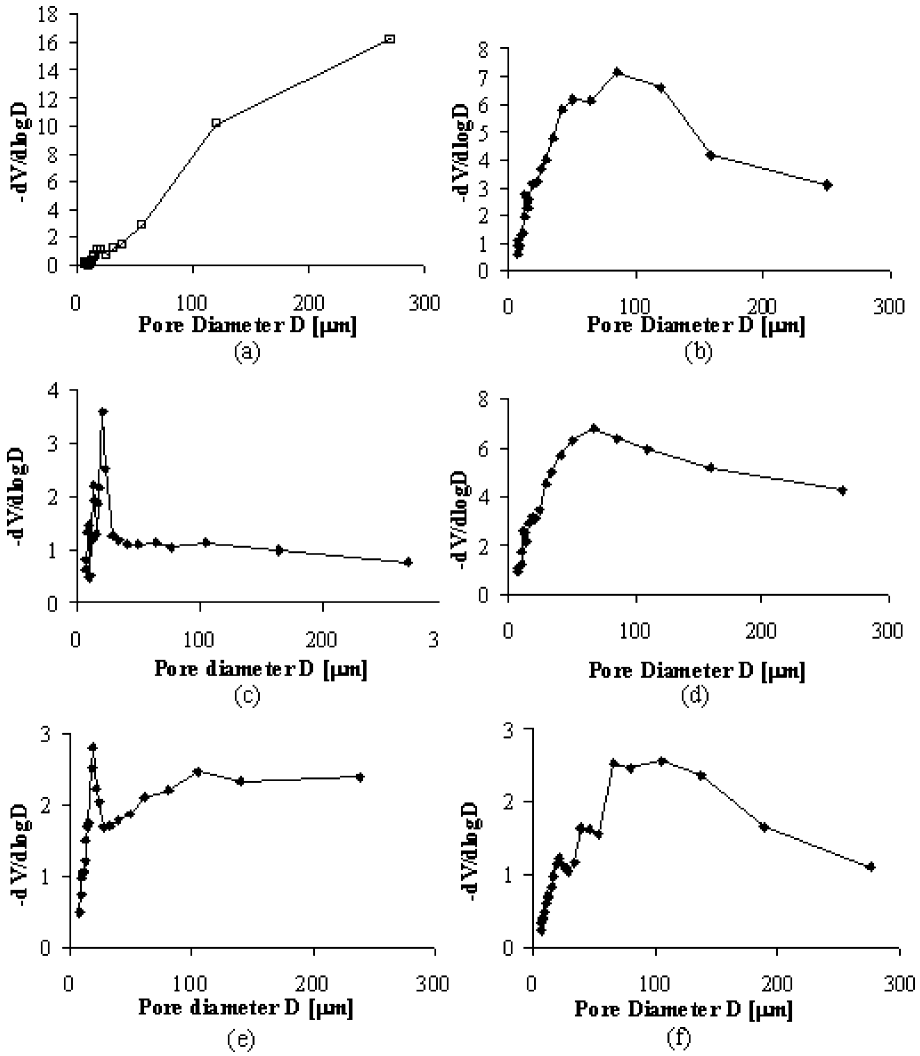


Figure 3. Mercury intrusion porosimetry (MIP) data for (a) unmodified foam (Sample 1), and the impregnated samples (b) Sample 4 (B-10), (c) Sample 6 (H-5), (d) Sample 7 (H-2.5), (e) Sample 11 (H-5 E-5) and (f) Sample 13 (H-5 T-5).

Fourier transform infrared (FTIR) spectroscopy was used to confirm the presence of chemical changes due to impregnation. Figure 4 and Table 3 shows the spectra and peak assignments of an unmodified sample, and of polymer composites with polyHEMA and polyBMA, respectively.

The presence of polyHEMA [38] and polyBMA [39,40], respectively, in the impregnated/polymerised samples was confirmed by the characteristic peaks located at 1728 cm^{-1} and 1726 cm^{-1}, respectively (designated by an asterisk in Figure 4), which are assigned C=O stretching. Other peaks can be assigned as follows: the broad peak at 3303 cm^{-1} is characteristic of O-H stretching (alginate), the peak at 2900 cm^{-1} is characteristic of C-H stretching; the change in intensity of this peak further

confirms the presence of the synthetic polymers. The asymmetric and symmetric stretches of the COO^- group of alginate are located at 1620 cm^{-1} and 1433 cm^{-1}, respectively [41-43]. The peaks at 1146, 1086 and 1045 cm^{-1}, correspond to C-C stretching, C-O stretching and O-H bending, respectively [42,44,45].

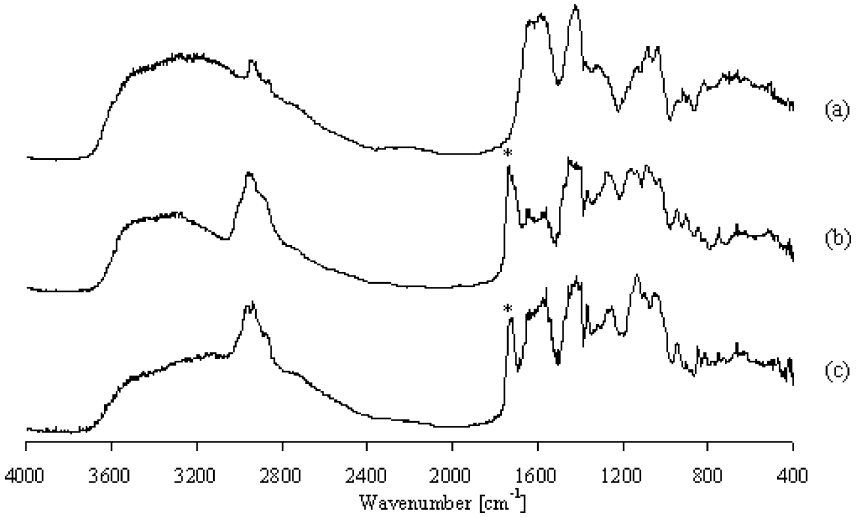


Figure 4. Fourier transform infrared (FTIR) spectra for (a) unmodified alginate foam, alginate foams impregnated with (b) 2-hydroxyethylmethacrylate (HEMA) [Sample 6] and (c) butylmethacrylate (BMA) [Sample 3]. * denotes the C=O bond found in HEMA (1728 cm^{-1}) and BMA (1726 cm^{-1}) respectively.

Table 3. Peak assignments of the infrared absorption bands for unmodified alginate foam, and alginate composites form from impregnation and polymerization of 2-hydroxyethylmethacrylate (HEMA) and butylmethacrylate (BMA) for Sample 6 (H-5) and 3 (B-5), respectively.

Wavenumber [cm^{-1}]	Peak Assignment	Reference
3303	O-H str	40
2949	C-H str	40
1620	COO^- asym str	40,41
1433	C-O sym str	39,40
1317	C-O str	40
1146	C-C str	40
1086	C-O str	42
1045	O-H bend	43
1728	C=O str (HEMA)	36
1726	C=O str (BMA)	37,38

Conclusions

In conclusion, it has been possible to successfully reinforce (emulsion templated) porous alginate hydrogels with synthetic polymers, using a sc-CO_2 assisted infusion approach. An increase in weight after impregnation strongly indicated that the monomer(s) had successfully infused into the alginate foam. Samples containing HEMA exhibited higher mass gains and were stiffer than those impregnated with

BMA after polymerisation. Samples with 5 % v/v HEMA and EGDMA, respectively, displayed the highest stiffness after polymerisation. SEM and MIP results revealed that at high monomer loadings, the subsequent synthetic polymer filled larger pores with a decrease in the total pore volume. At lower loadings of impregnated synthetic monomers, the synthetic polymer appeared to coat the inner surfaces of the large pores. This was rather unexpected as, although we are aware of the poor affinity of CO_2 for the alginate matrix, we had assumed that as a result of swelling under pressure, complete impregnation into the matrix would have been observed rather than coating of the walls. In lieu of the results, we conclude that the matrix may not be sufficiently swollen by the CO_2 to aid much or any impregnation into the matrix under these conditions (this would of course be perhaps be better achieved with a more hydrophilic solvent). The unexpected results are however interesting in that we are effectively able to increase in the stiffness of porous natural polymers and also to tune their macroporosity prior to possible cell seeding in TE applications. Further research work will focus on two aspects; the effect of substantially higher pressures on loading and the effects of modification upon cell surface interactions, the results of which will be reported in due course.

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