Non cell adhesive photopolymerized cross-linked layers (I): synthesis and characterization

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The aim of this study is to design an effective hydrogel, which has good potential for application in the field of tissue regeneration. Ultimately, the polymeric biomaterial will be used as a bottom, non-cell adhering layer of a polymeric scaffold, which will inhibit the adhesion and proliferation of fibroblast cells. A series of terpolymer hydrogels were prepared from MMA/HPOEM/MePOEM monomers based on varying formulations. The gels were polymerized via free radical polymerization under UV and characterized by TGA, DSC and FTIR studies. Furthermore, the double bond conversion, gel content and swelling properties of the polymer were analyzed and correlated to its crosslinking density, which revealed details about the hydrogel's character. Finally, cell culture experiments were used to compare the UV-cured polymer to its thermally derived non-functionalized polymer of the same formulation. Inverted optical microscopy was used to demonstrate cell-resistance of both materials with respect to a control Tissue Culture PolyStyrene plate.

1. Introduction

A biomaterial is a natural or synthetic substance that ''interfaces with living tissue and/or biological fluids'' [1]. They play a key role in engineering tissues as substitutes for functional replacements. These materials are used in devices for therapy and diagnosis, for drug delivery systems, and most importantly for use as scaffold systems for guided tissue growth.

Today's biomaterials could be composed of many different components such as metals, ceramics, natural tissues, as well as, polymers. Hydrogels, which will be examined closely in this paper, are a part of this last group. They are essentially hydrophilic crosslinked polymer gels that swell in water up to thousands of times their own dry weight. They generally exhibit good biocompatibility, degrade at optimal rates, are easily processed, and are similar to soft tissue [1–4].

Hydrogels tend to have low interfacial free energy at their hydrophilic surface, ''which results in a low tendency of proteins and cells to adhere to these surfaces'' [2]. This resistance to protein adsorption and cell adhesion makes the hydrogel base material a blank slate, free of biological interactions, upon which the desired biofunctionality can be built [4–9]. This will be very important in the design of an effective polymeric scaffold system, which will be explained later. Furthermore, the soft and rubbery nature of hydrogels helps to minimize irritation of the surrounding tissue [10]. The amount of water in hydrogels establishes absorption and diffusion of solutes through the hydrogel. The average pore size and pore distributions within the gel, the solute size and shape, its relative hydrophilic and hydrophobic character are also very important factors in solute permeation through any hydrogel. All of these above factors, however, are influenced by the polymerization composition and crosslinking density of the network, which will be analyzed in this paper as well [11].

The hydrogel biomaterial synthesized in this paper has significant potential for application in the field of tissue regeneration as mentioned above. Furthermore, it offers more advantages than the thermally derived polymer. It is a solvent free process, the curing time is very fast and well controlled, it is instantly started and shut down, and is environmentally friendly [12].

The hydrogel will ultimately be used as the bottom, non-cell adhering layer of a 3-D polymeric scaffold on which cells can grow selectively on a bioactive top layer [10]. Non-adherence of cells to the biomaterial surface will be shown using inverted optical microscopy, and will be compared to a thermally derived polymer of the same formulation, which is also non-cell adhering.

2. Experimental

2.1. Materials

Methyl methacrylate (MMA), FW ~ 100.12 g/mol, poly (ethylene glycol) methacrylate (HPOEM), M_n ~ 526 g/mol and ~ 360 g/mol, poly (ethylene glycol) methyl ether methacrylate (MePOEM), M_n ~ 300 g/mol

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and ~ 475 g/mol, Ethylene glycol dimethacrylate (EGDMA), FW 198.22 g/mol, monomers were obtained from Aldrich Chemical Co. Ebecryl 8210 urethane acrylate pre-polymer (UA), $M_w \sim 1383$ g/mol, was acquired from UCB Chemicals Corporation (M_w not given). Disposable columns, for removing the hydroquinone inhibitors and monomethyl ether hydroquinone were also purchased from Aldrich Chemical Co. Irgacure 184 (IRG 184) photoinitiator was obtained from Ciba Specialty Chemical. Ethyl acetate, ethanol and acetone solvents were obtained from Fisher Chemical Co. Glass tubes, petri dishes, 1-inch circular microscope cover glass slides, filter paper, and soxhlet extraction thimbles were also purchased from Fisher Chemical Co.

2.2. Methods

2.2.1. Polymer synthesis: photopolymerization of crosslinked $(MMA)_x(HPOEM)_y(MePOEM)_z$ terpolymer (POLY 1)

A comb polymer was synthesized by free radical polymerization of MMA, HPOEM, and MePOEM according to varying formulations. MMA and MePOEM, were both purified with an ethyl acetate wash using an inhibitor remover disposal column prior to use, while HPOEM was used as received. The formulations seen in Table I were made based on a total polymer mass of 5.0 g. The different experiments were conducted to achieve optimal polymerization results.

TABLE I Different formulations of the three monomers used to make terpolymer (w/w,%) (POLY 1)

Sample name	MMA (%)	HPOEM (%)	MePOEM (%)	IRG 184 (%)
D-1	70	15	15	0.2
D-2	60	20	20	0.2
D-3	50	25	25	0.2
D-4	40	30	30	0.2
D-4b	40	30	30	0.6
D-5	80	10	10	0.2
D-6	30	35	35	1.0
D-7	50	—	50	0.5

Each of the three monomers, as well as the photoinitiator required to initiate the UV-induced polymerization, were added to a beaker based on the formulations in Table I. The samples were stirred briefly to ensure proper miscibility of the three monomers and were added to a Petri dish to undergo UV polymerization

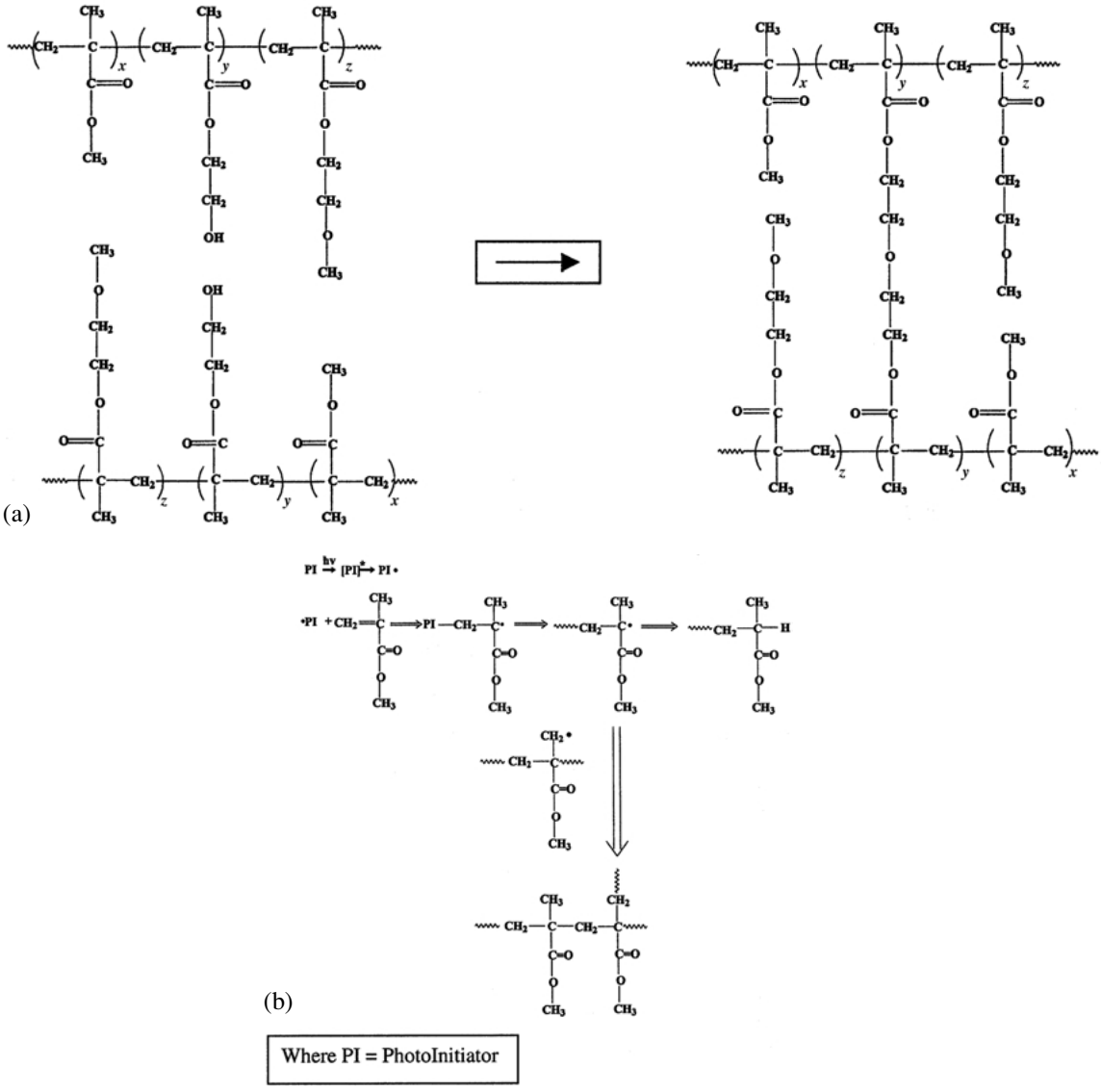

Figure 1 Plausible structure of POLY 1 and its possible crosslinking mechanisms.

for a period not exceeding 3 h. A B-100A model Blak-Ray Longwave Ultraviolet Lamp from UVP was used as the UV source. The lamp's power, frequency and current intensity were 115 V, 60 Hz, and 2.5 A, respectively, operating at a wavelength spectrum of 254–365 nm. The sample was added uniformly to the Petri dish, producing a polymer film of ~ 2 mm in thickness.

The proposed structure of the crosslinked POLY 1 can be seen in Fig. 1(a), where *x, y* and *z* represent the mole% of the MMA, HPOEM, and MePOEM monomers respectively. A crosslinked structure is proposed based on different mechanisms, as shown in Fig. 1. A mechanistic possibility occurs as the hydroxyl groups from two different polymer chains on the middle HPOEM monomer come together to form an ether linkage as water is lost, as seen in Fig. 1(a). Another probable explanation for the crosslinking occurring in the polymer system can be shown through free radical polymerization. Fig. 1(b) represents such a mechanism, illustrating the MMA monomer (as an example of one of hundreds of repeating units found in a polymer system) being attacked by a free radical. Following a series of propagation steps, a crosslink is formed with a random methyl methacrylate (MMA) repeating unit or with any other poly (ethylene glycol) methacrylate (HPOEM) or poly (ethylene glycol) methyl ether methacrylate (MePOEM) random repeat unit from the MMA/HPOEM/MePOEM polymer chain. Further studies are currently in progress to understand this.

2.2.2. Polymer synthesis: thermal polymerization of uncrosslinked $(MMA)_x(HPOEM)_y(MePOEM)_z$ terpolymer (POLY 2)

The non-functionalized thermally derived polymer was synthesized using the same three monomers as the above UV polymer. The reaction was performed in distilled THF in a nitrogen environment and refluxed for 18 h at 70 °C. The synthesis and characterization was completed according to Irvine *et al*. [6] and Hyun *et al*. [7]. The proposed structure of the linear, uncrosslinked POLY 2 is shown in Fig. 2, where *x, y* and *z* also represent the mole% of MMA = 86.21%, HPOEM = 7.89% and MePOEM = 5.90% monomers respectively, and $m = 6$ and $n = 9$ denote the ethylene oxide repeating units.

2.2.3. Polymer synthesis: UV polymerization of crosslinked $(MMA)_x\ (HPOEM)_y\ (MePOEM)_z\ (EGDMA)_m\ (PU)_n$ (POLY 3)

The UV derived polymeric hydrogel used in the cell attachment study was substantially modified from the UV material synthesized earlier (POLY 1). Following exposure to UV light, POLY 1 used above, was unable to withstand cell growth media and the subsequent introduction of 37 °C incubating temperatures. A modified formulation, as well as a new substrate were therefore utilized, which reduced shrinkage of the gel, improved the degree of crosslinking in the system, and enhanced the existing substrate–material interface.

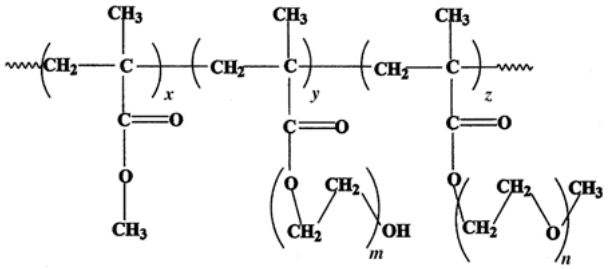

Figure 2 Plausible structure of POLY 2.

The new formulation was prepared as follows: 38% MMA, 25% HPOEM, 25% MePOEM, 1% IRG 184 photoinitiator, 1% EGDMA crosslinker, and 10% urethane acrylate (UA) pre-polymer (see table below). Ethylene glycol dimethacrylate (EGDMA) was added to increase the crosslinking density, and urethane acrylate (UA), to reduce the shrinkage of the gel and to improve its adhesion to the glass substrate. A low percentage of UA was added in order to decrease the brittle character of the hydrogel. It was necessary to maintain a rubbery, crosslinked material, which had limited shrinking properties as mentioned above. Furthermore, the monomer concentrations were kept virtually the same as the optimized POLY 1 sample discussed earlier.

	% of total polymer mass	MW (g/mol)	Moles	%
MMA	38	100.12	0.0189	72.41
HPOEM	25	526	0.00238	9.12
MePOEM	25	300	0.00417	15.98
EGDMA	1	198.22	0.000252	0.97
UA	10	1383	0.000362	1.39

Assuming 100% conversion.

This sample was uniformly coated on a 1-inch (25.4 mm) circular microscope cover glass and exposed to UV light for a 1 h period on a Fusion UV machine, Model I 300 MB from Systems Inc. This system operates at 3900 V, 2450 MHZ of microwave energy and 700 mA of d.c. current, which clearly was a substantial increase from the previously used UV lamp.

The resulting hydrogel produced a better quality film, 2 mm thick. This was qualitatively observed based on many varying curing techniques which manipulated the energy (0.0334 mJ/cm^2 per pass) and speed of the UV machine (7 ft/min), as well as the number of passes (2) that the gel underwent. The polymer was completely cured at a much faster rate, and was less sticky than the previous hydrogel, which also was indicative of a crosslinked material. FTIR further confirmed such a phenomenon. In addition, upon soaking in cell culture media, it did not shrink or lift off the surface of the glass cover slip, demonstrating greater physical strength than previously observed. The substrate–material interface was improved as well, since better adhesion of the polymer was observed to the glass substrate.

The proposed structure of POLY 3 is represented in Fig. 3, which illustrates the greater number of branching chains formed as a result of the monomeric vinyl groups polymerizing to allow crosslinking to occur. The

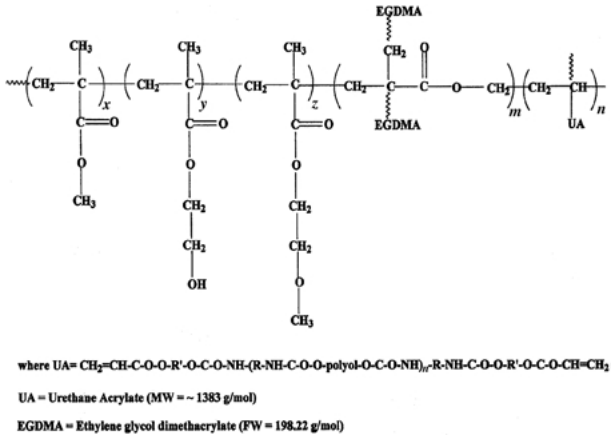

Figure 3 Plausible structure of POLY 3.

subscripts x, y and z represent the mole% of MMA = 72.41%, HPOEM = 9.12%, and MePOEM = 15.98%, monomers as earlier stated. The mole% of EGDMA, denoted by the subscript m was found to be 0.97%, and the mole% of the UA moiety at the end of the chain, n was 1.39% (see calculation above). These numbers were estimated based on feed ratios from the different components of the polymer system.

2.3. Characterization and Analysis

2.3.1. POLY 1

An extensive FTIR kinetics study was first performed, using a Thermo Nicolet Nexus 870 FTIR machine (resolution of 4) with OMNIC software, which monitored the C=C bond conversion. The study investigated only the sample that was considered to give the most optimal, uniform polymer film. About 40 mg of this optimum polymer sample D4b (POLY 1) (formulation found in Table I), was uniformly plated on a sodium chloride plate and analyzed using FTIR. Precisely after every half hour time interval beginning at time zero (where no UV exposure took place), up to a maximum of four hours (where 4 h of UV exposure took place), the polymer's IR was run immediately. The disappearance of the peak representing the C=C bond located at $\sim 1627\,\mathrm{cm}^{-1}$ in the IR spectra was qualitatively observed and then numerically determined using the following equation based on peak heights and shown in Table II,

$$\%\mathrm{DBC} = [100 - (x/y \times 100)] \qquad (1)$$

where x = peak intensity after UV exposure and y = peak intensity before UV exposure.

TABLE II C=C Double bond conversion with respect to UV dose in hours (POLY 1)

Dose (Hours)	Double Bond Conversion (%)
0.5	50.7
1.0	76.1
1.5	77.5
2.0	78.9
3.0	78.9
4.0	78.9

TABLE III Gel content (%) with respect to UV Dose in hours (POLY 1)

UV Dosage (Hours)	$\%P_{\mathrm{gel}}$
0.5	87.00
1.0	87.09
1.5	87.21
2.0	87.30
3.0	87.34

Gel content. The qualitative observations and characterizations were the defining parameters of relative film superiority among the different gel samples listed in Table I. Compared with the other formulations, sample D-4b (POLY 1) was less sticky and was cured at a faster rate, and was therefore chosen for further experimentation. The polymer was initially cured ~ 2 mm in thickness in a glass Petri dish according to UV dosage time ranging from 0.5 h to 3.0 h. After UV exposure, the sample was removed from the dish, placed in a thimble, weighed, and the homopolymer was removed using a soxhlet extraction technique. After extraction, the sample was again weighed and the percentage of sample that was converted into polymer was measured using the following equation,

$$\%P_{\mathrm{gel}} = \frac{\text{weight after extraction}}{\text{weight before extraction}} \times 100 \qquad (2)$$

The above equation represents the percentage of polymer gel content present in the sample, as seen in Table III.

Swelling properties. Four different soaking times were investigated in testing the polymer's swelling characteristics when immersed in distilled water for 0.5, 1, 1.5, and 2 h, respectively. Each sample containing the different exposure times was immersed in acetone in the soxhlet extraction thimble. The samples were each refluxed for 24 h and then vacuum dried for another 24 h at about 40 °C. 0.25–0.50 g of the samples each representing different exposure times, were then allowed to swell in vials filled with deionized water following ASTM 2795.15 swelling ratio guidelines for 6 h periods not exceeding 24 h. The samples were gently tapped dry with filter paper, and weighed immediately. The weight of the swollen gel (after soaking in deionized water) divided by the weight of the gel (before soaking in deionized water) gave the swelling ratio of the gel

$$\text{Swelling ratio} = \frac{W_{\mathrm{sg}}}{W_{\mathrm{g}}} \qquad (3)$$

where, W_{sg} is the weight of the swollen gel (after soaking), and W_{g} is the weight of the gel (before soaking) [12].

Solubility tests were also performed on POLY 1 to further characterize the thin film. The results are listed in Table IV. 10 mg portions of the terpolymer were tested with 10 ml of each solvent.

The crosslinked polymer film was finally characterized and analyzed by the following techniques; thermal gravimetric analysis (TGA) using a Hi-Res TGA 2950, TA Instruments at a heating rate of 20 °C/min, differential scanning calorimetry (DSC) using a modu-

TABLE IV Solubility of POLY 1

Solvent	Soluble
Toluene	No
Acetone	No
THF	No
DMSO	No
Chloroform	No

lated DSC 2920 at a heating rate of 10 °C/min and an inverse heat flow of 1/W/g performed in a nitrogen gas atmosphere, and FTIR analysis using a Thermo Nicolet Nexus 870 model.

2.3.2. POLY 2

This system in contrast with the first, represented a linear uncrosslinked system. The extensive analyses of double bond conversion, gel content and swelling properties were therefore unnecessary. POLY 2 was however characterized by TGA, DSC, and FTIR studies. Furthermore, its molecular weight was determined by gel permeation chromatography (GPC) using a Waters 1515 HPLC/GPC equipped with Waters HR 5E columns and a 2410 Detector, operating at 30 °C and at a flow rate of 1 ml/min. Polystyrene standards were used for calibration.

2.3.3. POLY 3

POLY 3 as mentioned above, was formulated simply to improve POLY 1 with regard to the cell work done on the polymer material. POLY 3 was considered to be even more crosslinked than the POLY 1 system. The extensive studies done on POLY 1 mentioned above to prove the crosslinking density of this system were not performed on the newly formulated material, because substantial evidence was already clearly found with the first system. POLY 3 was, however, characterized by TGA, DSC, and FTIR. Furthermore, a MALDI-TOF on a Voyager-DE Biospectrometry Workstation Time Of Flight Mass Spectrometer was performed to estimate the molecular weight of the urethane acrylate pre-polymer moiety. 2-[(4-Hydroxyphenyl) azo] benzoic acid (HABA) was used as a matrix, and a NaCI/THF solution was used to assist the ionizing process.

2.4. Cell culture

The cells used for assessment of the material were a male rat fibroblast cell line. The cells were grown in Dulbecco's modified Eagles medium DMEM (Sigma Chemical Co.) supplemented with 20% heat inactivated fetal bovine serum (GIBCO Laboratories), 1% fungizeone, 1% Penicillin/Streptomycin, 2.5% HEPES (1 M), and 1.25% L-glutamine. Cells were grown near confluence in T75 flasks (Fisher Chemical Co.) then subcultured every 3–5 days by dissociating with Trypsin-EDTA solution (GIBCO Laboratories). The cells were maintained at 37 °C in a fully humidified atmosphere at 8% CO_2 in air [5, 6].

2.5. Cell attachment

Following UV exposure with the Fusion UV machine, POLY 3 was removed from the cover glass microscope slides and soaked in sterile PBS buffer. After washing, the gel was placed in a 6 well tissue culture polystyrene (TCPS) Transwell plate (Corning Science Products) equipped with an insert to hold down the hydrogel.

Fibroblastic cells were collected after trypsinization by centrifugation at 1200 rpm for 5 min, re-suspended in cell culture medium, to a plating concentration of 3×10^5 cells per well in a total volume of 2 ml. After seeding, the cultures were incubated for 24 h at 37 °C in 8% CO_2 air. At the end of this period, pictures were taken using an Olympus IX 70 Optical Elements Corporation inverted optical microscope. All materials were present in triplicate, and as a reference material, cells seeded on TCPS were run in triplicate as well [13, 14].

Since the cells did not attach to the polymeric biomaterial, no cell attachment or proliferation assays were performed on the fibroblast cells. Microscopic qualitative analysis of cell culture was therefore the primary measure of non-cell adherence to the material.

3. Results and discussion

The crosslinking density of a polymer reveals a great deal about its character, and in this study will be an important factor in the design of an effective bottom layer material for use in a polymeric scaffold. The crosslinking density of the initial UV system (POLY 1) was therefore extensively characterized and analyzed. Of particular importance, was the kinetics IR study, which was performed on the POLY 1 system. The disappearance of the monomeric vinyl C=C over a 4 h period was observed by FTIR. The double bond conversion percentages as a function of UV exposure time measured in hours are listed in Table II and illustrated in Fig. 4.

Following 4 h of polymerization almost 80% of the C=C was converted to C–C as expected. As polymerization continued, the monomeric units containing the double bonds were being converted into a

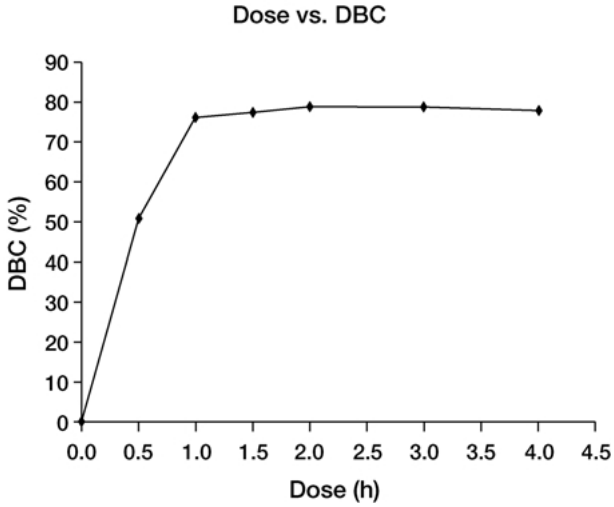

Figure 4 Double bond conversion (DBC) (%) of POLY 1 with respect to UV dose time in hours.

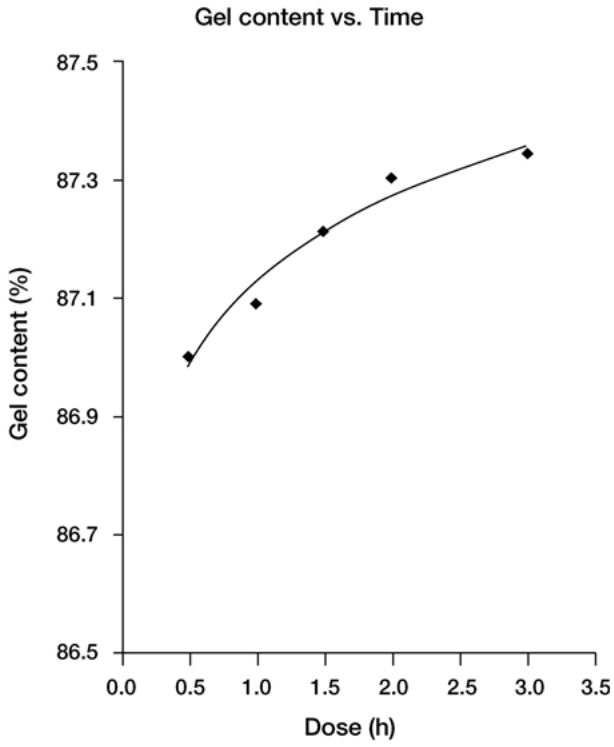

Figure 5 Gel content (%) of POLY 1 with respect to UV Dose time in hours.

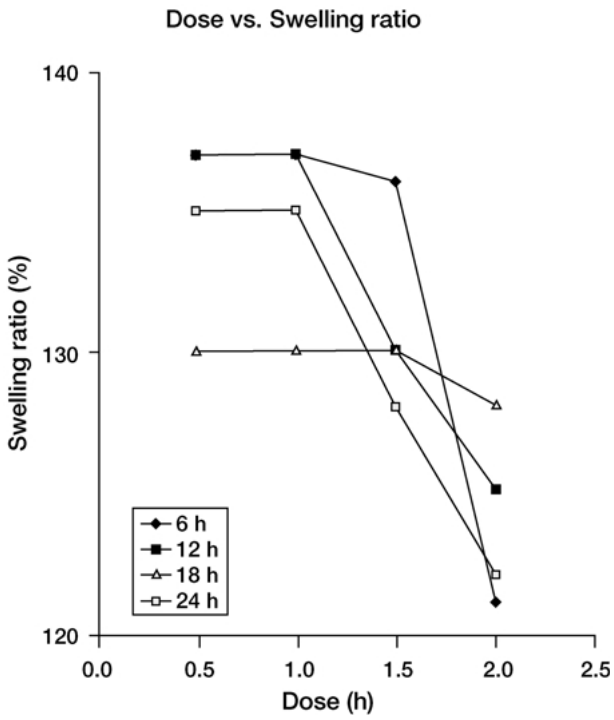

Figure 6 Swelling ratios (%) of POLY 1 at 6, 12, 18 and 24 h time points with respect to UV dose time in hours.

polymer system, which contained only single bonds, which was consistent with the literature [15, 16].

This above data could now be correlated to the gel content percentage of the POLY 1 crosslinked system at different exposure times. Table III represents the corresponding gel percentages.

As the dosage of UV exposure increased from 0.5 h to the maximum of 3 h, the gel content should have also increased, or remained the same. According to the literature, as UV polymerization increases, more of the sample should be converted into a gel polymer network or remain stable at its maximum gel content [12]. The greater the gel content of a polymer, the greater the degree of crosslinking that is occurring within the polymer. The relatively high constant gel content finding of approximately 87%, was not only indicative of almost complete conversion of the gel into a polymeric system, but it also demonstrated a high crosslinking density [15, 16]. This can be seen graphically in Fig. 5.

These findings can also be rationalized according to the swelling property data of the hydrogel, which was taken at 6, 12, 18 and finally 24 h. The swelling behavior of the hydrogels depends on the environmental conditions and the nature of the polymer. The latter involves the nature of the charge, ionic content, and the crosslinking density. Fig. 6 indicates a consistent pattern according to the literature and the above data [12, 15–21]. The data showed the swelling ratio staying relatively constant at over 100%. The hydrogel polymer material was therefore absorbing more than its total weight in water. Also consistent with the literature as seen in the graphical illustration, as the UV dose time increased, the swelling ratio accordingly decreased. As the sample was further polymerized, the gel content was increasing, and the C=C double bonded system was being converted to a C–C single bonded polymer system. The polymeric gel network was becoming more and more crosslinked, which allowed less and less water to be absorbed by the system, indicated by the decreasing swelling properties of the gel over time.

As mentioned earlier, extensive studies proving crosslinking density were not performed on POLY 2 or on POLY 3. POLY 2 was found to be an uncrosslinked system, so gel content and swelling ratio would not be applicable. Its molecular weight was found to be 59 000 Daltons by GPC. POLY 3, however, was found to be a highly crosslinked system, which did form a gel-like material similar to POLY 1. The addition of the urethane acrylate moiety to POLY 3 was undoubtedly the reason for this finding. The pre-polymer was found to have: $M_w = 1383$ g/mol, and a polydispersity index = 1.004 by MALDI-TOF analysis. Due to the increased crosslinking of this system, it would probably have a higher gel content and a lower swelling ratio than POLY 1. For these reasons, POLY 3 was found to be the more suitable material to be used as the potentially effective bottom layer of the polymeric scaffold.

Further characterizations were performed on POLY 1, as well as initial analyzes of POLY 2 and POLY 3. TGA analysis of all three polymer samples showed a relatively similar thermal decomposition temperature of 180 °C (98%) after soxhlet extraction as seen in Fig. 7. The polymer materials were stable until this temperature, and thereafter started to decompose as seen in the graph.

DSC analysis was also performed on all three polymers and is shown in Fig. 8. The UV derived POLY 1 did not show a distinct glass transition temperature (T_g) probably due to it being a highly crosslinked system. POLY 2 represents the uncrosslinked thermally derived polymer, which clearly showed a T_g at around 22 °C. POLY 3 also showed a T_g at approximately 22 °C. POLY 3 is however even more crosslinked than

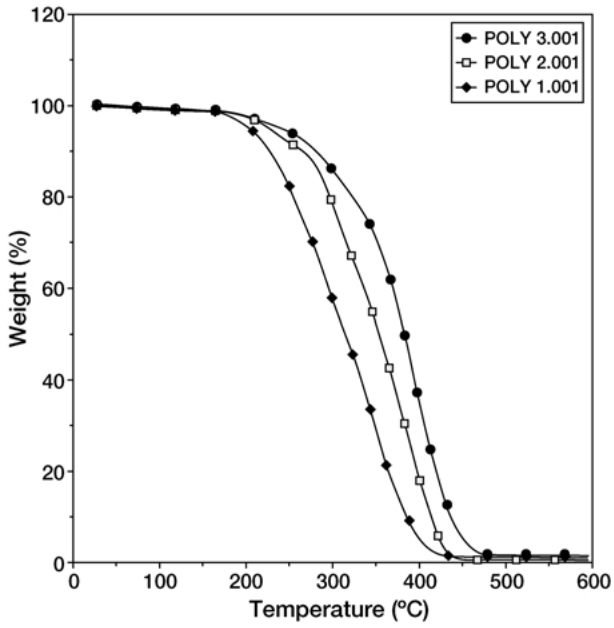

Figure 7 Thermogravimetric analysis of POLY 1, POLY 2, and POLY 3 at a heating rate of 20 °C/min.

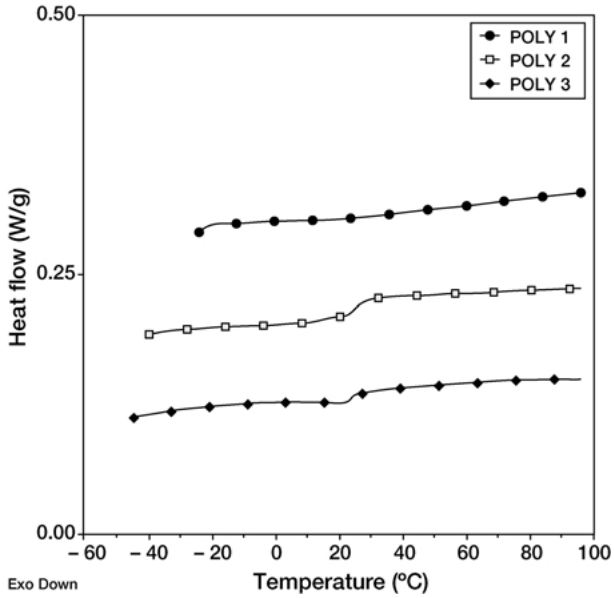

Figure 8 Differential scanning calorimetry (DSC) of POLY 1, POLY 2, and POLY 3 in a nitrogen atmosphere at a heating rate of 10 °C/min.

POLY 1, so a T_g was not anticipated. The reason for this finding can be explained by the introduction of the urethane acrylate (UA) moiety (M_w 1383 g/mol) that was added to POLY 3 in order to reduce the shrinkage of the gel and to increase its adhesion to the substrate. UA does itself have a T_g, which is a probable reason that one is observed for POLY 3.

The IR data illustrated in Fig. 9 of all three polymers, revealed noticeable peaks at about 3500 cm^{-1}, 1700 cm^{-1} and 1600 cm^{-1} representing O–H, C=O, and C=C stretching respectively, and indicative of all important functional groups present in the polymer chains. The only noticeable difference in all three spectra was the intensity of the C=C peak at 1600 cm^{-1}. In POLY 2, the peak was almost non-existent as compared to the other two polymer systems. The reason for this

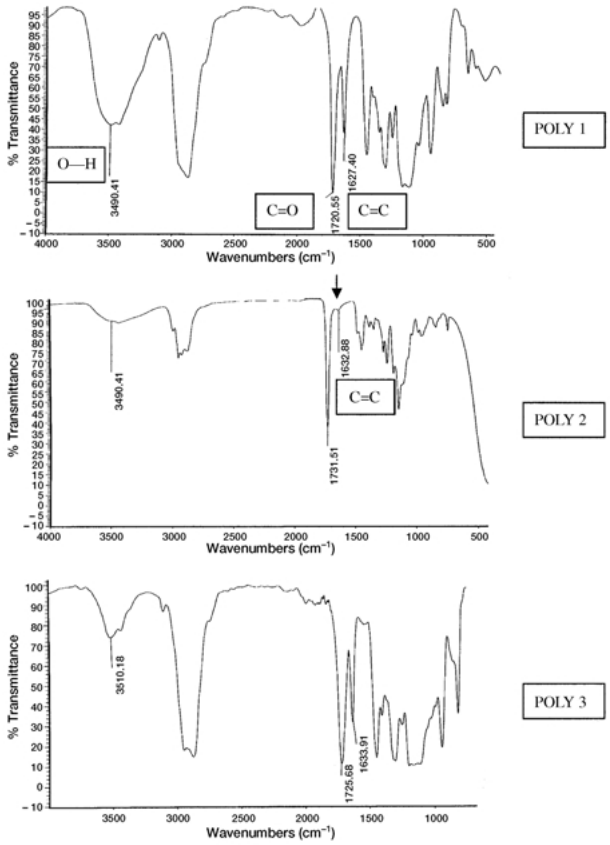

Figure 9 Infrared spectra of POLY 1, POLY 2 and POLY 3 at the zero time point under no UV exposure. Important functional groups shown: O–H at 3500 cm^{-1}, C=O at 1700 cm^{-1}, and C=C at 1600 cm^{-1}.

finding could be explained by the polymerization technique employed. POLY 2 was the product of a thermally derived system, which had a much higher energy throughput. This allowed the double bond to be more quickly and more effectively converted than its UV derived counterparts.

Peaks (cm^{-1})	Stretching
3490	O–H
1720	C=O
1627	C=C

POLY 2 and POLY 3 were then subjected to cell attachment studies. The cells were incubated for a 24-hour time period atop both POLY 2 and POLY 3 materials, and did not show any indication of attachment, elongation, or proliferation on either material. The cells remained circular in shape on both the UV cured polymer (POLY 3) as well as the non-functionalized thermally derived polymer (POLY 2). Fibroblastic cells were therefore found to be non-adherent to either material. It was also apparent that a larger number of cells on the non-functionalized polymer had detached from the surface, clearly confirming the lack of cell-surface attachment. The significant differences in morphology of the TCPS control, the UV polymer and the non-functionalized polymer are demonstrated in Fig. 10. It is evident by the optical micrographs that the control plate is allowing for proper cell adhesion, elongation and proliferation, judging from the healthy spindle-like shape of the cells. Upon re-seeding the cells that were exposed

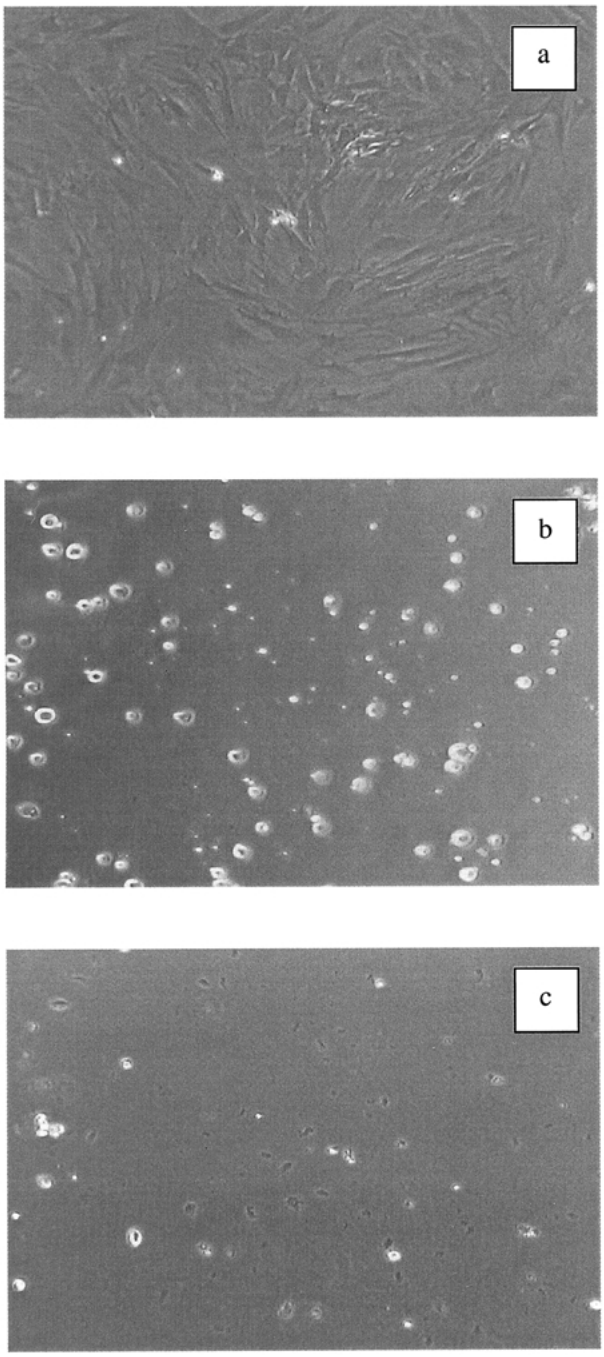

Figure 10 (a) Control on TCPS (b) UV polymer (POLY 1) (c) Non-functionalized polymer (POLY 2), 24 h incubation period (10 ×).

to the material on a tissue culture polystyrene plate, they seemed to grow again quite nicely. The cells did not appear to be dead, which confirmed that the formulated material, although cell-resistant, was non-toxic to the cells.

The non-adherence of the cells can be explained by the surface chemistry of the materials being used. Neither material contained any surface functional groups, which would act as effective binding sites for the fibroblast cells. There is no activated surface present that would allow the cells to adhere and consequently to grow. Future work will therefore involve tethering an activated Arg–Gly–Asp (RGD) amino acid sequence to the surface of the functionalized thermally derived polymer material, which would induce this cell attachment and proliferation.

4. Conclusion

After 4 h of polymerization, almost 80% of the C=C double bond was converted to C–C single bond indicative of the transformation from the monomeric units to the polymer system. This was also consistent with the gel content findings of 87% for the polymeric material. As UV exposure increased, the gel content increased as well, confirming the formation of a highly crosslinked polymer. All of the above data was then correlated to the swelling ratio of the hydrogel, which was found to remain over 100% at each time point. Furthermore, as the UV exposure time increased, the swelling ratio of the gel was found to decrease, also indicative of a highly crosslinked polymeric gel network.

Finally, the material was used as a substrate for cell culture, and compared to its thermally derived counter part. The materials were found to both be cell-resistant, as well as non-toxic to the fibroblast cells. No cell attachment nor spreading were observed, proving the formulated material to be an effective blank slate bottom layer of a future bilayered polymeric scaffold on which cells will eventually adhere, grow, and align into organized patterns.

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