# Enhanced Mechanical and Cell Adhesive Properties of Photo-crosslinked PEG Hydrogels by Incorporation of Gelatin in the Networks

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**Abstract**

Although synthetic polymers may have suitable physicochemical properties for biomedical applications, biological properties are generally lacking. Poly(ethylene glycol) (PEG) is a frequently used polymer for the preparation of hydrogels. Due to its hydrophilic character, however, cellular interactions with PEG hydrogels are minimal or absent. To improve the cell adhesive properties of PEG hydrogels, we developed hybrid hydrogels based on PEG and the natural polymer gelatin. PEG dimethacrylate (PEG-dMA) and gelatin methacrylate (GelMA) macromers were prepared, which were photo-crosslinked in water in different ratios (75:25, 50:50 and 25:75 % (v/v)). The obtained hybrid networks showed macrophase separation, which could be prevented by photo-crosslinking in 0.5 % (v/v) acetic acid in water. The toughness of 50:50 % PEG-dMA:GelMA hydrogels prepared in 0.5 % acetic acid was 2.5 times higher than that of single polymer hydrogels made of PEG-dMA or GelMA. Hybrid hydrogels crosslinked in 0.5 % acetic acid supported the proliferation of human mesenchymal stem cells to the same extent as compared to 100 % gelatin hydrogel, whereas the cells did not proliferate on 100 % PEG hydrogel. In conclusion, our results show that both the cell adhesive and mechanical properties of a photo-crosslinked PEG network can be improved by incorporation of gelatin in the network.

Keywords: Methacrylated PEG, Methacrylated Gelatin, Photo-crosslinking, Hydrogel.

## 1. Introduction

Synthetic as well as natural polymers are frequently used for biomedical applications. Generally, the physicochemical properties of synthetic polymers (e.g. mechanical properties, degradability), can be tuned for a certain application. It remains a challenge, however, to provide synthetic polymers with biological properties (e.g. cell adhesive sites or cell instructive properties). Conversely, natural polymers like extracellular matrix proteins possess biological information, but their mechanical properties are generally insufficient. Thus, preparation of polymer structures containing both synthetic and natural polymers may solve this problem.

Poly(ethylene glycol) (PEG) is a hydrophilic, non-toxic and non-immunogenic polymer, that exhibits relatively good mechanical properties. PEG is frequently used to prepare hydrogels, which are suitable materials for applications in drug delivery and regenerative medicine [1-6]. Hydrogels consist of polymeric networks that are able to swell in aqueous environments because of their hydrophilic character [1]. Methods to fabricate hydrogels are versatile, including physical and chemical crosslinking [7, 8]. Chemical crosslinking has advantages in terms of the formation of covalent bonds, e.g. using photo-crosslinkable polymers [7, 9, 10].

A major drawback of PEG hydrogels is the lack of biological moieties such as sites for cell binding [11, 12]. A solution to this problem may be the implementation of extracellular matrix components such as proteins or glycosaminoglycans during hydrogel preparation [13]. Gelatin is a water soluble natural polymer derived from the extracellular matrix protein collagen [14]. Gelatin-based hydrogels are enzymatically degradable, and cells can adhere to and spread within the gels [15, 16]. Hybrid hydrogels made of PEG and gelatin may have suitable properties for biomedical applications [12, 17].

Also other synthetic polymers like poly(ε-caprolactone) (PCL) or poly(trimethylene carbonate) (PTMC) could be used to make mechanically tough hybrid hydrogels with gelatin. As PCL and PTMC are biodegradable and PEG is not, this would provide better control over biodegradability. However, finding a common solvent for PCL, PTMC and gelatin is a challenge. Therefore, PEG was chosen as both PEG and gelatin are water soluble.

In this study, PEG and gelatin were functionalized with methacrylate groups. PEG-Gelatin hybrid hydrogels were formed by photo-crosslinking in water. The physical properties of the networks were determined by tensile testing, water uptake and gel content measurements. The biological properties were investigated by culturing of human mesenchymal stem cells (hMSCs) on the surface of the polymer networks. We hypothesized that the photo-crosslinked combination of PEG and gelatin would lead to beneficial properties of the hybrid hydrogels in terms of both the biological and mechanical characteristics.

## 2. Materials and Methods

### 2.1 Materials

Gelatin from porcine skin (type A) with Mw = 20-25 kg/mol and Mw = 50-100 kg/mol, poly(ethylene glycol) (PEG) (linear) with Mw = 4 kg/mol and Mw = 10 kg/mol, triethylamine (TEA), methacrylic anhydride (Maah), 2-hydroxy-1(4-(hydroxyethoxy)phenyl)-2-methyl-1-propanone (Irgacure 2959 or I 2959), deuterated chloroform and 2,4,6-trinitrobenzenesulfonic acid solution (TNBS, 5 % (w/v) in $H_2O$) were obtained from Sigma-Aldrich. Diethyl ether and dichloromethane (DCM) were purchased from VWR Chemicals. Sodium bicarbonate ($NaHCO_3$) was provided by Merck. Dialysis membrane (MWCO = 12-14 kDa) was purchased from Spectra/Por®. All compounds were used without further purification. Dulbecco's PBS (DPBS), Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), glutamax and penicillin/streptomycin were obtained from Gibco.

CyQuant cell proliferation assay and calcein-AM/ethidium homodimer-1 Live/Dead staining kit were purchased from Invitrogen.

2.2 Methacrylation of PEG

PEG was dissolved at 33 % (w/v) in DCM by magnetic stirring in an inert argon atmosphere at RT. After the PEG was fully dissolved, TEA (4 mol/mol PEG) and methacrylic anhydride (4 mol/mol PEG) were slowly added to the stirred solution. After reaction for 5 days, the PEG-dimethacrylate (PEG-dMA) was precipitated drop-by-drop in diethyl ether. The resulting white polymer suspension was vacuum filtrated and the precipitate was washed three times using diethyl ether. Next, the obtained polymer was vacuum dried for two days. The degree of functionalization was determined by proton nuclear resonance (1H-NMR) spectroscopy using a Varian Inova 300 MHz NMR spectrometer and d-chloroform as a solvent [2, 18]. The degree of functionalization (DF) of the PEG-dMA used in this study was 98 %.

2.3 Methacrylation of gelatin

Gelatin was dissolved at 10 % (w/v) in Millipore water at 50 ℃ by magnetic stirring. After the gelatin was fully dissolved, a certain amount of methacrylic anhydride was slowly added to the solution under intensive stirring. An emulsion was formed which was reacted for 3 hours. Subsequently, the solution was transferred to a centrifuge tube and the methacrylated gelatin (GelMA) and unreacted methacrylic anhydride were separated by centrifugation at 4000 g for 5 mins at RT. The GelMA (supernatant) was collected in a glass bottle and the solution was diluted two times with Millipore water of 40 ℃ to terminate the reaction. The resulting solution was transferred to a 12-14 kDa MWCO dialysis bag and dialyzed against water for 3 days at 40 ℃ to remove methacrylic acid byproduct. Finally, the GelMA was freeze dried and stored at -25 ℃ before use. GelMA preparations with a different DF were obtained by varying the amount of methacrylic anhydride (0.05, 0.1, 0.5 and 2.0 ml/g of gelatin). The DF was determined by a TNBS assay to quantify the amount of residual free amine groups in the functionalized GelMA relative to unreacted gelatin [16, 19].

2.4 Hydrogel preparation

PEG-dMA and GelMA were separately dissolved at a concentration of 20 % (w/v) in water or in 0.25 % or 0.5 % (v/v) acetic acid in water at 50 ℃. I 2959 (0.05 % (w/v)) was added as a photo initiator. Three different hybrid hydrogels were fabricated by preparing mixed solutions containing 75:25, 50:50 or 25:75 % PEG-dMA:GelMA (v/v). Next, a solution was placed between two quartz glass plates separated by a 1 mm spacer, and photo-crosslinked by irradiation for 30 mins at 365 nm in a UV box. Finally, the hydrogels were extracted in water for three days to remove uncrosslinked polymer.

2.5 Physical properties

*2.5.1 Water uptake and gel content.* Determination of water uptake and gel content of the hydrogels was based on gel weights in both swollen and dry states. After photo-crosslinking, the gels were dried for 2 days ($m_0$), extracted in water for 3 days ($m_s$) and dried again for 2 days ($m_1$). All steps were performed at 37 °C using three samples of equal size for each gel. The water uptake and gel content were calculated according to equations (1) and (2):

$$\text{Gel content} = \frac{m_1}{m_0} \times 100\% \quad (1)$$

$$\text{Water Uptake} = \frac{m_s - m_1}{m_1} \times 100\% \quad (2)$$

*2.5.2 Tensile properties.* Stress-strain measurements were performed according to ASTM D638 using a Zwick Roell tensile tester. Dumbbell-shaped specimens (50 x 9 mm) in the wet state were elongated at a speed of 10 mm/min at RT. Starting from the initial position (30 mm grip-to-grip separation), the stress and elongation of three samples for each gel were measured to obtain values for the tensile modulus ($E_{mod}$) and elongation at break ($\varepsilon_{max}$). Toughness ($W_{Tensile}$) was used as a parameter for the resistance to fracture of a hydrogel under stress, and determined by integrating the stress–strain curve (area under the curve). A tough hydrogel is characterized by a balance between strength and elongation [20].

2.6 Cell culture

Circular specimens (n=6) of single polymer hydrogels and hybrid hydrogels with a diameter of 12 mm and a thickness of 1 mm were placed in a 48 well cell culture plate. A rubber ring with an outer diameter of 12 mm was put on top of the specimens to prevent floating. After disinfection of the hydrogels by 30 mins incubation with 70 % (v/v) ethanol in water followed by washing with DPBS, human mesenchymal stem cells (hMSCs) were seeded on the specimens at a density of 10,000 cells/cm$^2$. Subsequently, the cells were cultured at 37 ℃ in humidified air containing 5 % (v/v) $CO_2$ for a period of 10 days. Three independent experiments were performed with hMSCs at passage 5-7. The culture medium consisting of DMEM containing 10 % (v/v) FBS, 1 % (v/v) glutamax and 1 % (v/v) penicillin/streptomycin was refreshed every two days. On day 1, 6 and 10, the specimens were rinsed with DPBS to remove culture medium and non-adhering cells. Next, the adhering cells were lyzed by 30 mins incubation with CyQuant lysing buffer after which the culture plate was stored at -25 ℃. Finally, the number of adhering cells was quantified by adding 20 µl lysate to 180 µl CyQuant GR dye solution diluted 1:400 in lysing buffer, and measuring the fluorescence at an excitation wavelength of 480 nm and an emission wavelength of 520 nm using a Tecan Saphire fluorometer.

Live/Dead staining was performed 24 hours after cell seeding. The specimens were rinsed with warm DPBS (37 ℃), and incubated with 2 µM calcein-AM/4 µM ethidium homodimer-1 solution for 1 hour at 37 ℃. After rinsing with warm DPBS, pictures were taken using an EVOS FL Cell Imaging System. Calcein-AM is retained

within living cells, producing an intense uniform green fluorescence upon hydrolysis by esterases. Ethidium homodimer-1 enters cells with damaged membranes and undergoes a 40-fold enhancement of fluorescence upon binding to nucleic acids, thereby producing a bright red fluorescence in dead cells.

## 3. Results and Discussion

### 3.1 Effects of macromer degree of functionalization (DF) on network properties

Linear PEG has only two functional groups at the end of the polymer chain, so the end groups should be fully functionalized with methacrylate groups to incorporate all the PEG chains in the network. The DF of PEG-4K ($M_w$ = 4 kg/mol) used in this study was 98 % as determined by NMR. The gel content of PEG hydrogels formed at 20 % (w/v) polymer concentration was 95 %, demonstrating a good conversion of the PEG macromers into networks. PEG with a $M_w$ of 10 kg/mol and a DF of 95 % was also tested. As hydrogels made at 20 % (w/v) polymer concentration had a gel content of only 71 %, because of a lower amount of methacrylate groups per volume, PEG-4K was chosen for further studies.

Functionalization of gelatin with methacrylate groups takes place at the primary amines of the amino acid side chains. The amount of available amine groups in gelatin type A is 0.0385 mol/100g [21]. In the present study, gelatin type A with a $M_w$ of 50-100 kg/mol was used. Gelatin type A with a $M_w$ of 25-50 kg/mol was also tested, but at a similar DF and concentration, no differences in gel contents and mechanical properties of the crosslinked hydrogels were found.

Four different GelMA preparations with a DF of 22, 42, 62 and 90 % were prepared (GelMA-22, GelMA-42, GelMA-62 and GelMA-90), with corresponding numbers of methacrylate groups per gelatin chain of 6, 13, 18 and 25. Photo-crosslinking at 20 % (w/v) polymer concentration resulted in gelatin hydrogels with different properties, as shown in Table 1. With increasing DF, the water uptake of the hydrogels decreased due to increasing crosslink density. The hydrogel made using GelMA-22 had the lowest gel content probably due to the low DF. The hydrogel made using GelMA-90 showed the highest E-modulus and the lowest elongation at brake. This can be explained by the high crosslink density of this hydrogel. Toughness is a parameter determined by strength as well as extensibility, and was the highest for the GelMA-42 hydrogel (4.80 N/mm$^2$). In view of the advantages of the use of tough biomaterials in biomedical applications, GelMA-42 was chosen for further experiments.

**Table 1** Properties of GelMA photo-crosslinked networks prepared with increasing GelMA DF. Mean and standard deviation are shown for 3 specimens.

| Network component | DF (%) | Gel content % | Water uptake % | $E_{mod}$ MPa | $\varepsilon_{brake}$ % | $W_{Tensile}$ N/mm$^2$ |
|---|---|---|---|---|---|---|
| **GelMA-22** | 22 | 78 (15) | 671 (57) | 0.039 (0.002) | 98 (5) | 3.31 (0.59) |
| **GelMA-42** | 42 | 92 (1) | 423 (12) | 0.118 (0.010) | 86 (7) | 4.80 (0.74) |
| **GelMA-62** | 62 | 88 (3) | 404 (10) | 0.140 (0.006) | 58 (6) | 3.15 (0.66) |
| **GelMA-90** | 90 | 89 (1) | 345 (60) | 0.148 (0.002) | 26 (5) | 0.80 (0.25) |

3.2 Effects of macromer concentration on network properties

The macromer concentration in solution during crosslinking will have an effect on the process of network formation and on the characteristics of the formed network. With increasing macromer concentration, the crosslink density of the network will increase whereas the extensibility will decrease [2, 22]. As shown in Tables 2 and 3, photo-crosslinking of PEG-dMA and GelMA at concentrations from 10 - 50 % (w/v) resulted in the formation of hydrogels, with gel contents ranging from 88 - 98 % demonstrating a good conversion of the macromers into networks. As expected, with increasing macromer concentration, and thus increasing crosslink density, the water uptake and the extensibility of the hydrogels decreased whereas the tensile modulus increased. The networks prepared from 10 % PEG-dMA and 10 % and 15 % GelMA were very fragile, resulting in relatively low values for the elongation at break. As the gelatin hydrogels prepared from 20 % GelMA showed the highest toughness, this concentration was chosen for further experiments. Although the PEG hydrogels prepared from 50 % PEG-dMA showed the highest toughness, their extensibility was relatively low. Therefore, also in the case of PEG-dMA a concentration of 20 % was chosen.

**Table 2** Properties of PEG-dMA photocrosslinked networks prepared with increasing PEG-dMA concentration. Mean and standard deviation are shown for 3 specimens.

| PEG-dMA Hydrogel | Gel content % | Water uptake % | $E_{mod}$ MPa | $\varepsilon_{brake}$ % | $W_{Tensile}$ N/mm$^2$ |
|---|---|---|---|---|---|
| **10 %** | 88 (1.4) | 947 (37) | 0.085 (0.002) | 58 (5) | 2.33 (0.51) |
| **15 %** | 92 (0.3) | 658 (22) | 0.147 (0.023) | 81 (7) | 4.05 (0.27) |
| **20 %** | 95 (0.9) | 598 (16) | 0.207 (0.002) | 73 (6) | 5.50 (0.34) |
| **30 %** | 93 (1.1) | 453 (9) | 0.380 (0.016) | 61 (5) | 5.00 (1.17) |
| **50 %** | 97 (1.8) | 356 (12) | 0.670 (0.009) | 50 (6) | 8.50 (0.60) |

**Table 3** Properties of GelMA-42 photocrosslinked networks prepared with increasing GelMA concentration. Mean and standard deviation are shown for 3 specimens. a: hydrogel too weak to put on tensile test machine

| GelMA Hydrogel | Gel content % | Water uptake % | $E_{mod}$ MPa | $\varepsilon_{brake}$ % | $W_{Tensile}$ N/mm$^2$ |
|---|---|---|---|---|---|
| **10 %** | 90 (2.4) | 683 (34) | -[a] | - | - |
| **15 %** | 89 (2.3) | 482 (21) | 0.065 (0.006) | 66 (12) | 1.69 (0.26) |
| **20 %** | 92 (0.8) | 423 (12) | 0.118 (0.010) | 86 (7) | 4.80 (0.32) |
| **30 %** | 98 (2.3) | 286 (10) | 0.256 (0.010) | 58 (5) | 3.47 (1.56) |
| **50 %** | 98 (0.9) | 224 (7) | 0.487 (0.026) | 42 (4) | 3.71 (0.20) |

3.3 Preparation and characterization of hybrid hydrogels

*3.3.1 Hydrogels formed by photo-crosslinking in water.* Three different hybrid hydrogels were fabricated by preparing mixed solutions containing 75:25, 50:50 or 25:75 % PEG-dMA:GelMA (v/v) in water. After mixing two macromer solutions, the solution became turbid resulting in opaque hydrogels. In contrast, the single polymer hydrogels consisting of only PEG or gelatin were transparent, see Figure 1a. The turbidity of the mixed macromer solutions is probably caused by aqueous two phase separation (ATPS) of PEG and gelatin (see below).

The tensile modulus and toughness of single macromer and mixed macromer networks prepared in water are shown in Figure 1b. The tensile moduli of the hybrid hydrogels were higher than those of the single polymer

hydrogels. The 25:75 % PEG-dMA:GelMA hydrogel showed the highest tensile modulus. In contrast to the E-moduli, the hybrid hydrogels had a similar toughness as compared to the single polymer hydrogels. As shown in Figure 1c, proliferation of hMSCs was absent on networks prepared from 100 % PEG-dMA, whereas the cells proliferated well on the 25:75 % PEG-dMA:GelMA and 100 % GelMA hydrogels. The other two hybrid hydrogels showed intermediate hMSCs proliferation.

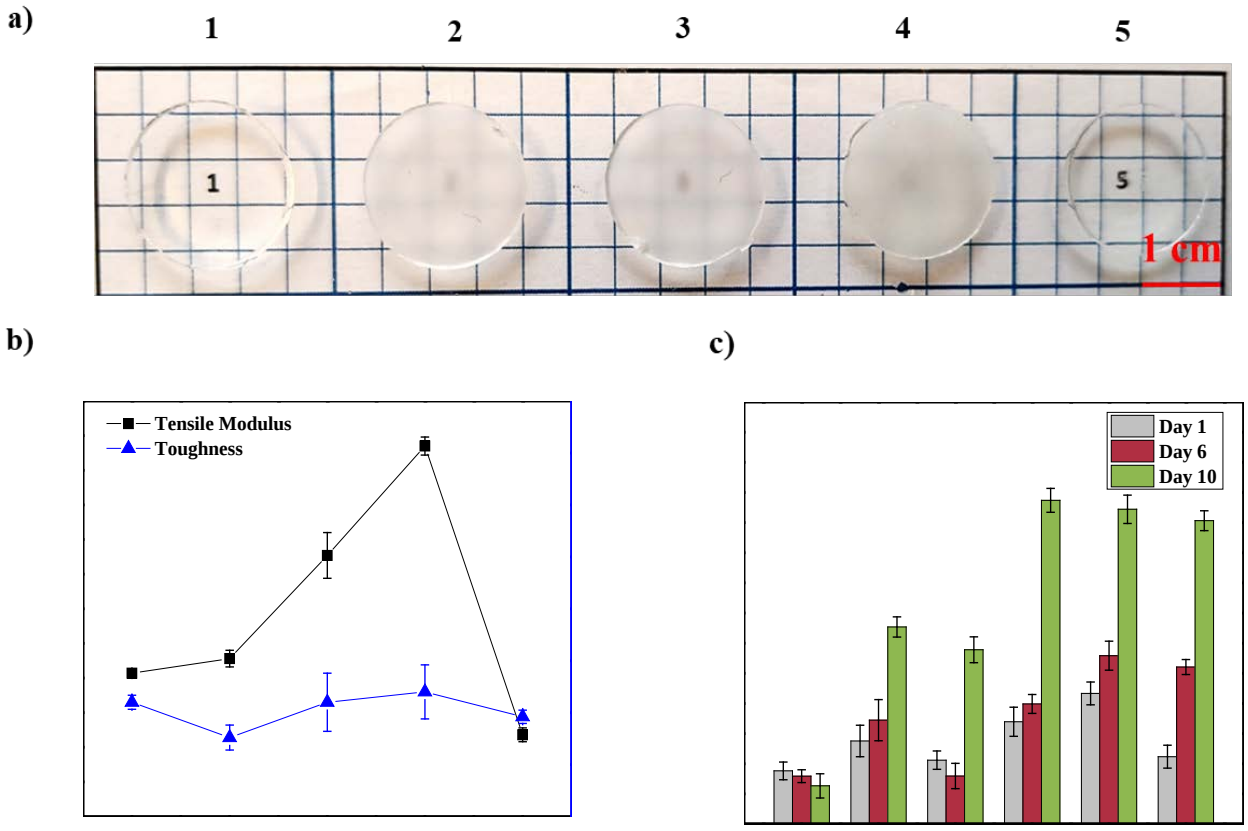


**Figure 1** a) Macroscopic appearance of networks formed in water. 1. PEG-dMA: GelMA = 100 %: 0; 2. 75 %: 25 %; 3. 50 %: 50 %; 4. 25 %: 75 %; 5. 0:100 %. b) Tensile modulus and toughness of single polymer hydrogels and hybrid hydrogels photo-crosslinked in water. c) hMSCs numbers after cell culturing for 1, 6 and 10 days on single polymer and hybrid hydrogels. Empty: well surface without hydrogel specimen, coated with 0.1% (w/v) gelatin solution in water.

*3.3.2 Hydrogels formed by photo-crosslinking in acetic acid solutions.* When aqueous solutions of two different polymers are mixed, ATPS may occur, as shown for PEG/gelatin as well as other mixtures [23-25]. ATPS has found valuable applications in food industry, fabrication of polymeric microparticles [26], and extraction and separation of biological molecules and cell subtypes [27]. Moreover, it was shown that ATPS in hydrogels had a positive effect on the morphology of embedded cells as well as new tissue formation [28]. In our study, ATPS also occurred as shown in Figure 2a for 50:50 % PEG-dMA:GelMA hydrogel photo-crosslinked in water. Droplet-like structures of around 100 µm are clearly visible. Although microphase separation improves the mechanical properties of materials, macrophase separation as shown in Figure 2a has the opposite effect [29].

ATPS can be influenced by different parameters, such as the composition of the mixture, the polymer concentrations, the temperature as well as the solubility of the polymers. As compared to water, the solubility of gelatin is higher in aqueous acid solutions, which may decrease ATPS [30, 31]. In our work, acetic acid was used to minimize phase separation of the mixed solutions. As shown in Figure 2b, macrophase separation was absent in a 50:50 % PEG-dMA:GelMA hydrogel photo-crosslinked in 0.5 % (v/v) acetic acid in water. Figure 3 shows the macroscopic appearances of the single polymer and hybrid hydrogels prepared in 0.25 % (v/v) and 0.5 % (v/v) acetic acid. As compared to the turbid hybrid hydrogels shown in Figure 1a, the 75:25 % PEG-dMA:GelMA hydrogel prepared in 0.25 % acetic acid had become transparent, demonstrating that phase separation was decreased. When the concentration of acetic acid was increased to 0.5 %, all hybrid hydrogels were transparent.

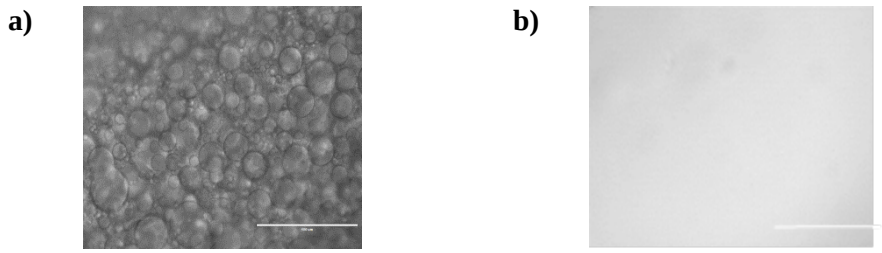


**Figure 2** Microscopic pictures of PEG-dMA: GelMA = 50 %: 50 % hybrid hydrogels. a) photo-crosslinked in water. b) photo-crosslinked in 0.5 % acetic acid. Scale bar is 400 µm.

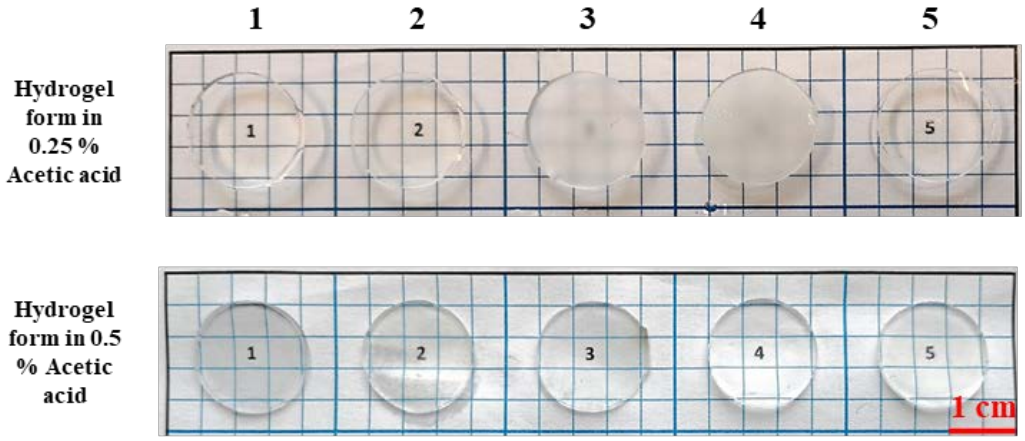


**Figure 3** Macroscopic appearance of networks formed in 0.25 % and 0.5 % acetic acid. 1. PEG-dMA: GelMA = 100 %: 0; 2. 75 %: 25 %; 3. 50 %: 50 %; 4. 25 %: 75 %; 5. 0:100 %.

Tensile properties of hydrogels made in acetic acid solutions are shown in Figure 4. The tensile moduli of gelatin hydrogels formed in 0.25 % and 0.5 % acetic acid were 0.226 MPa and 0.200 MPa, respectively, whereas the tensile modulus of the gelatin hydrogel formed in water was 0.118 MPa (shown in Figure 1b). In contrast to the tensile moduli, the maximum elongations of gelatin hydrogels were lower when crosslinked in 0.25 % and 0.5 %

acetic acid as compared to water (55 %, 69 % and 89 %, respectively), see Table 4. This can be explained by the better solubility of gelatin in acetic acid solutions [31]. As compared to water, in aqueous acetic acid the entanglement of gelatin peptide chains in the network increases and the length of peptide chains between crosslinks decreases due to the increased solubility of gelatin. This resulted in gelatin hydrogels with a lower extensibility and a higher stiffness. Unlike gelatin, the mechanical properties of PEG hydrogels made in acetic acid solutions did not substantially differ from those of the hydrogel prepared in water (shown in Figures 1b and 4, and Table 4). The values for the tensile modulus of the hybrid hydrogels prepared in acetic acid solutions showed the same trend as those for the hybrid hydrogels made in water, see Figures 1b and 4. In all solvents, the 25:75 % PEG-dMA:GelMA hydrogel had the highest E-modulus. Due to the high value for the elongation at break of the 50:50 % PEG-dMA:GelMA hydrogels crosslinked in 0.5 % acetic acid solution (Table 4), this composition resulted in the highest toughness. Thus, the absence of macrophase separation as well as a certain balance between stiffness and extensibility result in good mechanical properties, which is in agreement with literature [20] [29].

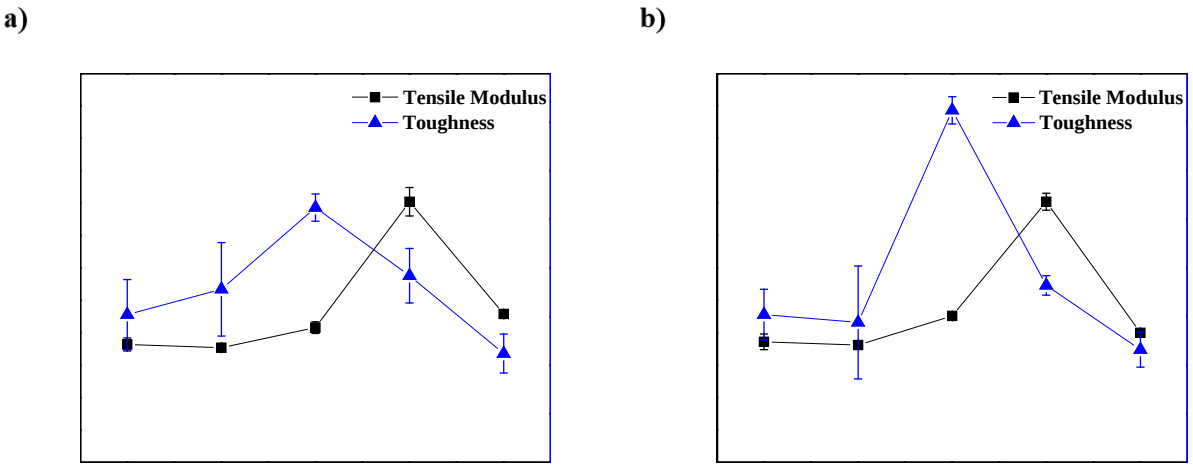


**Figure 4** Tensile modulus and toughness of single polymer hydrogels and hybrid hydrogels photo-crosslinked in a) 0.25 % acetic acid and b) 0.5 % acetic acid.

**Table 4** Maximum elongation of single polymer hydrogels and hybrid hydrogels photo-crosslinked in 0.25 % acetic acid and 0.5 % acetic acid.

| Network component PEG-dMA: GelMA | Maximum elongation % | | |
|---|---|---|---|
| | In water | In 0.25 % Acetic acid | In 0.5 % Acetic acid |
| **100 %: 0** | 73 (2) | 78 (10) | 82 (6) |
| **75 %: 25 %** | 45 (4) | 92 (11) | 79 (8) |
| **50 %: 50 %** | 43 (5) | 80 (6) | 98 (3) |
| **25 %: 75 %** | 41 (5) | 54 (6) | 60 (1) |
| **0: 100 %** | 86 (7) | 55 (6) | 69 (5) |

The hybrid hydrogels prepared in acetic acid solutions supported the proliferation of hMSCs to a greater extent than the hybrid hydrogels made in water, see Figures 1c and 5. All hybrid hydrogels prepared in 0.5 % acetic acid showed the same cell proliferation as the 100 % gelatin hydrogel. This can be explained by the better mixing of PEG-dMA and GelMA in aqueous acetic acid, as discussed above, resulting in a more homogeneous distribution of gelatin in the hybrid networks and thus a better cell proliferation. After 24 hours of culturing, hardly any dead cells were observed on all surfaces, as shown in Figure 6. All hybrid hydrogels prepared in 0.5 % acetic acid showed similar cell attachment as on 100 % gelatin hydrogel, whereas cell attachment on the hybrid hydrogels prepared in water and 0.25 % acetic acid was lower. Hardly any cell attachment was observed on the 100 % PEG hydrogels. These results are in line with the other results discussed above.

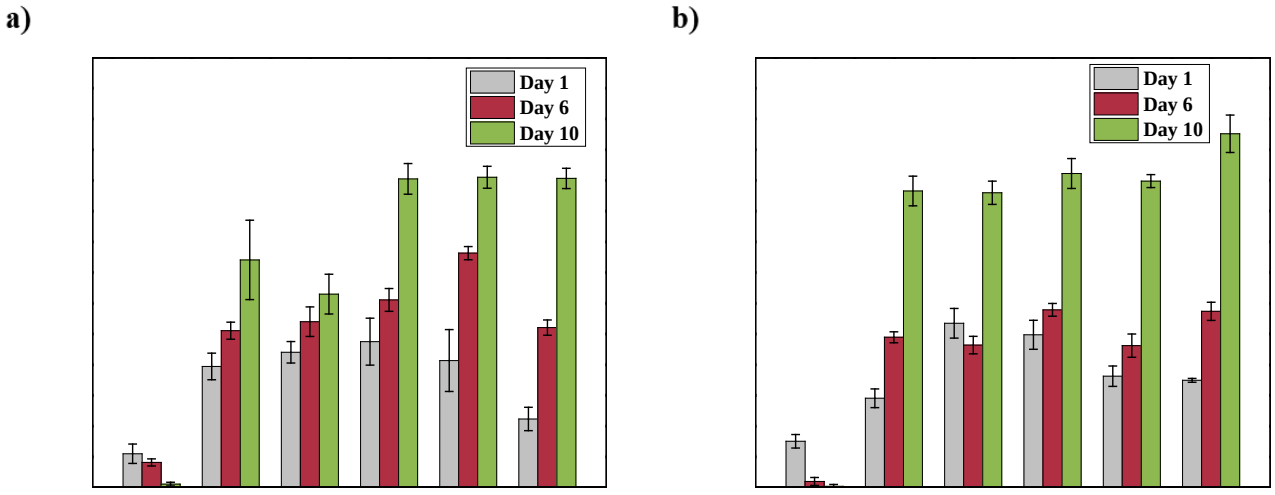


**Figure 5** Numbers of adherent hMSCs on hydrogels after culturing for 1, 6 and 10 days, a) hydrogels prepared in 0.25 % acetic acid and b) hydrogels prepared in 0.5 % acetic acid.

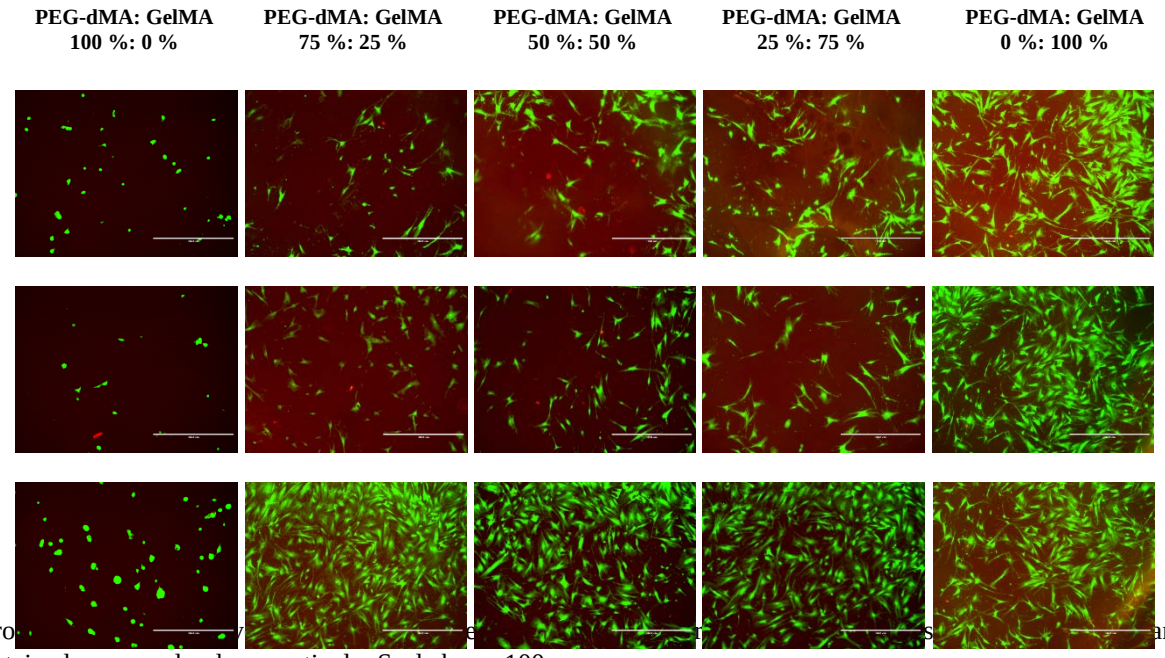


**Figure 6** Micro[illegible] and dead cells are stained green and red, respectively. Scale bar = 100 µm.

## 4. Conclusions

In this study, we prepared and characterized photo-crosslinked single polymer hydrogels based on different concentrations of PEG-dMA or GelMA. Higher macromer concentrations influenced the physical characteristics of the hydrogels in terms of increased tensile modulus, reduced water uptake and decreased elongation at break. Macrophase separation of hybrid hydrogels could be reduced by photo-crosslinking of PEG-dMA and GelMA mixtures in aqueous acetic acid solutions. Hybrid hydrogels prepared from 50:50 % PEG-dMA:GelMA mixtures in 0.5 % acetic acid had a higher toughness than single polymer hydrogels made of PEG-dMA or GelMA. Hybrid hydrogels of various compositions crosslinked in 0.5 % acetic acid supported the proliferation of hMSCs to the same extent as 100 % gelatin hydrogel, whereas the cells did not proliferate on 100 % PEG hydrogel. Our results show that both the cell adhesive and mechanical properties of a photo-crosslinked PEG network can be improved by incorporation of gelatin in the network.

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