Published in final edited form as:

J Biomed Mater Res A. 2010 May ; 93(2): 558–566. doi:10.1002/jbm.a.32563.

Mechanical and Cell Viability Properties of Crosslinked Low and High Molecular Weight Poly(ethylene glycol) Diacrylate Blends

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Abstract

There is a strong need for tissue engineering scaffolds that are mechanically robust, exhibit good biocompatibility, and can be made from readily available materials. To this end, blends of commercially available poly(ethylene glycol) diacrylate (PEGDA) with molecular weights of 400 and 3400 were UV-crosslinked at total polymer concentrations that varied systematically from 20 to 40 wt %. The compressive strength and cell viability were determined for each PEGDA mixture. The compressive modulus of the blends was maximized when the wt % ratio PEGDA3400/400 was about 40/60, with the compressive strength reaching 1.7 MPa. Cell viability results with a LIVE/DEAD fluorescence assay show an average viability of ~ 80% at a total PEGDA concentration of 20 wt %, for all blends. Increasing the total polymer concentration increased the compressive modulus of a polymer, but adversely affected cell viability for all the PEGDA blend compositions. The blend composition affected the mechanical behavior of the discs, where a higher degree of crosslinking was achieved by increasing the concentration of shorter chained PEGDA400, whereas elasticity was gained by incorporating longer chained PEGDA3400 into the blends. These results can be exploited for use in tissue engineering applications, where a mechanically robust scaffold is advantageous.

Keywords

Cell encapsulation; Hydrogel; Mechanical properties; Photopolymerization; Tissue Engineering

1. Introduction

Poly(ethylene glycol) diacrylate (PEGDA) has been widely used in many biomedical applications due to its cytocompatibility, non-toxicity, and ease of use [1–5]. Some of these applications include hydrogel development for drug delivery, tissue engineering, wound healing, and electrospinning processes for bioapplications [6–9]. Hydrogels are constructed from water soluble polymers or monomers (such as PEGDA or hydroxyethyl methacrylate), that are crosslinked into insoluble three-dimensional polymer networks that are comprised mostly of water and resemble human tissue [10]. Water and nutrients can penetrate into the hydrogel, providing the necessary means to support cellular growth, and ultimately tissue development. It is the particular use of low and high molecular weight crosslinked PEGDA blends as a biocompatible cellular matrix that is of interest in this paper [11–13].

Beyond biocompatibility, the mechanical attributes of a polymeric matrix are equally important and should be designed to address the requirements of the tissue being replicated. The compressive modulus and other mechanical attributes of crosslinked hydrogel materials

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can vary greatly [14,15]. In choosing materials that have desirable, intrinsic mechanical properties, one can alter the processing conditions during polymerization or combine various polymers to achieve a final biomaterial with desired mechanical characteristics [16–18]. While work has been done on the mechanical strength of PEGDA hydrogels or PEGDA blends, there has been little or no work done on the compressive strength and cell viability associated with low and high molecular weight PEGDA hydrogel blends.

In this research, our goal was to control mechanical properties and cell viability of PEGDA hydrogels. This was achieved by photocrosslinking blends of commercially available low and high molecular weight PEGDA polymers. By changing the ratio of the low and high molecular weight components in a polymer blend, we can control the mechanical properties of the polymer matrix. To determine whether the total polymer concentration as well as the ratio of the different polymers in the blend has an adverse affect on cell viability, cells of human epithelial origin (MD-MBA-231) were encapsulated as analog cells in these PEGDA blends and their viability was determined at fixed total polymer concentrations. The results provide the basis for future work involving the use of human mesenchymal stem cells for the development of cartilage tissue, and highlight the use of low and high molecular weight PEGDA blends for tissue engineering applications.

2. Materials and Methods

2.1 Materials

Low molecular weight PEGDA (MW 400) was purchased from Polysciences Inc. (Warrington, PA), and a higher MW PEGDA (MW 3400) was purchased from Glycosan Biosystems (Salt Lake City, Utah). The two PEGDA components henceforth will be referred to as PEGDA400 and PEGDA3400. The PEGDA polymers were used as received. Irgacure[®] 2959 (CIBA, Basel, Switzerland) was purchased as the photoinitiator. The aqueous component of the hydrogel precursor solution (prior to polymerization) was comprised of a 1X PBS solution (GIBCO[®] Dulbecco's Phosphate Buffered Saline, Sigma-Aldrich, St. Louis, MO).

MDA-MB-231 human breast cancer cells were cultured in a 10 vol % fetal bovine serum comprised of, HyQ[®] DMEM/High Glucose powder (Logan, Utah), penicillin/streptomycin, and sodium bicarbonate cell growth media in BD Biosciences[®] T-75 (Sparks, MD) flasks at 37 °C in a 5% CO₂ in air environment. Cell viability was determined by using a LIVE/DEAD fluorescence bioassay purchased from Molecular Probes[®] (Carlsbad, CA).

2.2 Crosslinking

The purpose of the experiments was to assess cell viability in, and the mechanical properties of, crosslinked discs of low and high molecular weight PEGDA blends. Polymer discs were prepared by photocrosslinking PEGDA400, PEGDA3400, or blends of the two. The PEGDA polymer(s) were dissolved in a 1X PBS solution and then agitated in an ultrasonic bath (Fisher Scientific, Model B2200R-1, Pittsburgh, PA) for 15 min. Once the PEGDA polymer was completely dissolved, a photoinitiator solution composed of Irgacure[®] 2959 dissolved in 1X PBS at a concentration of 0.6 wt %, was added to the PEGDA/PBS solution. For every precursor solution, the final concentration of the photoinitiator was 0.05 wt %, to minimize any adverse affects on cell viability [19]. The solution was then mixed again using the ultrasonic bath, and the pH was adjusted to 7.4.

The polymer discs used in the mechanical testing were crosslinked in a 35 mm diameter petrie dish, on a level surface, using a 365-nm Blak-Ray[®] long-wave ultraviolet lamp (Cole Parmer, Vernon Hills, IL) for 15–20 min. The intensity of the UV light was approximately 15 W/cm² as measured by a Cole-Parmer Series 9811 Radiometer (Vernon Hills, IL). The

cylindrical shaped samples used in compression tests were obtained by using a 10 mm Acu-Punch® (Ft. Lauderdale, FL) biopsy punch, to cut out the discs from the petri dish. The discs were then placed in a 1X PBS solution overnight to achieve full hydration and then the thickness of each disc was measured using a Chicago® digital micrometer (Fremont, CA). The average thickness of all the discs was 2.82 ± 0.35 mm.

2.3 Compressive Testing

Once the thickness of the discs was measured, the compressive modulus of each disc was determined by placing it in a Rheometrics Solids Analyzer II compression device (Piscataway, NJ). Each disc was compressed for 3 s at a strain rate of 0.5 s^{-1} . These conditions insured that the discs were not permanently deformed and limited water loss from one successive test to the next. A stress-strain curve was then recorded by a computer data acquisition system, and the modulus of the sample was calculated via a linear fit in Excel®, based on Young's Equation [20]. Compression tests were repeated four times per disc, so that an average compressive modulus could be ascertained for each composition.

2.4 Viability Assessment of Crosslinked Discs

Once the compression tests were completed, polymer solutions of similar compositions were created for determining initial cell viability. Cells were separated by centrifugation from the growth media and added at a concentration of 2×10^6 cells per ml of polymer solution to a sterilized, filtered ($0.45 \mu\text{m}$ filter) PEGDA solution at a pH of 7.4. The cells were resuspended in the PEGDA solution by pipetting the cell/PEGDA solution several times. 130 μL of the cell/PEGDA solution was then added to sterilized molds created from the detached caps of 1.5 ml polypropylene centrifuge tubes (Fisher Scientific, Pittsburgh, PA) and subjected to 10 min of long-wave UV light (365 nm) at 15 W/cm^2 . The discs had a final thickness of approximately 2 mm and a diameter of 9 mm, the dimensions of the mold. After crosslinking, the newly formed discs were removed from the mold and added to the wells of a 48 multi-well plate (BD Biosciences, Sparks, MD) and cultured in cell culture media (250 μL).

Cell viability was determined by using a LIVE/DEAD (Molecular Probes®, Carlsbad CA) fluorescence bioassay consisting of calcein AM to track living cells, and ethidium homodimer-1 to track dead cells. The time between encapsulation and addition of the dye was about 30 min. A small amount (0.5 μL) of each dye at a concentration of 1 mg/ml was added to each well containing the photoencapsulated cells. The multiwell plate containing the constructs was placed in a cell incubator for 30 min at 37°C and a CO_2 level of 5%. After incubation, the discs were examined using an Olympus® Model IX71S1F fluorescence microscope (Center Valley, PA) at a magnification of $10\times$. Green and red fluorescent images were captured using ImagePro® software (Media Cybernetics, Inc., Bethesda, MD). Adobe Photoshop CS3® (San Jose, CA) was used to count the number of green and red illuminated cells to determine the average cell viability percentage for a given polymer composition. At least five samples were analyzed for each PEGDA blend composition.

2.5 Statistical Methods

To quantitatively assess differences in the compressive strength and cell viability amongst the blends, ANOVA and Tukey tests were performed using Origin Lab software (OriginLab Corporation, Northampton, MA) [21].

3. Results

3.1 General Observation of Blends

Six different PEGDA blends of varying wt % ratios of PEGDA3400 to PEGDA400 (0/100, 20/80, 40/60, 60/40, 80/20, 100/0) at total polymer concentrations of 20 wt % and 40 wt % were used to create the crosslinked constructs needed for the compression tests. Digital camera images of the discs at a total polymer concentration of 20 and 40 wt % are shown in Figures 1 and 2, respectively. The discs created from pure PEGDA400 (Fig 1-a and 2-a) were opaque white. As the amount of PEGDA3400 was increased in the blend, the discs became more transparent. In the 20 wt % samples, the discs were transparent at a 40/60 blend (Fig. 1-c.), whereas the discs were not fully transparent until the amount of PEGDA3400 in the 40 wt % blends reached 80 wt % (80/20 blend, Fig 2-e).

3.2 Compressive Testing

Figure 3 illustrates a characteristic, single stress-strain measurement for a blend comprised of PEGDA3400, at total polymer concentrations of 20 wt % and 40 wt %. For a given polymer blend, increasing the total polymer concentration from 20 to 40 wt % resulted in a marked increase in the compressive modulus (from 94 to 711 kPa), as highlighted in Figure 3. For the entire blend population, the percent strain (with standard deviation, SD) induced on the 20 wt % samples was $10.95 \pm 9.2\%$ and $8.63 \pm 11.1\%$ for the 40 wt % samples. Figure 4 shows an example of typical behavior exhibited by all of the PEGDA blends where little energy loss is observed over four successive tests comprising a compression cycle.

When the overall polymer concentration was held constant, it was also possible to increase the compressive modulus of a crosslinked disc by altering the ratio of the low and high molecular weight PEGDA in the polymer precursor solution. Figure 5 shows the compressive modulus of crosslinked PEGDA blends at total polymer concentrations of 20 and 40 wt %. As the wt % of PEGDA 3400 in the blend increased, a maximum average compressive modulus was achieved, at ~ 40/60 wt % PEGDA3400/400. The modulus at this composition (with standard error, SE) was 0.42 ± 0.02 MPa at 20 total wt % and 1.71 ± 0.13 MPa at 40 total wt %.

ANOVA analyses were performed on the data sets presented in Figure 5. It was found that the average compressive moduli of each population were significantly different ($p < 0.05$) from each other. Tukey tests were also carried out on individual blends. At 20 wt % total PEGDA concentration, the pure PEGDA3400 blend (100/0) was significantly different, when compared to all other blends, having the lowest modulus (with SE) of 0.13 ± 0.009 MPa. The 80/20, PEGDA3400/400 blend was statistically different than all other blends, except the pure PEGDA400 (0/100) blend, which is evident in the trend of the 20 wt % curve in Figure 5. The pure PEGDA3400 blend (100/0) at 40 wt % loading had the lowest compressive modulus (with SE) of the population at 0.52 ± 0.06 MPa and was statistically equivalent with the all blends except for the 20/80 and 40/60 samples. Similarly, the pure PEGDA400 blend was statistically equivalent to all blends except for 20/80 and 40/60, which had the highest compressive (with SE) moduli of the group at 1.60 ± 0.19 and 1.71 ± 0.13 MPa, respectively. The 20/80 and 40/60 blends were considered statistically equivalent. This trend was similar to that observed in the 20 wt % population.

Figure 6 extrapolates the compressive strength of the blends as a function of the blend composition and the total polymer concentration over the entire range of 20 to 40 wt %. As the total polymer content increases, the exact blend composition becomes more critical for producing the strongest hydrogel. At a total polymer concentration of 20 wt %, the maximum compressive modulus is achieved when the PEGDA3400 content is between 20

and 60 wt %. However, when the total polymer content has reached 40 wt %, the range of the PEGDA3400 producing the strongest material has been reduced to ~35 to 40 wt %.

3.2 Cell Viability Assessment

The photoencapsulated cells were analyzed for cell viability using LIVE/DEAD® staining. The average initial cell viability (with SD) for all blends encapsulated at 20 wt % total polymer concentration was $79 \pm 7.9\%$ and was $36 \pm 7.0\%$ when the total polymer concentration was increased to 40 wt %. The viability results of each PEGDA composition are presented in Figure 7. Two control experiments were also performed where the bioassay was applied to cells suspended in HyQ® cell growth media with and without exposure to 10 min of UV light (Noted respectively as MEDIA and MEDIA-UV on the x-axis in Fig. 7) to determine any ill effects associated with the UV treatment. ANOVA analyses were conducted for the 20 and 40 wt % data sets. The means of the blends within the two populations were significantly different from each other ($p < 0.05$).

Tukey tests were applied to compare the means within the 20 and 40 wt % populations and with the MEDIA and MEDIA-UV controls samples. The pure PEGDA3400 (100/0) at 20 wt % had an average viability (with SE) of $84 \pm 4.1\%$ and was statistically equivalent to all other samples. The control samples MEDIA and MEDIA-UV were also statistically equivalent with average viabilities (with SE) of $97 \pm 1.0\%$ and $97 \pm 1.4\%$, respectively, indicating that UV light itself had little effect on cell viability. None of the polymer samples were statistically equivalent to the control samples. The pure PEGDA400 (0/100) discs at 20 wt %, had the highest average viability (with SE) of all the polymer blends at $88 \pm 1.9\%$, which was equivalent to all samples except for the 60/40 and 80/20 blends. The 60/40 and 80/20 blends were statistically different than the control samples and had the lowest viability (with SE) at $70 \pm 4.6\%$ and $72 \pm 4.9\%$, respectively.

Tukey tests were also applied to the 40 wt % data. In this case, none of the blends were equivalent to the media controls. This indicated that increasing the total polymer concentration from 20 to 40 wt % had a significant negative impact on the overall cell viability. The pure PEGDA3400 (100/0) blend had the highest viability (with SE) at $60 \pm 4.7\%$ and was statistically different than all other polymer blends. The pure PEGDA400 (0/100) had an average viability (with SE) of $35 \pm 5.2\%$ and was statistically equivalent to all blends except the 100/0 blend. The 20/80, 40/60, and 60/40 blends were statistically identical and had the lowest average viability (with SE) amongst the 40 wt % data at $20 \pm 2.0\%$, $28 \pm 4.0\%$ and $31 \pm 4.1\%$, respectively. Taking into account the statistical differences of the 20/80 and 80/20 blends, it appears that the addition of PEGDA3400 at a loading up of to 60 wt % did not dramatically increase cell viability. However, viability did improve when the amount of PEGDA3400 was raised to 80%, with a further increase in cell viability when the entire matrix was composed of only PEGDA3400.

3.3 Mechanical Testing and Cell Viability of 20:80 PEGDA3400/400 Blend

Results from Figures 5 and 7 showed that the 20/80 wt %, PEGDA3400/400 blend exhibited strong mechanical properties and good cell viability when the total polymer concentration was 20 wt %. Consequently, additional PEGDA constructs of this particular composition (20/80 wt %) were created at total polymer concentrations of 25, 30, and 35 wt %. The cell viability data and compressive mechanical strength data were obtained and the results are shown in Figure 8 as a function of the total PEGDA concentration.

The results indicate a strong dependence of cell viability and compressive modulus on the total polymer content of the PEGDA matrix. The average compressive modulus (with SE) increased from 0.4 ± 0.02 to 1.6 ± 0.2 MPa, when the total polymer concentration of the

precursor solution was increased from 20 to 40 wt %. The average cell viability (with SE) decreased from $81 \pm 2.1\%$ to $20 \pm 2.0\%$ when the total polymer concentration increased from 20 to 40 wt %. Consequently, cell viability was sacrificed for compressive strength. The preceding results were verified by ANOVA analysis which indicated that the mean of each population was significantly different from one another ($p < 0.05$).

4. Discussion

Crosslinked discs, composed of blends of low and high molecular weight PEGDA, were tested for both compressive strength and cell viability at total polymer concentrations of 20 and 40 wt %. It was found that the mechanical strength increased by a factor of 3 to 4, when the overall polymer concentration was increased from 20 to 40 wt %, however cell viability was sacrificed as the polymer loading increased. It is possible to control the mechanical properties of the discs, by varying the ratio of PEGDA3400 and 400 in the blends. The 20/80, PEGDA3400/400 blend provided a balance of mechanical strength and cell viability at total polymer loadings of 20–40 wt %.

4.1 Mechanical Strength Data

Increasing the total polymer concentration of the samples increased the crosslink density of the polymer matrix due to a larger number of reactive diacrylate groups, which in turn, increased the compressive modulus of experimental samples, as shown in Figures 3 and 5. There is a thermodynamic relationship between the modulus and the crosslink density of a given polymer, where the modulus is directly proportional to changes in the crosslink density [22]. The values of the average compressive modulus of pure PEGDA400 (0/100, as shown at the far left in Figure 5) are 0.26 and 1.02 MPa at 20 and 40 wt %, respectively. There was also an increase in compressive modulus in the pure PEGDA3400 (100/0 blends, as shown at far right in Fig. 5) from 0.13 and 0.52 MPa at 20 and 40 wt %, respectively. Additionally, a higher ratio of PEGDA3400 was needed in the blend at 40 wt % total PEGDA to create a transparent construct due to the increased number of crosslinks within the polymer network at 40 wt %, relative to that at 20 wt %, as shown in Figure 2.

The arching behavior of the compressive modulus vs. composition plot of Figure 5, where the modulus peaks at roughly 40–60 wt % PEGDA3400 at 20 and 40 wt % total polymer loading are worth noting. While the number of crosslinks increased in discs that were composed of higher amounts of short-chained PEGDA400 molecules, a disc comprised solely of this shorter length material did not produce the most robust material [23]. To achieve further gains in compressive modulus, a “rubber-like” behavior is required in addition to stiffness. This requires the presence of longer polymer chains which increase chain mobility during mechanical deformation and the recovery processes [24]. The addition of the long-chain PEGDA3400 imparted such mobility and maximized the compressive modulus. Thus, the combination of long and short chained PEGDA monomers is required to achieve a high degree of crosslinking (for overall strength) and the proper degree of polymer elasticity to withstand compressive loads. The importance of cross-linking and blend composition is also apparent in Figure 6, as the ratio of PEGDA3400 to 400 becomes more critical at higher total polymer loadings. When the overall polymer concentration is increased, the cross-link density increases, and the impact of individual components of the PEGDA blend becomes more pronounced. Consequently, more attention has to be given to the specific blend composition.

When comparing the mechanical test data to results from other studies in the literature, there is a fair degree of variation in the observed compressive modulus depending on the overall polymer concentration or molecular weight of the material. For example, increasing the concentration of polyethylene glycol dimethacrylate hydrogels, designed for cartilage

scaffolding, (PEGDM, MW=3000) from 10 to 20 wt % increased the compressive modulus from 0.060 to 0.670 MPa at a strain rate of 0.2 mm/s [25]. In this case, doubling the total polymer concentration resulted in a 10 fold increase in compressive modulus. For the PEGDA blends of this study, a 4 fold increase in the compressive modulus was observed when the total polymer concentration was doubled from 20 to 40 wt %, with an increase in modulus from about 0.4 MPa to 1.7 MPa. In another instance, PEGDA hydrogels were created for vocal fold development. The crosslinked hydrogels were composed of 10, 20, and 30 wt % 10,000 MW PEGDA and had compressive moduli of 30, 90, and 110 kPa at a strain of 1 %, respectively [26]. The lower modulus values of the vocal fold hydrogels can be likely be attributed to the larger 10,000 PEGDA molecular weight they were composed of.

For comparative purposes, the 20/80 mixture of PEGDA3400/400 at a total polymer concentration of 20 wt % produced a hydrogel with a compressive modulus of ~ 400 kPa in this paper, which is on par with the 20 wt % PEGDM scaffold material, and several times larger than the 20 wt % 10,000 MW PEGDA vocal fold hydrogel. At total polymer loadings of 30 % or more, the PEGDA blends reached ~ 1 MPa, which is on par for what is needed for engineering-designed tissues such as articular cartilage [27]. The strain percentages experienced by the 20 and 40 wt % samples of ~ 10%, are also similar to the range of deformation exhibited by native cartilage after various types of activity [28]. Based on the results in this paper, a compressive modulus of about 1 MPa, with a cell viability of ~55–75 %, can be achieved using a PEGDA polymer blend, with a 3400/400 weight ratio of 20/80 at a total polymer concentration of 25–30 wt %.

4.2 Cell Viability Data

From the curves in Figure 7, it appears that the presence of PEGDA400 in the blends is detrimental to the cells when the total polymer concentration is 40 wt %. A possible reason for this is an increase in the free radical concentration from the shorter chained PEGDA400 during the crosslinking process can adversely affects the cells [19,29]. The effect is not seen in the 20 wt % PEGDA material, presumably because the total polymer concentration is sufficiently low. The composition of the cell/polymer suspension may affect the cells in other ways. A higher molecular weight species, like the PEGDA3400, will have a lower diffusion rate into the cells and thus cause less damage, as compared to PEGDA400. For example, it was found the that the permeability of polyethylene glycol (PEG) with a MW of 4000 across cell monolayers was 1.44×10^{-6} cm/s, whereas the permeability of a water soluble drug called Propranolol[®] with a MW of ~ 300 had a permeability rate of 30.2×10^{-6} cm/s, a 20 fold increase [30].

When the total polymer concentration is varied, it can change the scaffold topology and morphology, which will affect cell adhesion and viability. In our experiments, however, the cells are more likely to be affected by the chemical effects caused by increased net polymer concentration rather than by the variation of the cell-polymer interface or changes in pore size. The time between the initial viability measurements and scaffold formation was less than an hour, so the role of topology, relative to the chemical effects, on initial cell viability is expected to be minimal. While it would be advantageous to investigate the topology of the hydrogel, current methods do not allow direct mapping and even indirect characterization via dextran diffusion experiments do not yield information about the ultrastructure that exists between the cells and the polymer [26]. Additionally, the cells are part of the unpolymerized polymer precursor solution and are encapsulated as the scaffold is formed, so phenomena like decreased cell entrapment from pore size reduction should not be an issue in PEGDA. Ultimately, to optimize cell viability, our results indicate that the lowest total polymer concentration should be used to capsule the cells. In such a case, the cells would

be more likely to develop into a functional tissue and deposit extracellular matrix of their own contributing to the overall strength of the polymer.

5. Conclusions

The goal of this project was to show that mixed molecular weight PEGDA blends could produce a hydrogel system with a compressive modulus on par with human tissues like articular cartilage, and maintain acceptable levels of initial cell viability. The 20/80 blend of PEGDA3400/400 provided a balance of mechanical strength and cell viability over a total polymer concentration range of 20–40 wt %. A modulus of 1 MPa was obtained at a total polymer concentration of 25–30 wt % while maintaining a cell viability of 55–75 % for the 20/80 blend. Generally, the cell viability and mechanical strength were strongly dependent on the total polymer concentration. Cell viabilities were >80% at a total polymer concentration of 20 wt %, but the compressive modulus was only about 0.4 MPa.

In the future, it should be possible to improve cell viability by reducing the overall polymer concentration and allow cells to deposit extracellular matrix in order increase the compressive modulus of the system. This work provides an important first step in the development of mixed molecular weight PEGDA hydrogels for tissue engineering applications.

Acknowledgments

We thank Dr. Joseph Mansour, Professor of Mechanical and Aerospace Engineering of Case Western Reserve University for the compression tests. The research was supported in part by a grant from the National Institutes of Health (EB006203).

References

1. Hahn M, Miller JS, West JL. Three-dimensional biochemical and biomechanical patterning of hydrogels for guiding cellular behavior. *Adv Mater.* 2006; 18(18):2679–2685.
2. Patel P, Smith CK. Rheological and recovery properties of poly(ethylene glycol) diacrylate hydrogels and human adipose tissue. *J Biomed Mater Res A.* 2005; 73A(3):313–319. [PubMed: 15834933]
3. Arcaute K, Mann BK, Wicker RB. Stereolithography of three-dimensional bioactive poly(ethylene glycol) constructs with encapsulated cells. *Ann Biomed Eng.* 2006; 34(9):1429–1441. [PubMed: 16897421]
4. Cruise G, Scharp DS, Hubbell JA. Characterization of permeability and network structure of interfacially photopolymerized poly(ethylene glycol) diacrylate hydrogels. *Biomaterials.* 1998; 19(14):1287–1294. [PubMed: 9720892]
5. Halstenberg S, Panitch A, Rizzi S, Hall H, Hubbell J. Biologically engineered protein-graft-poly(ethylene glycol) hydrogels: A cell adhesive and plasmin-degradable biosynthetic material for tissue repair. *Biomacromolecules.* 2002; 3(4):710–723. [PubMed: 12099815]
6. Bikram M, et al. Hydrogel encapsulation of adenovirus-transduced cells expressing BMP2 for local osteoinduction. *Mol Ther.* 2005; 11L:S263–264.
7. Ghosh K, et al. Fibronectin functional domains coupled to hyaluronan to stimulate adult human dermal fibroblast responses critical for wound healing. *Tissue Eng.* 2006; 12(3):601–613. [PubMed: 16579693]
8. Ji Y, et al. Electrospun three-dimensional hyaluronic acid nanofibrous scaffolds. *Biomaterials.* 2006; 27(20):3782–3792. [PubMed: 16556462]
9. Hahn S, et al. Photolithographic patterning of polyethylene glycol hydrogels. *Biomaterials.* 2006; 27(12):2519–2524. [PubMed: 16375965]
10. Seidell J, Malonge SM. Synthesis of polyHEMA hydrogels for use as biomaterials; bulk and solution initiated polymerization techniques. *Materials Research.* 2000; 3(3):79–83.

11. Shu X, et al. Synthesis and evaluation of injectable, in situ, crosslinkable synthetic extracellular matrices (sECMs) for tissue engineering applications. *J Biomed Mater Res A*. 2006; 79A(4):901–912.
12. Nguyen KT, et al. Photopolymerizable hydrogels for tissue engineering applications. *Biomaterials*. 2002; 23(22):4307–4314. [PubMed: 12219820]
13. Antonio, X.; Gaydos, C.; Mao, JJ. In vitro degradation behavior of photopolymerized PEG hydrogels as a tissue engineering scaffold. *Proceedings of the 28th IEEE EMBS Annual International Conference*; New York: IEEE; 2006.
14. Guoxin T, Yingjun W, Guang Y. Synthesis and properties of polyethylene glycol diacrylate 2-hydroxyethyl methacrylate hydrogels. *Key Eng Mater*. 2008; 368–372:1175–1177.
15. Saltzman, W. *Tissue Engineering*. Oxford University Press; New York: 2004. p. 400
16. Valles E, et al. Equilibrium swelling and mechanical properties of hydrogels. *Polym Bull*. 2000; 44(1):109–114.
17. Butler M, Clark MH, Adams S. Swelling and mechanical behavior of biopolymer hydrogels containing chitosan and bovine serum albumin. *Biomacromolecules*. 2006; 7(11):2961–2970.
18. Barcellos O, Pires ATN, Katime I. Physical properties of hydrogels of poly(2-hydroxyethyl methacrylate) and copolymers with mono-methyl itaconate synthesized by bulk and solution polymerization. *Polym Int*. 2000; 49(8):825–830.
19. Bryant S, Nuttelman CR, Anseth KS. Cytocompatibility of UV and visible light photoinitiating systems on cultured NIH/3T3 fibroblasts in vitro. *J Biomater Sci, Polym Ed*. 2000; 11(5):439–457. [PubMed: 10896041]
20. Riley, W.; Sturges, DL.; Morris, DH. *Statics and Mechanics of Materials: An integrated approach*. John Wiley and Sons, Inc; New York: 1996. p. 91
21. Montgomery, D. *Design and Analysis of Experiments*. 6. John Wiley and Sons, Inc; New York: 2005. p. 94
22. Sperling, L. *Introduction to Physical Polymer Science*. 2. John Wiley and Sons, Inc; New York: 1992. p. 382–401.
23. Schmedlen, R.; Nyugen, KT.; West, JL. Tissue engineered vascular grafts: Elastic polyethylene glycol scaffolds for culture under pulsatile flow conditions. *Proceedings of the Second Joint EMBS/BMES Conference*; Houston. 2002.
24. Flory, P. *Principles of Polymer Chemistry*. Cornell University Press; London: 1969. p. 433
25. Bryant SJ, et al. Crosslinking density influences the morphology of chondrocytes photoencapsulated in PEG hydrogels during the application of compressive strain. *J Orthop Res*. 2004; 22:1143–1149. [PubMed: 15304291]
26. Huimin L, Munoz-Pinto D, et al. Influence of hydrogel mechanical properties and mesh size on vocal fold fibroblast extracellular matrix production and phenotype. *Acta Biomater*. 2008; 4:1161–1171. [PubMed: 18515199]
27. Saltzman, W. *Tissue Engineering*. Oxford University Press; Oxford: 2004. p. 348–349.
28. Eckstein F, Lemberger B, et al. In vivo cartilage deformation after different types of activity and its dependence on physical training status. *Ann Rheum Dis*. 2005; 64(2):291–295.
29. Burdick J. Controlled degradation and mechanical behavior of photopolymerized hyaluronic acid networks. *Biomacromolecules*. 2006; 6(1):386–391. [PubMed: 15638543]
30. Reynolds C, Tedeschi H. Permeability properties of mammalian cell nuclei in living cells and *in vitro*. *J Cell Sci*. 1984; 70:197–207. [PubMed: 6501436]

Figure 1. Photopolymerized PEGDA discs at a total polymer concentration of 20 wt % containing blends of PEGDA3400/400 (wt % ratio): (a) 0/100, (b) 20/80, (c) 40/60, (d) 60/40, (e) 80/20, (f) 100/0. The diameter of the discs is 1 cm.

Figure 2. Photopolymerized PEGDA discs at a total polymer concentration of 40 wt % containing blends of PEGDA3400/400, (wt % ratio): (a) 0/100, (b) 20/80, (c) 40/60, (d) 60/40, (e) 80/20, (f) 100/0. The diameter of the discs is 1 cm.

Figure 3.

Typical, single stress-strain measurements for discs composed of pure PEGDA3400 (100/0) blend at 20 and 40 wt % total polymer concentration, with the slope of each line given as the compressive modulus, E .

Figure 4.

Typical curves of compressive modulus vs. successive test number in a compression cycle to determine potential energy losses during compressive loading, with standard error. Sample size was $n \geq 5$.

Figure 5. Effect of blend polymer composition on the compressive modulus of PEGDA3400/400 blends for an initial polymer loading of 20 and 40 wt % with standard error. Sample size was $n \geq 5$.

Figure 6. Continuous, 2-D contour plot detailing expected compressive modulus behavior for PEGDA blends as a function of total polymer loading and blend composition.

Figure 7. Cell viability as measured using fluorescent LIVE/DEAD assay of photopolymerized PEGDA3400/400 (wt % ratio) blends after 10 min of UV exposure at 15 W/cm^2 at a total polymer concentration of 20 and 40 wt %, with standard error. Sample size is $n \geq 5$.

Figure 8. Cell viability and compressive modulus of PEGDA3400/400 (wt % ratio), 20/80 blends, as a function of total polymer concentration, with standard error. Sample size was $n \geq 5$ for all samples.