Synthesis and Characterization of PEG Dimethacrylates and Their Hydrogels

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Facile synthesis and detailed characterization of photopolymerizable and biocompatible poly(ethylene glycol) dimethacrylates (PEGDM) and poly(ethylene glycol) urethane-dimethacrylates (PEGUDM) are described. Poly(ethylene glycol)s of various molecular masses (M_n = 1000 to 8000 g/mol) were reacted with methacrylic anhydride or with 2-isocyanatoethyl methacrylate to form PEGDMs and PEGUDMs, respectively. PEGDMs were also prepared by a microwave-assisted route to achieve fast reaction conversions under solvent free conditions. Combined analyses of ^{1}H NMR and MALDI-TOF MS confirmed the formation of prepolymers of high purity and narrow mass distribution (PD < 1.02). Aqueous solutions of the PEGDMs and PEGUDMs (10% and 20% by mass fraction) were photopolymerized to yield hydrogels. Bovine chondrocytes, seeded in the hydrogels, were used to assess the biocompatibility. Preliminary rheology and uniaxial compression measurements showed varied mechanical response, and biocompatibility studies showed that cells are completely viable in both types of hydrogels after two weeks.

Introduction

Hydrogels produced by photopolymerization have been investigated extensively as biomaterials in applications such as scaffolds for tissue engineering, drug delivery carriers, in the prevention of thrombosis, post-operative adhesion formation, and as coatings for biosensors.[1] The photopolymerization process allows the hydrogel to be generated in vitro or in vivo from a low viscosity solution of monomer, oligomer, or low molecular mass polymer (macromer) by a free radical pathway in a minimally invasive manner. The chemical cross-linking results in hydrogels that contain a high water content yet possess mechanical properties similar to those of soft tissues. Another advantage of hydrogels is their high permeability to oxygen, nutrients, and other water-soluble metabolites, making them particularly attractive as scaffolds in tissue engineering applications.

Although hydrogels have been studied as potential materials for bone, tendon, and nerve regeneration, it is cartilage tissue engineering that has shown the most promise. Chondrocytes encapsulated in hydrogels retain their native form, and over time can generate native cartilage tissue. The use of photopolymerized hydrogels as opposed to natural physical gels such as alginate also allows for the material properties to be more easily adjusted. For example, a typical approach to control the hydrogel mechanical properties is to tailor the network cross-link density. This can be achieved by adjusting the molecular mass of the macromer or by varying the mass percent of macromer in the solutions. The cross-link density in fully cross-linked networks is directly proportional to the gel modulus and inversely proportional to the swelling. These are important considerations for tissue engineering in which the former affects transport properties and the latter determines the materials functional practicality and influences cell behavior. In drug delivery applications, the pore or mesh size can be adjusted to control the drug release rate by varying the content, density, and length of the cross-linking groups.

Several types of photopolymerizable hydrogels have been investigated for use as biomaterials. These include poly(ethylene glycol) (PEG) acrylate derivatives, PEG methacrylate derivatives, poly(propylene fumarate-*co*-ethylene glycol)[2] and oligo(poly(ethylene glycol) fumarate)[3] that contains cross-linkable sites in the polymer backbone, poly(vinyl alcohol) derivatives,[4] modified polysaccharides such as those from hyaluronic acid, and dextran methacrylates. We are particularly interested in PEG dimethacrylates (PEGDM) and similar PEGDM derivatives as model systems because PEG alone is bio-inert but can be easily modified to become bioactive.[5] Cross-linking by dimethacrylates have been shown to be biocompatible with the unreacted dimethacrylates having relatively low cytotoxicity.[6,7] In addition, PEGDMs and their copolymers and derivatives have been successfully used by several groups both in vitro and in vivo as scaffold materials.[8]

There is a general consensus that the material properties and external stimulation strongly affect the cell response. The importance of PEGDM hydrogel cross-link density (controlled by PEGDM mass fraction in solution) on me-

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chanical properties and on the chondrocytes' ability to produce cartilaginous tissues has been demonstrated by Bryant and Anseth.[9] In addition, PEGDM co-photopolymerized with a degradable macromer, acrylate endcapped poly(lactic acid)-*b*-poly(ethylene glycol)-*b*-poly(lactic acid) in the presence of chondrocytes shows that biodegradable moieties in hydrogels have significant effects in tissue generation. Despite the large number of studies currently available, there is still a lack of a clear understanding of the correlation between material properties and cell response. Furthermore, after years of research, the physical properties of hydrogels are still difficult to predict by theories due to nonidealities of the gel formation. These nonidealities include conversion dependent reactivity, cyclization and multiple cross-linking, and defects and nonhomogeneous cross-linking (also known as spatial gel inhomogeneity). Well-defined model materials are necessary for the preparation of hydrogels with high reproducibility and easily adjustable properties.

We have prepared a series of controlled molecular mass (MM) PEGDMs and poly(ethylene glycol) urethane dimethacrylates (PEGUDM) of high purity and low polydispersity. PEGDMs were prepared both in solution and under solvent free conditions via a microwave-assisted route. The synthetic approaches described herein are particularly straightforward. The dimethacrylate products were characterized by proton nuclear magnetic resonance (^{1}H NMR) and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). PEGDMs and PEGUDMs of different molecular masses were photocrosslinked to form hydrogels, and preliminary cell viability studies were conducted. The material structure−property relationships and detailed cell response studies will be described in a later paper.

Experimental Section[15]

Materials. PEG (MM ≈ 1000 (1k) to 8000 g/mol (8k)), methacrylic anhydride (MA), 2-isocyanatoethyl methacrylate (IEM), ethyl ether, and triethylamine (TEA) were purchased from Sigma-Aldrich and used as received. Dichloromethane was purchased from Sigma-Aldrich and dried over activated molecular sieves (4 Å) prior to use. Photoinitiator Irgacure 2959 (I2959) was obtained from Ciba Specialty Chemicals and used as received. Primary bovine chondrocytes were cultured in growth medium composed of Dulbecco's modified Eagle medium, 10% fetal bovine serum, 1% minimum essential medium (GIBCO, Invitrogen Corp), 50 μg/mL L-ascorbic acid 2-phosphate (Sigma), and 1% antibiotics (penicillin/streptomycin) (Mediatech, Inc.). Cell viability was measured using Live/Dead Viability/Cytotoxicity Kit (L-3224) purchased from Molecular Probes Inc.

Synthesis of PEGDM and PEGUDM. PEGDM and PEGUDM were prepared from the reaction of various PEGs and MA or IEM, respectively. An example of the synthesis of a 5k PEGDM is as follows. PEG (5 g, ≈0.001 mol), 2.2 equiv of MA (0.34 g, 0.0022 mol), and TEA (0.2 mL) were reacted in ≈15 mL of dichloromethane over freshly activated molecular sieves (≈3 g) for 4 d at room temperature. The solution was filtered over alumina and precipitated into ethyl ether. The product was filtered and then dried in a vacuum oven overnight at room temperature.

Microwave-Assisted Synthesis of PEGDM. PEG (0.2 g) and a large excess of MA (up to 5-fold excess) were mixed in a capped scintillation vial and placed in a commercial domestic microwave (GE, 1100 W) for various reaction times ranging from 2 to 10 min. Once the vial was cooled to room temperature, approximately 2 mL of ethyl ether was added and the vial was gently shaken to allow the PEGDM to precipitate. For the 1k PEGDM, the vial was placed in a freezer to facilitate the precipitation process. Product was collected by filtration and dried in a vacuum oven.

Characterization PEGDM and PEGUDM. High-resolution, 270 MHz proton NMR spectra were taken on a 6.35 T JEOL GX270 spectrometer manufactured by JEOL, Ltd. (Akishima, Japan). Deuterated chloroform was used as a solvent, and the polymer concentrations were varied between 2.5% and 3.0% by mass fraction. All spectra were run at room temperature, 15 Hz sample spinning, 45° tip angle for the observation pulse, and a 10 s recycle delay, for 64 scans. The standard relative uncertainty for molecular mass calculated via ^{1}H NMR arises from the choice of baseline and is estimated to be 8%.

The MALDI matrix, dihydrobenzoic acid (DHB), and the PEGDM and PEGUDM were dissolved in 1 mL of THF. Sodium was used as the cationizing reagent in a 1:1 by volume ratio of THF solution (0.5 mg/mL solution in THF) and PEGDM or PEGUDM/DHB solution. All MALDI samples were deposited on the target by electrospray. The MALDI-TOF MS was performed on a Bruker (Billerica, MA) REFLEX II in reflectron mode using delayed extraction and low-mass (i.e., matrix-ion) blanking as previously described.[10] Each spectrum shown is the sum of 75 discrete laser shots and is shown without smoothing or background subtraction. Estimated expanded uncertainty reported for MM moments arises from the choice of baseline and laser power (5%). The estimated standard uncertainty in overall signal intensity from repeatability studies is 15%.

Preparation of Hydrogels. Photopolymerized hydrogels were prepared according to a previously described procedure.[9] PEGDM or PEGUDM (10% or 20% by mass fraction) and aqueous I2959 solution (0.05% by mass fraction) were mixed in distilled deionized water or growth medium when chondrocyte is encapsulated in the hydrogel. Cylindrical samples of 3 mm in height and 6 mm in diameter were cured with a long wavelength UV source (365 nm, 300 μW/cm^2) for 10 min to obtain hydrogels. All hydrogels maintain their structural integrity for the entire time under static culture. Bovine chondrocytes were seeded into hydrogels at a cell density of 17 000 cell/mL to 100 000 cell/mL gel. Cell viability within the cell-hydrogel scaffolds under static cultures was measured at 14 d.

Characterization of Hydrogels. FTIR was used to measure the bulk reaction kinetics. The methacrylic vinyl contents of PEGDM were analyzed by Fourier transform infrared spectroscopy. The infrared samples were films, approximately 0.3 mm thick, prepared by solvent casting a film from a solution of PEGDM and photoinitiator in dichloromethane. Spectra were recorded at 2 cm^{-1} resolution on a Magna System 550 FTIR (Nicolet Instrument Tech-

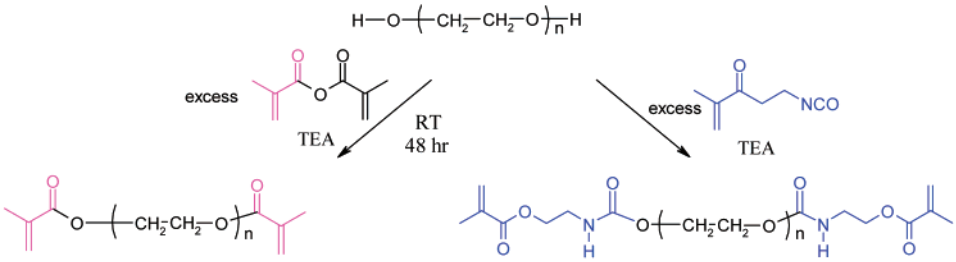

Figure 1. Synthesis of PEGDM and PEGUDM.

nologies, Madison, WI) equipped with a DTGS detector. The co-addition of 64 scans gave adequate signal-to-noise.

In situ rheology measurements were used for assessing the reaction kinetics and shear modulus. Rheological measurements were performed on a stress-controlled Rheometrics SR-5000 rheometer in a parallel plate configuration (quartz plates, 40 mm diameter). The instrument was raised onto a platform, and a quartz Pen-Ray 5.5 W mercury UV lamp was mounted under the bottom plate, allowing in-situ monitoring of the hydrogel formation. For these studies, a multi-wave UV lamp was used. Aqueous PEGDM or PEGUDM solutions containing 0.05% I2959 photoinitiator by mass fraction were loaded into the rheometer. The sample was exposed to UV for a short time (1−2 min) to allow the sample to cure without external perturbation. The late time cure was monitored by measuring the storage and loss modulus (G' and G'', respectively) at 1 rad/s and 1 Pa as a function of reaction time. Duplicate experiments showed excellent reproducibity with relative standard uncertainty of 3%.

The shear modulus was also determined using uniaxial compression measurements performed using a TA.XT2I HR Texture Analyzer (Stable Micro Systems, U.K.). This apparatus measures the deformation (±0.001 mm) as a function of an applied force (±0.01 N). Cylindrical hydrogels (height 3 mm, diameter 6 mm) were deformed (at constant volume) between two parallel glass plates. The shear modulus, G, was calculated from the nominal stress, σ (force per unit undeformed cross-section), using the equation[11]

$$\sigma = G(\Lambda - \Lambda^{-2})$$

where Λ is the macroscopic deformation ratio ($\Lambda = L/L_0$, L and L_0 are the lengths of the deformed and undeformed specimen, respectively). Measurements were carried out in triplicate at deformation ratios $0.6 < \Lambda < 1$. No volume change or barrel distortion was detected.

Cell viability was determined as follows. The Calcein AM and Ethidium homodimer-1 were added to chondrocyte media at a concentration of 2 μM/mL. This live/dead assay was then added to the cell-seeded hydrogels and incubated at 37 °C in 5% CO_2 for 5 min. Live and dead cells were imaged using an inverted microscope Eclipse TE 300 with a TE-FM Epi-Fluorescence attachment (Nikon, Inc.). Digital pictures were taken using a Nikon Coolpix 990 digital camera.

Results and Discussions

Synthesis and Characterization of PEGDM and PEGUDM. PEGDMs and PEGUDMs of high purity and low polydispersity are prepared as model materials for the formation of photocrosslinkable hydrogels. The PEG hydroxyl endgroups react with methacrylic anhydride to form PEGDM or with 2-isocyanatoethyl methacrylate to form PEGUDM (Figure 1). Urethane linkages in the PEGUDM are incorporated as an approach to enhance the hydrogen bonding in hydrogels, thus providing an additional adjustable parameter controlling the material properties.

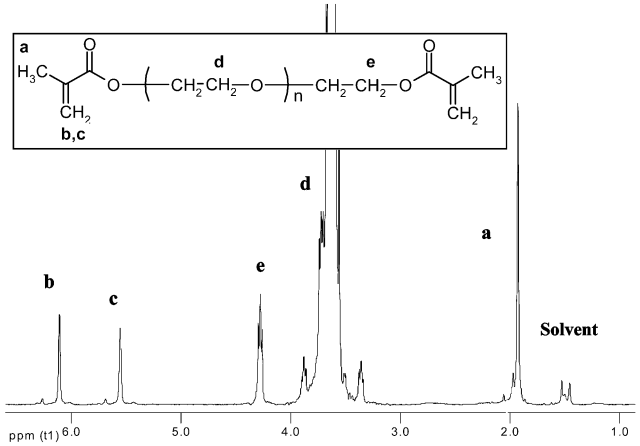

Figure 2. ^{1}H NMR of 3k PEGDM.

For the solution synthesis of PEGDM, we choose to use the less reactive methacrylic anhydride as opposed to the more commonly used methacryloyl chloride. The byproduct formed in the reaction of PEG with methacrylic anhydride is methacrylic acid, rather than the triethylamine/HCl salt formed in the reaction with methacrylic chloride. Since methacrylic acid can be removed more easily than the salt byproduct, methacrylic anhydride was used in these reactions. This allows for a straightforward oligomer purification process, which consists of one filtration through alumna followed by a single precipitation with diethyl ether. Given sufficient reaction time, only a slight excess of methacrylate anhydride relative to PEG hydroxyl is necessary to achieve quantitative conversion.

Proton NMR and MALDI-TOF MS together provide comprehensive information regarding the degree of methacrylate conversion and product purity. Figure 2 shows a typical ^{1}H NMR spectrum of PEGDM. PEG has one main chemical shift at $\delta \approx 3.64$. Under the current measurement conditions, the ethylene glycol penultimate endgroups cannot be differentiated from those of internal ethylene glycol segment. The chemical shift of methylene protons on MA are $\delta \approx 5.80$ and 6.21. Upon reaction of PEG, these protons shift to $\delta \approx 5.57$ and 6.13 (peaks b and c), respectively. Moreover, the protons adjacent to the methacrylate groups

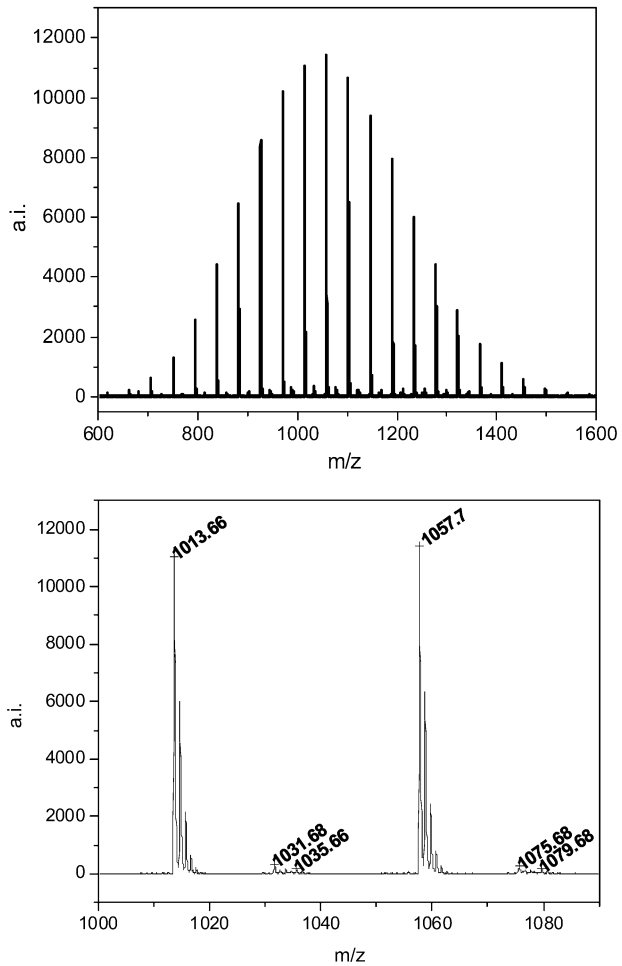

Figure 3. MALDI-TOF MS of 1k PEGDM, (a) full spectra and (b) expansion showing one main set of peaks 44 u apart due to Na^+ cationized PEGDM and two minor sets of peaks of PEGDM cationized by K^+ and H^+, respectively.

shift to $\delta \approx 4.30$. The ^{1}H NMR spectra for PEGDM shows the expected peaks, but the lack of additional peaks suggests that unreacted methacrylic anhydride, methacrylic acid byproduct, and triethylamine all have been quantitatively removed.

MALDI-TOF MS is a powerful technique from which the molecular mass, molecular mass distribution, and endgroup functionalities can be determined. Since MALDI detects all species within a discrete molecular mass range, it can be used to determine the amount of PEGDM versus the amount other impurities, such as PEGs with only one hydroxyl reacted (PEG mono-methacrylate) and unreacted PEG in a mixture. A MALDI-TOF MS spectrum of a typical 1k-PEGDM (Figure 3a) clearly illustrates both the high degree of methacrylate conversion and narrow polydispersity. Upon a closer examination (Figure 3b), three sets of peaks are observed. The main series corresponds to Na^+ cationized PEGDM. A minor second series of peaks corresponds to K^+ cationized PEGDM, and the third minor series may be attributed to H^+ cationized species. H^+ and K^+ contamination may occur during sample preparation and is common for the MALDI analysis of PEG and PEG derivatives.[12] H^+ is due to the matrix acid, and K^+ is in the matrix salt. Although the molecular mass calculation for the third series of peaks agrees with H^+ cationized PEGDM, it also agrees with those

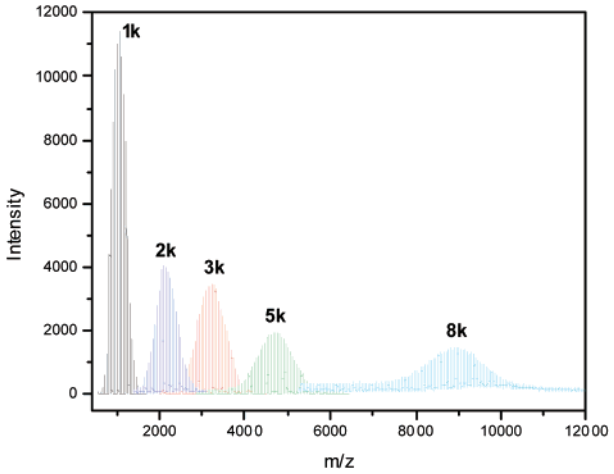

Figure 4. MALDI-TOF MS of a series of PEGDMs.

calculated for Na^+ cationized PEG mono-methacrylate. One approach to qualitatively differentiate the origin of these peaks is by comparing the M_n of these different series. In the scenario that the peaks represent H^+ cationized PEGDM, the M_n of the minor series should only differ from the main series by 22 u (MM difference between Na^+ and H^+), where as the M_n difference should be higher (MM difference of $\approx$56 u between PEGDM and PEGM) if the third series correspond Na^+ cationized PEG mono-methacrylate.

The MALDI-TOF MS spectra of PEGDMs prepared from different molecular mass PEGs are shown in Figure 4. Intrinsic to MALDI analysis, the relative signal intensities decrease and the breadth of the peak appears to increase as the molecular mass increases. Each molecular mass can be clearly distinguished with all oligomers displaying the expected molecular mass distribution. The degree of conversion is quantitatively assessed for each product.

As mentioned previously, the combination of ^{1}H NMR and MALDI-TOF MS is necessary to gather the full picture of the product purity and degree of methacrylate conversion. From ^{1}H NMR analyses, the molecular mass of PEGDMs can be calculated by comparing the peak intensities of ethylene glycol protons adjacent to the methacrylate (peak e) to internal ethylene glycol protons (peak d) or by comparing the peak integrations of a methacrylate proton (end group proton) to an ethylene glycol proton. However, since the unreacted PEG hydroxyl groups cannot be distinguished by ^{1}H NMR due to overlapping with PEG protons, the molecular mass calculation must assume stoichiometric conversion. This is not necessarily true depending on the reaction conditions employed in the synthesis. MALDI provides complementary information as to the amount of dimethacrylate species as well as those of PEG mono-methacrylate and unreacted PEG. On the other hand, PEG and methacrylated PEG derivatives are fragile in the MALDI analysis and could fragment during the laser desorption. The fragmentation may lead to biasing and, therefore, affect the MM calculations. Proton NMR provides confirmation to the MALDI calculations. It is only when the two techniques agree that we can conclude that high reaction conversions have been achieved. The molecular mass results of all PEGDMs are listed in Table 1. For all PEGDMs, the number average molecular masses (M_n) obtained by ^{1}H NMR match

Table 1. Molecular Mass Results (g/mol) of PEGDM Obtained Using ^{1}H NMR and MALDI-TOF MS

PEG	M_n (NMR)	M_n (MALDI)	M_w (MALDI)	PDI
1k	1047	1064	1085	1.02
2k	2222	2150	2178	1.01
3k	3424	3236	3283	1.01
5k	5057	4630	4681	1.01
8k	8333	8680	8776	1.01

Table 2. Reaction Conversion of PEGDMs Synthesized by the Microwave Process Calculated by ^{1}H NMR

PEG M_n	MA/PEG (mol:mol)	reaction time (min)	% conversion
1k	2.2	2	29
	2.2	5	40
	2.2	10	68
	4	5	84
	10	5	96
4k	2.2	5	37
	4	5	79
	10	5	99

closely to those calculated by MALDI. Complementary techniques thus conclusively demonstrate the high reaction conversion and low impurity in these dimethacrylates.

We have also explored the use of microwaves to prepare PEGDMs. Microwave reactions have gained significant interest recently due to their ability to achieve fast reaction rates often without the need for an organic solvent.[13,14] In the conventional thermal reaction, energy is transferred to the material through convection, conduction, and radiation. Energy transfers thus rely on diffusion of heat from the surfaces of the material, which leads to nonuniform heating and may cause excess heating at the surface leading to side reactions. Microwave energy can be delivered directly to material through molecular interaction with the electromagnetic field and can increase the local reaction kinetics, which leads to significantly reduced reaction time. Microwave reactions are becoming more widely used in combinatorial chemistry and green (solvent free) chemistry. The use of microwave reaction for the synthesis of PEGDM is particularly straightforward. The reaction requires 5 min to reach completion under microwave irradiation as opposed to 4 d for solution reactions. In addition, microwave-assisted reactions do not require a solvent or catalyst, and the product can be precipitated simply by adding diethyl ether.

For the microwave preparation of PEGDMs, the ratio of PEG to MA and optimized reaction times are important in achieving high reaction conversions. Whereas the solution reaction requires only a slight excess of MA relative to PEG, a near stoichiometric conversion by the microwave reaction is facilitated by a larger amount of MA relative to PEG. Table 2 shows the effect of reaction time and reagent ratio on the reaction conversion. The effect of reaction time can be compared for the microwave reactions of 1k PEGDM with the same reagent stoichiometric ratio of MA to PEG as the solution reaction, i.e., 2.2. The conversion increases with increased reaction time, but does not reach high conversion even after 10 min reaction time. Clearly the dominating effect in achieving high conversion is the MA to PEG ratio where

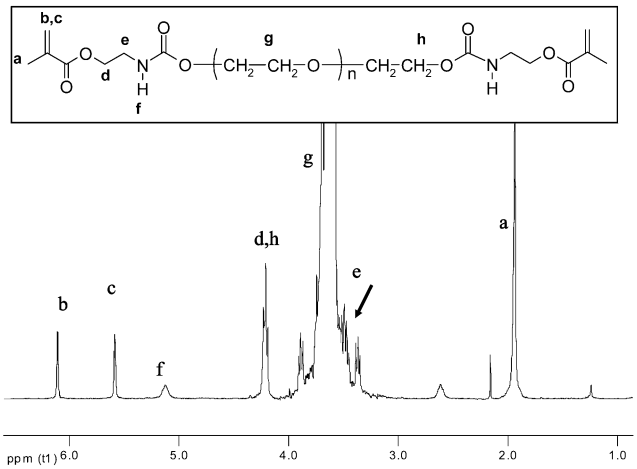

Figure 5. ^{1}H NMR of a 3k PEGUDM.

a monotonic increase in reaction conversion is observed with an increased MA to PEG ratio. At a MA to PEG ratio of 10, a near stoichiometric conversion can be achieved after 5 min. Since the microwave reaction is carried out neat, the lack of molecular mobility may require an excess of MA to be present locally. It is interesting to note that, although the reaction temperature becomes elevated during the microwave reaction, we do not detect cross-linking or any other side reaction in the product. Both the ^{1}H NMR and MALDI are nearly identical for PEGDMs prepared by the solvent approach or the microwave-assisted route.

The effect of PEG molecular mass on conversion is also evaluated for the microwave-assisted reactions. Table 2 shows the percent conversion calculated from the ^{1}H NMR results for 1k and 4k PEGDM reacted using various MA to PEG ratios for the same length of reaction time. Although PEGs are crystalline at room temperature, reaction mixtures are heated above the melting temperatures during microwave irradiation; thus, no significant differences between the reaction conversion for 1k and 4k PEG are observed.

Solution preparation of PEGUDMs is done in a similar manner as the PEGDMs. The isocyanate group on IEM is reacted with PEG hydroxyl groups. Polyurethane and related polyurethane copolymers have been used in various biological applications, such as heart valve implants; therefore, the urethane linkages used in the current study are expected to have relatively low cytotoxicity. The urethane spacer provides additional hydrogen bonding sites that may enhance the mechanical properties. Moreover, urethane linkages may be degradable under certain conditions. The PEGUDMs prepared from various PEG precursors are also characterized using a combination of ^{1}H NMR and MALDI-TOF MS. Figure 5 shows a typical ^{1}H NMR of 3k PEGUDM. The expected peaks are observed for the PEGUDM products.

The MALDI-TOF MS of PEGUDMs synthesized from various molecular mass PEGs are shown in Figure 6. Similar to the PEGDM spectra, the intensities generally decrease and breadth of distribution increases with increased molecular mass. The intensity is slightly higher for the 3k PEGUDM than for the 2k PEGUDM since a higher reaction conversion is obtained for the 3k polymer. The insert of Figure 6 shows the expanded spectra of the 3k PEGUDM. Two series of

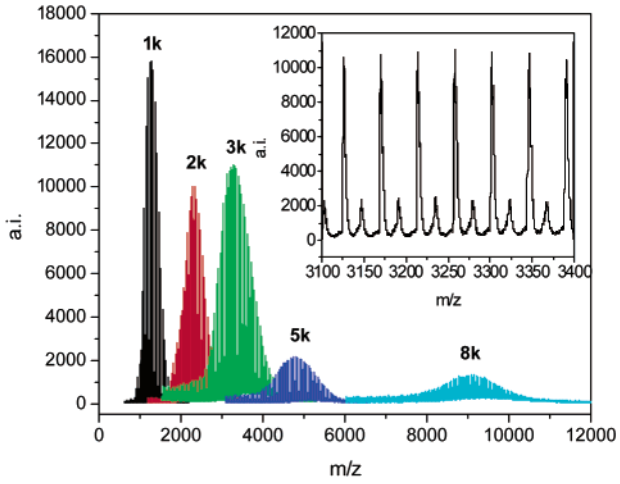

Figure 6. MALDI-TOF MS of PEGUDMs prepared from different molecular mass PEGs. The insert shows an expanded spectrum of 3k PEGUDM.

Table 3. Percent Reaction Conversion of PEGUDM Synthesis Calculated by ^{1}H NMR and MALDI-TOF MS

	NMR	MALDI % conversion		
PEG	% conversion	S1	S2	total
1k	95	87	14	93
2k	80	64	36	82
3k	89	83	17	92
5k	93	87	13	94
8k	85	66	34	83

peaks are clearly distinguishable. The main series with higher peak intensities (S1) corresponds to Na^+ cationized PEGUDM. Endgroup analysis using the Polymerics software suggests that the minor series (S2) corresponds to Na^+ cationized PEG mono-urethane methacrylate. This is in agreement with the difference in M_n calculated for the two series, which correlates well with the molecular mass difference of an endgroup. ^{1}H NMR confirms this peak assignment, and this is described in detail in the following paragraph.

Table 3 lists the reaction conversions for PEGUDM calculated by ^{1}H NMR and MALDI-TOF MS. From ^{1}H NMR, we can calculate an apparent molecular mass by comparing the methylene protons adjacent to the urethane (peak d) vs internal PEG protons (peak g). Since the molecular mass of PEG is known, we can calculate a theoretical M_n of PEGUDM at 100% reaction conversion. It is thus possible to back calculate the reaction conversion by comparing the apparent M_n to the theoretical M_n. To calculate the reaction conversion from MALDI, the peak integrations of the two series (S1 and S2) are first calculated. The total reaction conversion is then the sum of S1 and half of S2. As shown in Table 3, the reaction conversions obtained from the very different techniques are statistically identical. It is noted that although the reaction conversions are relative high, the synthesis of PEGUDM does not reach a near stoichiometric conversion as is the case for the PEGDM reactions.

Preparation and Characterization of Hydrogels. The reaction kinetics of the cross-linking of bulk PEGDM and PEGUDM are monitored by FTIR. From the FTIR spectrum, a decrease in the C═C stretch and a shift in the C═O stretch are observed as the methacrylate groups react. As shown in Figure 7a, high vinyl group conversions can be achieved after 15 min irradiation for the bulk reactions of PEGDM. Similar studies are carried out for PEGUDMs (Figure 7b). The bulk reaction kinetics appear to be similar for PEGDM and PEGUDM.

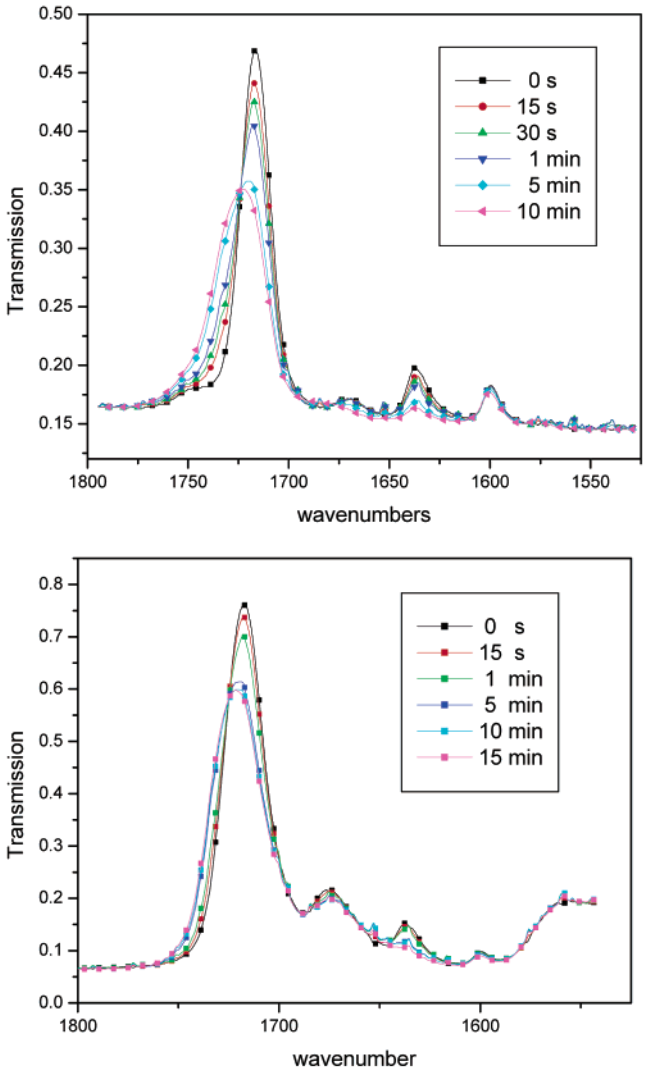

Figure 7. FTIR of bulk photocure of (a) 4k PEGDM and (b) 5k PEGUDM monitored as a function of UV exposure time.

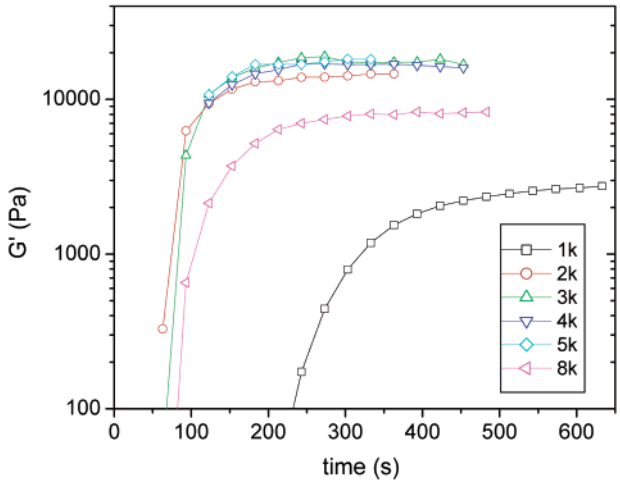

Figure 8. Storage modulus measured as a function of UV irradiation time for aqueous PEGDM (10% by mass fraction).

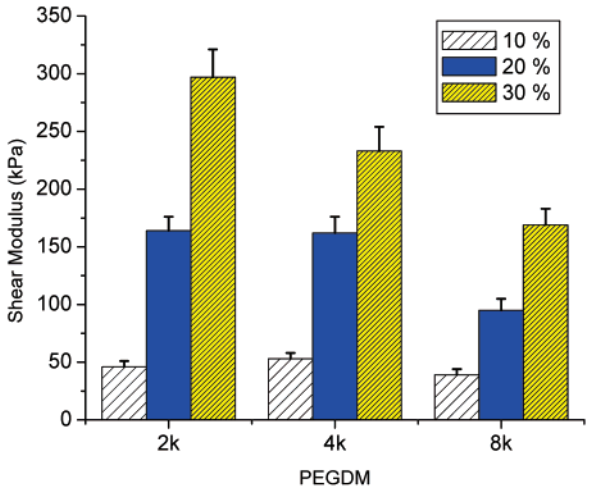

Figure 9. Shear modulus of hydrogels prepared using various PEGDM molecular masses and at various mass fractions.

Reaction kinetics and gel mechanical properties of hydrogels are studied by in situ rheological studies. Hydrogels are prepared by loading low viscosity, photoinitiator-containing, aqueous PEGDM or PEGUDM solutions between two parallel plates followed by irradiation to cross-link the dimethacrylates. Since the solutions are confined between parallel plates, a certain amount of stress is always built into the as-prepared hydrogels. When submersed in water or growth medium, these hydrogels can swell or contract depending on their thermodynamic state. In addition, PEG hydrogels produced by the free radical polymerization have high spatial inhomogeneity. Relationships between gel structures and properties will be the subject of a future paper.

In the present study, only preliminary data determined by rheology on the gel modulus as-prepared in the confined state is discussed. Two measurements are examined for each sample: a time-sweep measurement in which the storage modulus (G') and loss modulus (G'') are monitored as a function of irradiation time and a subsequent frequency sweep measurement at low strain amplitude. From the time sweep (Figure 8, only G' is shown for clarity), the reaction kinetics can be qualitatively evaluated. During the course of the reaction, both G' and G'' increase, and G' becomes greater than G'' once gelation occurs. The storage modulus G' increases as the reaction progresses for several orders of magnitude and eventually plateaus. The time at which the G' reaches a plateau provides a rough estimate of the time it takes to complete the photocrosslinking reaction. It should be noted that the light source used in the rheological measurements was of a different intensity and wavelength; therefore, although the trends in the curing rate are correct, the kinetics determined here cannot be used directly to predict the rate of curing under long UV wavelength. The subsequent frequency sweep verifies the formation of a chemically cross-linked gel. It is also of interest to examine the slope of G' as a function of frequency which provides qualitative information regarding the strength of the gel. All PEGDM and PEGUDM gels show relatively weak frequency dependence in the frequency range examined, confirming that the gels are in fact cross-linked and that gels as prepared are mechanically robust.

The shear moduli of hydrogels were also measured using a uniaxial compression test and calculated using equations derived from the strain-energy function. Figure 9 shows the shear modulus of PEGDM hydrogels prepared from different molecular mass oligomers (2k, 4k, and 8k) and as a function of PEGDM mass fraction (varying from 10% to 30%). As

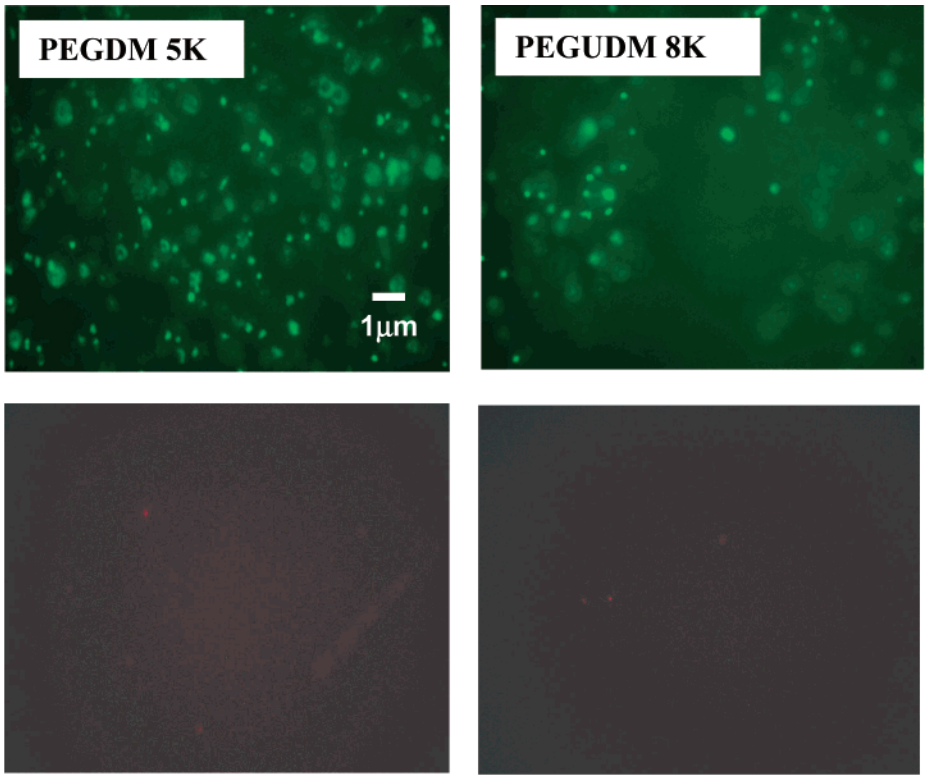

Figure 10. Live stain (top) and dead stain (bottom) of PEGDM (left column) and PEGUDM (right column) hydrogels containing bovine chondrocytes. The cell density is 100 000 cell/mL.

expected, the shear modulus monotonically increased as the oligomers mass fraction increased for PEGDMs of all molecular masses. The effect of molecular mass on shear modulus appears to depend on the oligomer content. For hydrogels high in the PEGDM mass fraction (30%), the shear modulus decreased as the molecular mass increased, presumably due to a decrease in the cross-link density as the molecular mass increased. However, the variation between molecular mass and shear modulus is less pronounced for hydrogels with lower PEGDM contents (i.e., 10% and 20%). Whereas hydrogels prepared from the 8k PEGDM do have a lower shear modulus than those prepared from the 2k and 4k PEGDM, there are no significant differences in the shear modulus of hydrogels prepared from the 2k and 4k PEGDM at lower PEGDM mass fractions.

Bovine chondrocytes seeded in PEGDM and PEGUDM hydrogels (Figure 10) are used as preliminary assessment for determining the biocompatibility of these materials. Given the high purity of the product and assuming networks with a relatively high degree of conversion can be achieved, we expect the cells to be viable in the hydrogels. Live cells are distinguished by their intracellular esterase activity, which is determined by the enzymatic conversion of nonfluorescent cell-permeable Calcein AM to an intensely green fluorescent calcein. Live cells enzymatically activated the fluorescent calcein. The dead stain Ethidium homodimer-1 is excluded by intact membranes of live cells. Therefore, Ethidium homodimer-1 only enters cells with damaged membranes and attaches to nucleic acids within dead cells to produce red fluorescence. The cell viability is thus measured through these physical biochemical properties. Figure 10 shows the live/dead cell stain for PEGDM and PEGUDM at 10% and 20% by mass fraction. Live and dead cell stains show that cells are completely (or nearly completely) viable in both types of hydrogels after two weeks. It should be noted that, since the PEGDM hydrogels are transparent and the chondrocytes are encapsulated within the hydrogel at different depths, only a fraction of the cells could be imaged in focus at a particular focal plane. The majority of the cells appear to have poor resolution since they are imaged out the focus due to the three-dimensionality of the hydrogel scaffold.

Conclusions

Facile synthesis and detailed characterization of PEGDMs and PEGUDMs are presented. A near quantitative conversion can be achieved by the reaction of PEG with MA, and relatively high reaction conversions, between 82% and 93%, can be obtained for the synthesis of PEGUDM. The combination of ^{1}H NMR and MALDI-TOF MS proves to be extremely effective in providing detailed characterization of reaction conversion, molecular mass, and molecular mass distribution. PEGDMs can be prepared both in solution and in bulk via a microwave-assisted route. Both approaches yield easily recoverable products of high purity and high conversion, but the reaction time is significantly reduced for the microwave reaction, i.e., 5 min for the microwave-assisted reaction versus 4 d for the solution reaction.

PEGDMs and PEGUDMs photopolymerize in water or growth medium to form hydrogels. Preliminary studies show varied mechanical response but that cells are completely viable in both types of hydrogels after two weeks. Hydrogels prepared from these dimethacrylates can provide a basis for better understanding how the material properties influence the cell response.

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References and Notes

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(15) Certain commercial materials and equipment are identified in this paper in order to specify adequately the experimental procedure. In no case does such identification imply recommendation by the National Institute of Standards and Technology nor does it imply that the material or equipment identified is necessarily the best available for this purpose.

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