Synthesis and Characterization of PEG and PEG Urethane Dimethacrylate Hydrogels

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INTRODUCTION

Hydrogels produced by photopolymerization have recently attracted significant interest as biomaterials in applications such as scaffolds for tissue engineering, drug delivery carriers, thrombosis prevention devices, post-operative adhesion promoters, and coating 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 (MM) functional polymer (macromer) by a free radical pathway in a minimally invasive manner. Photochemical induced crosslinking results in hydrogels that contain large water contents and that have mechanical properties similar to those of soft tissues. In addition, hydrogels have high permeability for oxygen, nutrients, and other water-soluble metabolites. Another advantage of photo-generated hydrogels as opposed to natural physical gels such as alginate is that their material properties can be more easily adjusted to fit the desired application.

Several types of photopolymerizable hydrogels, including those derived from poly(ethylene glycol) (PEG) diacrylate and dimethacrylate derivatives, and PEG copolymerized with fumarate groups, have been investigated for use as biomaterials.[1] We are interested in well-characterized PEG dimethacrylates (PEGDM) and similar reactive PEG derivatives as model systems because, although PEG alone is bio-inert, it can be easily modified to become bioactive. In addition, hydrogels from PEGDM and their copolymers and derivatives have been shown to be promising as scaffold materials.[2]

Despite the large number of studies currently available, there is still a lack of a clear understanding of the correlation between various material properties and cell responses. Furthermore, the physical properties of hydrogels are still difficult to predict by theories due to non-idealities of gel formation. Well-defined model materials are necessary for the preparation of hydrogels with highly reproducible and easily adjustable properties. To this end, we have prepared a series of controlled MM PEGDMs and PEG urethane dimethacrylates (PEGUDM) of high purity and low polydispersity. PEGDMs and PEGUDMs of different molecular masses have been photo-crosslinked to form hydrogels and preliminary cell viability studies have been conducted.

EXPERIMENTAL

Reagents. PEG (MM ≈ 1000 g/mol (1k) to 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 was obtained from Ciba Specialty Chemicals and used as received. Primary bovine chondrocytes were obtained from NIH. The growth medium is composed of Dulbecco's modified Eagle medium, 10 % fetal bovine serum, 1 % minimum essential medium 50 µg/mL L-ascorbic acid 2-phosphate and 1 % antibiotics (penicillin / streptomycin). Cell viability was measured using Live/Dead Viability / Cytotoxicity Kit (L-3224) purchased from Molecular Probes.

Synthesis of PEGDM and PEGUDM. PEGDM and PEGUDM were prepared from the various PEGs and MA or IEM, respectively. An example of the synthesis of 5k PEGDM is as follows. PEG (5 g, ≈ 0.001 mol), a 2.2 equivalence of MA (0.34 g, 0.0022 mol) and TEA (0.2 mL) was reacted in ≈ 15 mL dichloromethane and freshly activated molecular sieves for 4 d. 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.

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). All spectra were run in chloroform-d at 15 Hz sample spinning, 45° pulse tip angle, and a 10 s relaxation delay, for 64 scans. The relative uncertainty for MM calculation is less than 5 %. The MALDI-TOF MS was performed on a Bruker (Billerica, MA) REFLEX II using 2,5-dihydrobenzoic acid (DHB) as the matrix in reflectron mode with delayed extraction and low-mass (i.e. matrix-ion) blanking.[3] 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 choice of baseline and laser power (5 %). The estimated standard uncertainty in overall signal intensity from repeatability studies is 15 %.

Preparation of Hydrogels: PEGDM or PEGUDM (10 or 20) % by mass fraction and aqueous Irgacure 2959 solution (0.05 % by mass fraction) were mixed in distilled deionized water or growth medium when chondrocyte is encapsulated in the hydrogel.[4] Samples were cured with a long wavelength UV source (365 nm, 300 mW/cm^2) for 10 min to obtain hydrogels. Bovine chrondrocytes were seeded into hydrogels at a cell density of 17 000 to 100 000 cell/mL. Cell viability within the cell-hydrogel scaffolds under static cultures was measured at 14 d.

RESULTS AND DISCUSSION

PEGDMs and PEGUDMs of high purity and low polydispersity were prepared as model materials for the formation of photo-crosslinkable hydrogels. Urethane linkages were incorporated in PEG macromer (PEGUDM) as an approach to enhance the hydrogen bonding in hydrogels, thus providing an additional adjustable parameter for controlling material properties.

Proton NMR and MALDI-TOF MS together provide information as to the degree of methacrylate conversion and product purity. Figure 1 shows a typical ^{1}H NMR spectrum of PEGDM. PEG has one main chemical shift at $\delta \approx 4.0$ and the PEG endgroups protons cannot be differentiated from those of internal protons. The MA methylene chemical shift are $\delta \approx 5.55$ and 6.1. Upon reaction of PEG, the methylene protons shift to $\delta \approx 5.8$ and 6.1, respectively. Moreover, the PEG protons adjacent to the methacrylate groups shifted to $\delta = 4.2$. The ^{1}H NMR spectra for PEGDM showed the expected peaks, and the lack of additional peaks suggest that unreacted MA, methacrylic acid by-product, and TEA all have been quantitatively removed.

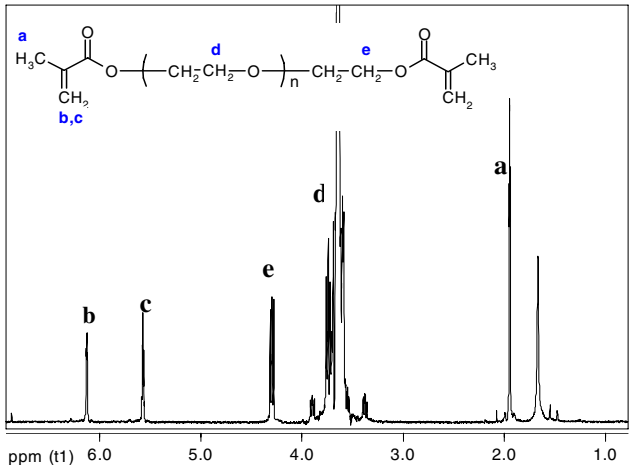

Figure 1. ^{1}H NMR of 2k PEGDM

The MALDI-TOF MS spectra of PEGDMs prepared from different MM PEGs are shown in Figure 2. Intrinsic to MALDI analysis, the

* *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*

relative signal intensities decrease and of the peak distribution breadth appear to increase as the MM increases. Each MM can be clearly distinguished with all oligomers showing the expected MM distribution. The MM, polydispersity, and endgroup functionalities can be calculated by MALDI. Since MALDI detects all species within a discrete MM range, it quantitatively determines the amount of dimethacrylates versus the amount other impurities, such as PEGs with only one hydroxyl reacted and unreacted PEG. A MALDI spectrum of 1k-PEGDM (insert of Figure 2) clearly illustrates both the high degree of methacrylate conversion and the narrow polydispersity. Similar results were obtained for the PEGUDM.

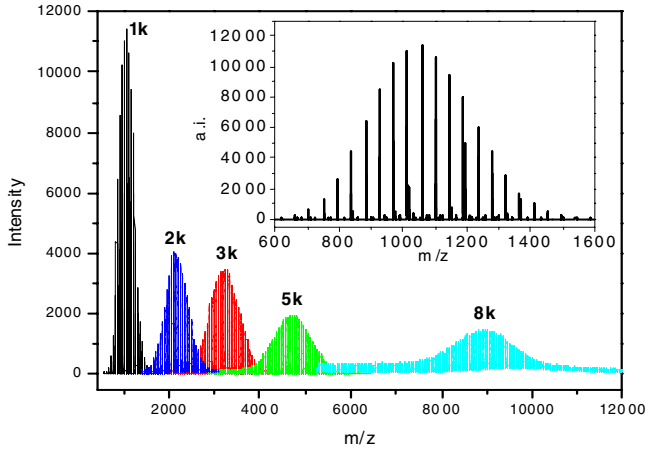

Figure 2. MALDI-TOF MS of PEGDMs, insert shows the1k PEGDM

As mention previously, the combination of ^{1}H NMR and MALDI-TOF MS is necessary for the complete characterization of the product with respect to purity and degree of methacrylate conversion. From ^{1}H NMR analyses, the MM of PEGDMs can be calculated by comparing the peak intensities of ethylene glycol protons adjacent to the methacrylate to internal ethylene glycol protons. However, since the unreacted PEG hydroxyl groups can not be distinguished by ^{1}H NMR due to overlapping with PEG protons, the MM calculation must assume stoichiometric conversion. This is may not be true depending on the reaction conditions employed in the synthesis. MALDI provides complementary information as to the amount of dimethacylates species as well as those species with one side reacted and unreacted. 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 of the MALDI calculations. It is only when the two techniques agree that we can conclude that high reaction conversions have been achieved. The MM 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 closely to those calculated by MALDI. Complementary techniques thus conclusively demonstrate the high reaction conversion and low impurity in the reaction product.

Table 1. Molecular Mass Results for PEGDMs

PEG MM	*M_n (NMR)*	*M_n (MALDI)*	*M_w (MALDI)*	*PDI*
1 k	1047	1064	1085	1.02
2 k	2222	2150	2178	1.01
3 k	3424	3236	3283	1.01
4 k	4486	4165	4199	1.01
5 k	5057	4630	4681	1.01
8 k	8333	8680	8776	1.01

The reaction kinetics of the crosslinking of bulk 4k PEGDM was monitored by FTIR. The reaction kinetics and gel mechanical properties were studied by *in situ* rheological studies (not discussed in detail here). 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. In bulk reactions, high vinyl group conversions can be achieved after 15 min irradiation. Similar studies were carried out for PEGUDMs (not shown). The bulk reaction kinetics appear to be similar for PEGDM and PEGUDM.

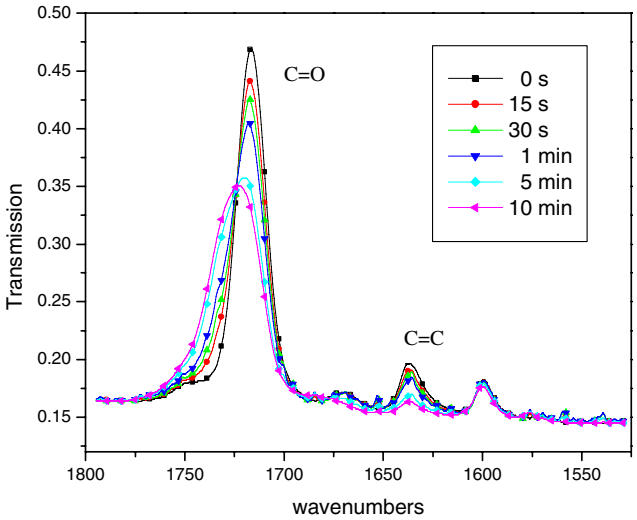

Figure 3. FTIR of a bulk 5k-PEGDM crosslinking reaction

Bovine chrondrocytes, seeded in PEGDM (Figure 4) and PEGUDM hydrogels (not shown), were used as preliminary assessment for determining the cell responses to the hydrogels. Live cell stain (Calcein AM) and dead cell stains (Ethidium homodimer-1) show that cells were completely viable in both types of hydrogels after two weeks.

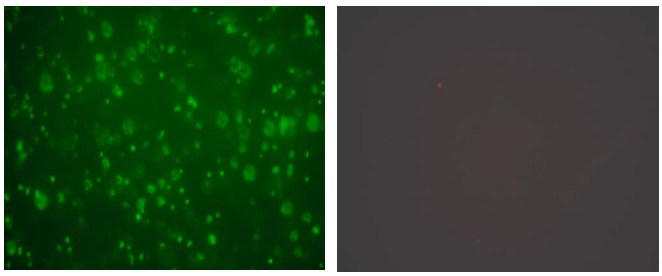

Figure 4. Bovine chrondrocytes seeded in PEGDM with the live cell stain (left) and dead cell stains (right)

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