The Use of MALDI-TOF MS and ^{1}H NMR as Complimentary Methods for Confirming Composition and Purity of Hydrogel Prepolymers

Feature
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Photopolymerizable poly(ethylene glycol) dimethacrylates (PEGDM) and similar PEGDM derivatives of various molecular mass were synthesized and characterized. PEGDM hydrogels were studied as model tissue engineering scaffolds for soft tissue regeneration because PEG alone is bio-inert, but can be easily modified to become bioactive. In addition, photopolymerization of dimethacrylates are relative fast reaction, and the resulting hydrogels have been shown to be biocompatible with the unreacted methacrylates having relatively low cytotoxicity. Cells that generate cartilage, chondrocytes, encapsulated in hydrogels retain their native form and over time can generate native cartilage tissue. 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.

The PEG hydroxyl endgroups react with methacrylic anhydride to form PEGDM or with 2-isocyanatoethyl methacrylate to form poly(ethylene glycol) urethane-dimethacrylates PEGUDM (Figure 1). Triethylamine (TEA) was used to catalyze the reaction. PEGDMs were also prepared

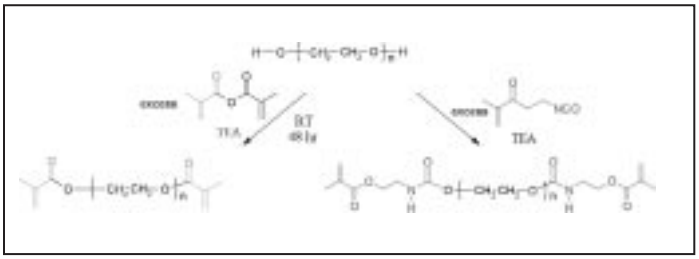

Figure 1. *Synthesis of PEGDM and PEGUDM.*

by a microwave-assisted route to achieve fast reaction conversions (five minutes for microwave reactions vs. four days for solution reactions) under solvent-free conditions. Hydrogels were prepared by photopolymerization. PEGDM or PEGUDM and aqueous initiator (Irgacure 2959) solution were mixed in distilled, deionized water or growth medium when chondrocyte is encapsulated in the hydrogel. Cylindrical samples were cured with a long wavelength UV source (365 nm, 300 μW/cm2) for 10 minutes to obtain hydrogels.

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). The two techniques together confirmed the formation of prepolymers of high purity and narrow mass distribution (PD < 1.02). The ^{1}H NMR spectra for PEGDM shows the expected peaks, but the lack of additional peaks suggests that unreacted methacrylate anhydride, methacrylic acid by-product, and triethylamine all have been quantitatively removed (Figure 2). MALDI-TOF MS is a powerful technique from which the molecular mass, molecular

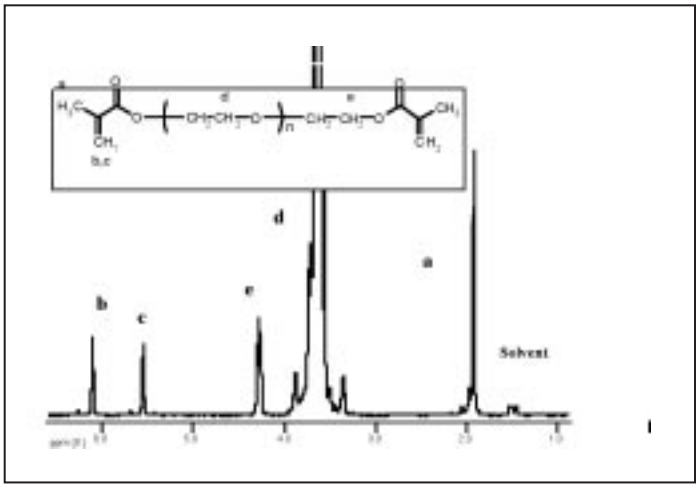

Figure 2. *^{1}H NMR of 3k-PEGDM (left), and MALDI-TOF MS of a series of PEGDMs. Insert shows the MALDI-TOF MS of 1k PEGDM (right).*

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 of other impurities, such as PEGs with only one hydroxyl reacted (PEG mono-methacrylate) and unreacted PEG in a mixture. The MALDI-TOF MS spectra of PEGDMs prepared from different molecular mass PEGs are shown in Figure 2. 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. The MALDI-TOF MS spectrum of 1k-PEGDM (Figure 2) clearly illustrates both the high degree of methacrylate conversion and narrow polydispersity. Upon a closer examination, three sets of peaks are observed. The main series corresponds to Na+ cationized PEGDM. The two minors sets correspond to H+ and K+ cationized PEGDM.

Bovine chondrocytes, seeded in PEGDM and PEGUDM

hydrogels, are used as preliminary assessment for determining the biocompatibility of these materials. Live cells are distinguished by their intracellular esterase activity and are enzymatically activated the fluorescent calcein (green). The dead stain 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

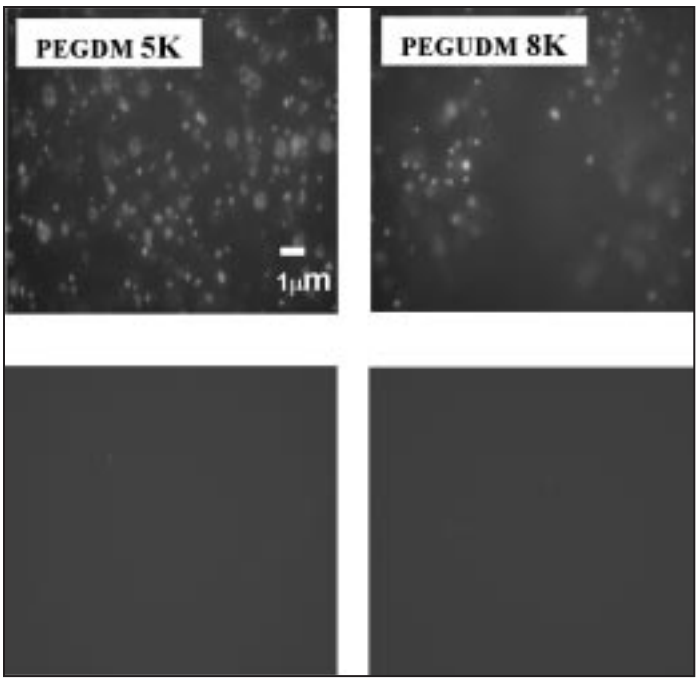

Figure 3. *Live/dead stain (top and bottom, respectively) of PEGDM and PEGUDM hydrogels containing bovine chondrocytes. The cell density is 100 000 cell/mL.*

measured through these physical biochemical properties. Figure 3 shows the live/dead cell stain for PEGDM and PEGUDM. Live and dead cell stains show that cells are completely (or nearly completely) viable in both types of hydrogels after two weeks.

The PEGDM hydrogel structure and mechanical properties were determined using small-angle neutron scattering (SANS) and uniaxial compression tests, respectively. Figure 4 shows the two-dimensional SANS patterns for the 4k-PEGDM solutions and gels obtained at the 2m detector distance. A marked difference is evident as the solutions photo-crosslink to form hydrogels. At high PEGDM mass fractions, a ring develops in the scattering pattern indicating the presence of a well-defined structural length scale (correlation length ξ). Both the gel structure and shear modulus depend on the PEGDM molecular mass as well as the oligomer mass fraction. For PEGDM of all molecular masses, the shear modulus increased as ξ_{gel} decreased. These observations are consistent with the theory of rubber elasticity. The effect of molecular mass is less apparent for lower mass fraction hydrogels (10 percent). For the 30-percent hydrogels, the expected trend of increased shear modulus with decreased molecular mass is observed. Hydrogels prepared from these dimethacrylates can provide a basis for understanding the effect of material structures and properties influence on cell response. For more information on this topic, contact the author at slgibson@nist.gov (NIST Polymers Division) and see "Synthesis and Characterization of PEG

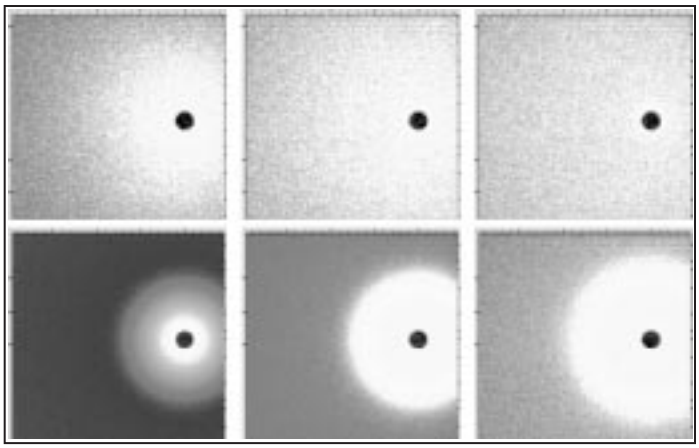

Figure 4. *Two-dimensional scattering patterns of 4k-PEGDM solutions (top row) and corresponding hydrogels (bottom row) at various PEGDM mass fractions (10 percent, 20 percent, and 30 percent from left to right).*

Dimethacrylates and Their Hydrogels," which can be accessed at http://polymers.nist.gov/uploads/lin-gibson0204.pdf.

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