Communications to the Editor

Lactide-Based Poly(ethylene glycol) Polymer Networks for Scaffolds in Tissue Engineering

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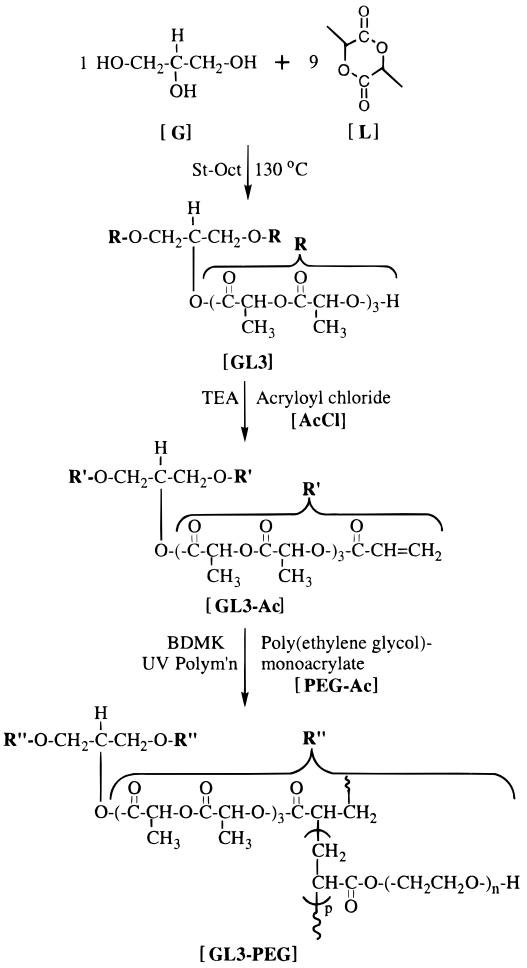
Introduction. Tissue engineering has recently attracted a great deal of attention for its potential in repairing, reconstructing, regenerating, or replacing tissues in damaged or diseased organs.[1] A number of tissues and organs have already been investigated using this approach, including cartilage, liver, skin, bone, tendon, ureter, intestine, pancreas, and blood vessels.[2] One approach in tissue engineering employs a hybrid system combining biomaterials as structural scaffolds to organize living cells into a desired structure *in vitro* or *in vivo*.[3] Polymers for these scaffolds require good biocompatibility, suitable biodegradability, and the ability to interact specifically with appropriate cells.

Biodegradable homo- and copolymers of lactic acid and glycolic acid have been explored as scaffolds in several of the tissue engineering applications mentioned above. These materials are reasonably biocompatible and possess appropriate biodegradability, but they lack the ability to interact biospecifically with cells. To address this, Langer and co-workers have synthesized poly(lactic acid-*co*-lysine) by copolymerizing the heterodimer of lactic acid and lysine with the homodimer of lactic acid, lactide.[4] This biodegradable copolymer contains an amine group on the side chain of the lysyl residues to which a bioactive factor, such as the peptide arginine-glycine-aspartic acid (RGD),[5] can be grafted as a ligand to induce cell adhesion. To be most useful as bioactive scaffolds, the base polymer should support little cell adhesion on its own; in this manner, the cell adhesive properties of the scaffold can be determined exclusively by the immobilized bioactive factor, and the possibility exists to develop materials that are adhesive to only targeted cell types.[3,6] Cima has approached this issue using cross-linked poly(ethylene glycol) (PEG) star hydrogels with high ligand capacity as matrices for coupling the desired cell-binding ligands.[7]

Our laboratory has previously prepared low swelling, highly hydrophilic, cell adhesion resistant, and/or biospecific cell adhesive materials by incorporating PEG diacrylate and acrylic acid within highly cross-linked trimethylolpropane triacrylate (TMPTA) networks. PEG in the network resisted protein adsorption and cell adhesion, but the very high cross-linking density from TMPTA prevented swelling that would be induced by the high amount of PEG. The acrylic acid groups incorporated into the network were useful as sites to immobilize RGD and other peptides to promote cell adhesion. These materials exhibited ideal cell interaction characteristics for tissue engineering, but they were nondegradable.[6] In this study, we have extended our approach to networks that biodegrade into nontoxic products taking into account the above prerequisites. These polymer networks have been developed as materials for scaffolds in tissue engineering using nontoxic glycerol, biodegradable L-lactide, and biocompatible and ligand-immobilizable PEG, and the characteristics of these materials have been examined.

Scheme 1



Experimental Section. Scheme 1 shows the synthetic reactions leading to the formation of GL3-PEG networks. Networks were formed from two reactive precursors, a 3-armed acrylated lactic acid oligomer emanating from a glycerol center (GL*n*-Ac: G, glycerol; L*n*, *n* dimeric repeats of lactic acid; Ac, acrylate) and an α-monoacrylate-*ω*-monohydroxy PEG (PEG-Ac). The typical procedure is as follows: first, 3.19 g of glycerol (G, Aldrich), 45 g of L-lactide (L, 9 mol per G mol; Aldrich), and 0.63 g of stannous octoate (St-Oct, 1/200 of L mol; Sigma) were reacted in melt at 130 °C for 6 h

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Table 1. Characteristics of GL3-PEG Networks

material[a]	gel content (%)	T_g[b] (°C)	atomic %[c]		contact angle (deg)[d]		water absorption (%)
			C	O	θ_{adv}	θ_{rec}	
GL3 network	88.4	61	69	31	83	54	3.6
GL3-PEG1K-15	91.6	40	64	36	80	51	12.8
GL3-PEG4K-5	91.2	39	63	37	73	44	16.6
GL3-PEG4K-15	88.3	35	63	37	50	29	24.0

[a] *n*K indicates the molecular weight of the PEG-Ac, 1000 or 4000; -*m* indicates the mol % of PEG-Ac per GL3 mol. [b] Calculated from DSC thermograms by first heating from −20 to +200 °C. [c] Determined by ESCA narrow scan spectra. [d] Obtained from the result of first cycle in water; θ_{adv}: advancing contact angle, θ_{rec}: receding contact angle.

under argon. The melt was allowed to cool and was dissolved in chloroform, microfiltered, precipitated in hexane, and vacuum-dried to yield GL3 triol.[8] Second, 17.2 g of acryloyl chloride (AcCl, 6 mol per GL3 triol mol; Aldrich) dissolved in 60 mL of dichloromethane (DCM) was slowly dropped into 44 g of GL3 triol and 19.23 g of triethylamine (TEA, 2 mol per AcCl mol; Aldrich) dissolved in 400 mL of DCM and reacted at 0 °C for 6 h and at room temperature for 42 h. The solution was microfiltered, precipitated in diethyl ether, and vacuum-dried to yield 3-arm acrylated GL3 (GL3-Ac).[9] Finally, a 25% w/v solution of 1 g of GL3-Ac, PEG-Ac [MW = 1000 (1K) and 4000 (4K), 5 and 15 mol % of GL3-Ac mol; Monomer-Polymer & Dajac, Feasterville, PA], and 1% w/w 2,2-dimethoxy-2-phenylacetophenone (benzyl dimethyl ketal, BDMK; Aldrich) dissolved in DCM were photocopolymerized by ultraviolet (UV) irradiation (UV Products, San Gabriel, CA) on a Petri dish to obtain GL3-PEG networks.

The GL3-PEG network films produced as described above were dried under vacuum at 60 °C for 1 day, weighed (W_1), and then extracted with chloroform at room temperature for 1 day. The films were dried again and weighed (W_2). The gel content was calculated as $(W_2/W_1) \times 100\%$. Subsequently, the films were immersed in phosphate-buffered saline (PBS, pH 7.4) at room temperature for 1 day and weighed (W_3). The water absorption was calculated as $[(W_3 - W_2)/W_3] \times 100\%$. Extracted films were used for further surface and bulk characterization by differential scanning calorimetry (DSC; Perkin-Elmer DSC 7), electron spectroscopy for chemical analysis (ESCA; M-Probe Surface Science), and dynamic contact angle (DCA; Cahn Instruments). Human foreskin fibroblasts were seeded onto the films at 30 000 cells/cm^2 in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum at 37 °C for 1 day in a 5% CO_2 incubator. The number of adhered cells was counted at 200× by phase contrast microscopy, and the fraction of adhered cells was calculated based on the seeding density as described.[6]

Results and Discussion. L-Lactide (9 mol per glycerol mol) was ring-opening polymerized using nontoxic glycerol as trifunctional initiator and stannous octoate as a catalyst to yield biodegradable nontoxic GL3 triol with 3 lactidyl units or 6 lactyl repeats per arm. The conversion to GL3 triol from glycerol was found to be 100% by ^{1}H NMR, specifically by disappearance of **G**-CH_2OH (3.73 ppm) and by ratio of **L**-C*H* (5.16 ppm) to **G**-CH_2 (4.35 ppm). This observation demonstrates that each of the three hydroxyl groups of glycerol initiated the polymerization of L-lactide, that each GL3 triol had at least 1 lactidyl unit attached, and that the average number of lactidyl units attached was 3. In GL3-Ac, it was found by ^{1}H NMR that the degree of substitution of GL3 triol to acrylate was also 100%, specifically by disappearance of **L**-O*H* (2.85 ppm) and by ratio of CH_2═C*H* (5.91−6.44 ppm) to **L**-CH_3 (1.56 ppm). This observation demonstrates that each terminal hydroxyl group in GL3 triol was completely derivatized with a polymerizable acrylate group. GL3-Ac and monoacrylated PEG were photocopolymerized using a simple UV source with the initiator BDMK to form cross-linked networks. Durations of UV irradiation for GL3-PEG networks ranged from 30 s to 5 min, depending on the film thickness. The films obtained were glassy and transparent, and the gel content was approximately 90% (Table 1).

The thermal behavior observed by DSC indicated that all GL3 networks had no melting endotherms (T_m), but they displayed a glass transition temperature (T_g) due to the loose cross-linking by means of the oligolactyl spacer. All GL3-PEG networks showed lower T_g than did the GL3 homonetwork and the T_g of GL3-PEG decreased with increasing PEG molecular weight and PEG content (Table 1). This observation reveals that as PEG is incorporated in the networks, the bulk becomes rubbery and soft to a certain degree. Moreover, the lack of a PEG T_m indicates that the PEG is distributed throughout the network and is not separated into PEG-rich phases. The atomic surface compositions of the films were determined by ESCA. As shown in Table 1, after the incorporation of PEG-Ac in GL3 networks, the oxygen (O) atomic % of GL3-PEG increased at the expense of a decrease in the carbon atomic %. The theoretical value for the homonetwork is 44% O, and the theoretical value for the copolymeric networks is 44% O for GL3-PEG1K-15, 42% O for GL3-PEG4K-5, and 40% O for GL3-PEG4K-15. This observation indicates that while the PEG is phase-mixed in the network bulk, it is enriched at the network surface. This has been observed with other systems as well.[10]

To compare the surface and bulk properties of the films in contact with water, their dynamic contact angles and water absorption were evaluated (Table 1). Contact angle measurements showed that GL3-PEG4K-15 had the most hydrophilic surface among the network films tested due to the introduction of tethered, but otherwise water-soluble PEG. Water absorption of GL3 networks increased as PEG was incorporated and as PEG molecular weight and PEG content increased. However, the change in water absorption was small. These results show that all GL3-PEG networks have relatively low swelling and good bulk properties as compared to hydrogels and that only their surfaces, especially that of GL3-PEG4K-15, are more hydrophilic because the pendent PEG chains at the surfaces are highly mobile and are capable of rearrangement to minimize their interfacial free energy depending on the environment, such as air or water.[11]

Human fibroblasts were seeded onto the films to evaluate the resistance to cell adhesion. The results of cell culture are shown in Figure 1. All GL3-PEG networks showed less cell adhesion and spreading than

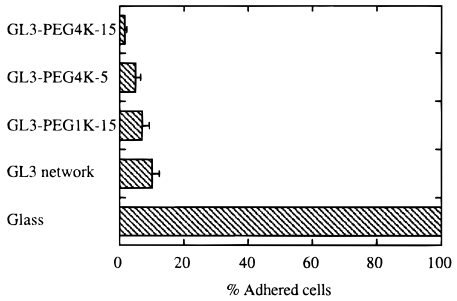

Figure 1. Human foreskin fibroblast adhesion onto GL3-PEG networks ($n = 3-5$).

a GL3 homonetwork and much less than glass used as a positive control. In particular, GL3-PEG4K-15 was most resistant to cell adhesion. This is attributed to the incorporation of the most PEG, which has high mobility and volume exclusion effect.[12] Generally, PEG is well-known to have low protein and platelet adsorption[13] and cell adhesion,[14] and it has been used to reduce the antigenicity and immune clearance of therapeutic proteins.[15]

Biodegradation of polymer scaffolds is an important factor for tissue engineering applications. The biodegradation of GL3-PEG networks can be controlled from several days to months by changing the amount of L-lactide and PEG-Ac and the cross-linking density and by employing other degradable segments such as oligo(glycolide) or oligo(ϵ-caprolactone).[16] In addition, to induce the selective cell adhesion toward cell-resistant GL3-PEG networks, reactive functional pendent groups on PEG-Ac can be directly employed for grafting ligands like RGD, REDV, and YIGSR peptides.[17] These coupled oligopeptides, which are present in extracellular matrix (ECM) proteins, represent the minimum sequence necessary for cell attachment with specifically targeted cells for tissue engineering.[18]

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(8) Yield 92%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.56 (m, 3H, CH_3), 2.85 (s, 1H, **L**-O*H*), 4.35 (m, 1H, **L**-C*H*OH and 2H, **G**-CH_2), 5.16 (m, 1H, **L**-C*H* and 1H, **G**-C*H*).

(9) Yield 83%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.56 (m, 3H, CH_3), 4.35 (m, 2H, **G**-CH_2), 5.16 (m, 1H, **L**-C*H* and 1H, **G**-C*H*), 5.91−6.44 (m, 3H, CH_2=C*H*).

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