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ScienceDirect
Acta Biomaterialia 5 (2009) 1872–1883
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

End-group effects on the properties of PEG-*co*-PGA hydrogels

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Received 29 May 2008; received in revised form 6 February 2009; accepted 10 February 2009
Available online 28 February 2009

Abstract

A series of resorbable poly(ethylene glycol)-*co*-poly(glycolic acid) (PEG-*co*-PGA, 4KG5) macromonomers have been synthesized with the chemistries from three different photopolymerizable end-groups (acrylates, methacrylates and urethane methacrylates). The aim of the study is to examine the effects of the chemistry of the cross-linker group on the properties of photocross-linked hydrogels. 4KG5 hydrogels were prepared by photopolymerization with high vinyl group conversion as confirmed by ^{1}H nuclear magnetic resonance spectrometry using a 1D diffusion-ordered spectrometry pulse sequence. Our study reveals that the nature of end-groups in a moderately amphiphilic polymer can adjust the distribution and size of the micellar configuration in water, leading to changes in the macroscopic structure of hydrogels. By varying the chemistry of the cross-linker group (diacrylates (DA), dimethacrylates (DM) and urethane dimethacrylates (UDM)), we determined that the hydrophobicity of a single core polymer consisting of poly(glycolic acid) could be fine-tuned, leading to significant variations in the mechanical, swelling and degradation properties of the gels. In addition, the effects of cross-linker chemistry on cytotoxicity and proliferation were examined. Cytotoxicity assays showed that the three types of hydrogels (4KG5 DA, DM and UDM) were biocompatible and the introduction of RGD ligand enhanced cell adhesion. However, differences in gel properties and stability differentially affected the spreading and proliferation of myoblast C2C12 cells.

Keywords: PEG-*co*-PGA; End-group chemistry; Micelle; Biodegradable; Hydrogel properties

1. Introduction

Hydrogels produced by photopolymerization from a variety of polymers have been used extensively for a number of biomedical applications such as scaffolds for tissue engineering and controlled release systems for drug delivery [1–4]. The rapid advances in these fields require not only the development of biocompatible and biodegradable scaffolds but also a versatile system in which we can controllably fine-tune their properties with respect to the specific goal. To provide a delivery system adaptable to many applications, several groups have reported that hydrogel properties can be adjusted by a number of parameters, such as nature of macromonomers, molecular weight, composition, cross-link density or external stimuli [5–8]. However, little is known about the effect of polymerizable end-groups on gel structure and properties, such as mechanics, swelling, degradation profiles and cell responses. These end-groups, which in this case are cross-linkers, have different chemistries and can affect the microscopic as well as the macroscopic structure of hydrogels. Consequently, the end-groups can be used to adjust the amphiphilicity of polymeric networks.

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doi:10.1016/j.actbio.2009.02.030

Although many variations of synthetic polymers can form hydrogels via chemical cross-linking, poly(ethylene glycol) (PEG) has been one of the most investigated systems. PEG has been functionalized with diacrylate or dimethacrylate groups and cross-linked to form nondegradable hydrogels via UV photoinitiation [9–11]. Nondegradable hydrogels have been used for diffusion-controlled delivery devices [12,13]. It has been shown that for high molecular weight drugs, such as peptides and proteins, a degradable hydrogel is more suitable for controlled delivery. To obtain degradable hydrogels, PEG has been copolymerized with degradable polymers such as poly(lactic acid), poly(glycolic acid) (PGA) or enzymatically resorbable polysaccharides [14–16]. Erosion of water-soluble polymers occurs by a combination of mechanisms. Linkages in the polymer may be labile to hydrolysis and enzymes, thereby producing oligomeric scission products soluble in water. For hydrophobic polymers, degradation is limited to the surface of the materials. For hydrophilic polymers, on the other hand, water may become entrapped in the polymer and degradation ensues via bulk erosion. PGA is one of the most thoroughly investigated poly(α-hydroxy esters). This class of polymers is bioerodible as a consequence of hydrolyzable bonds along the polymer backbone.

Hydrogels based on PEG-*co*-poly(α-hydroxy acid) diacrylates and their derivatives contain large amounts of water and have a hydrophilic, nonionic surface. This lowers the driving force for protein absorption on these surfaces from the physiological environment. Nonadhesive hydrogels have been modified with bioactive moieties such as GRGDS peptide sequences to facilitate cell adhesion, spreading and organization. The RGD sequence is a ubiquitous adhesive peptide, responsible for the integrin–ligand interaction between cells and the surface of RGD-modified hydrogels. Cell adhesion is often a necessary first step in basic cellular processes such as cell proliferation, cellular trafficking and tissue development.

The primary goal of this research was to elucidate how end-group chemistry might be exploited to fine-tune the properties of degradable hydrogels. We hypothesized that by varying the chemistry of the cross-linking group we could tune the gel microstructure, leading to a significant peculiarity in the hydrogels. The synthesis, characterization and evaluation of PEG-*co*-PGA-based hydrogels are described. Based on the method of Hubbell and co-workers, the macromonomers of interest in this research were synthesized based on a water-soluble central block of PEG extended with biodegradable oligomers of PGA and terminated with different cross-linkable end-groups [17,11]. The degree of polymerization of the hydrolytically labile extension was relatively small in order to conserve the water solubility of the PEG component. Therefore, the properties of the macromonomer in solution and the gel properties were determined primarily by the water-soluble central domain of PEG. Further, the amphiphilic nature of these macromonomers caused them to assume micellar conformations, enabling gelation. Acrylates, methacrylates and urethane methacrylates were chosen as terminal end-groups because they enabled photopolymerization, resulting in the formation of a cross-linked network. In addition, varying terminal vinyl group chemistry can influence the physical properties of hydrogels. These water-soluble macromonomers were photopolymerized via a light-sensitive initiation mechanism using long-wave UV irradiation of aqueous solutions (10% and 20% by mass fraction). The gels degraded by hydrolysis of the oligo(α-hydroxy acid) regions into PEG, α-hydroxy acid and acidic oligomers [17].

We report that the nature of photopolymerizable end-groups affects the amphiphilicity and micellar configuration of PEG-*co*-PGA precursors. Such a subtle effect has a significant impact on the physical properties of hydrogels and cell responses. The utility of using various end-group chemistries to cross-link synthetic and biocompatible polymers to form versatile hydrogels has the potential to produce a wide range of scaffolds appropriate for use in a number of biomedical applications.

2. Materials and methods

2.1. Materials

PEG ($MM \approx 4000\ g\ mol^{-1}$), acryloyl chloride (AC), methacrylic anhydride (MA), 2-isocyanatoethyl methacrylate (IEM), triethylamine (TEA), stannous octoate, dibutyltin dilaurate (DBTD) and Hoechst dye were purchased from Sigma–Aldrich and used as received. Dichloromethane and ethyl ether were obtained from Fischer, and dichloromethane was dried over activated molecular sieves prior to use. Glycolide was purchased from E.I. du Pont de Nemours. Acryloyl-PEG-*N*-hydroxysuccinimide (ACRL-PEG-NHS, $3400\ g\ mol^{-1}$) was purchased from Nektar Therapeutics. GRGDS peptide was purchased from Bachem Bioscience Inc. Photoinitiator Irgacure 2959 (I2959) was obtained from Ciba Specialty Chemicals and used as received. All other chemicals used were of reagent grade and were used without further purification. For in vitro cell culture, murine myofibroblast cells (C2C12) were obtained from the American Type Culture Collection (Manassas, VA) and cultured in Dulbecco's modified Eagle's medium purchased from Invitrogen (Carlsbad, CA), supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin, all obtained from Invitrogen. Phosphate-buffered saline (PBS) was purchased from Invitrogen. FITC-labeled Phalloidin were purchased from Sigma–Aldrich (St. Louis, MO). Live/Dead® Viability/Cytotoxicity Kit was purchased from Molecular Probes, Inc. (Eugene, OR).

2.2. Methods

2.2.1. Synthesis of degradable macromonomers

4KG5 copolymer was prepared from the reaction of PEG ($4000\ g\ mol^{-1}$) and glycolide at a ratio of 5 mol glyco-

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