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Cell-adhesive and mechanically tunable glucose-based biodegradable hydrogels

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ABSTRACT

The development of materials with biomimetic mechanical and biological properties is of great interest for regenerative medicine applications. In particular, hydrogels are a promising class of biomaterials due to their high water content, which mimics that of natural tissues. We have synthesized a hydrophilic biodegradable polymer, designated poly(glucose malate)methacrylate (PGMma), which is composed of glucose and malic acid, commonly found in the human metabolic system. This polymer is made photocrosslinkable by the incorporation of methacrylate groups. The resulting properties of the hydrogels can be tuned by altering the reacting ratio of the starting materials, the degree of methacrylation, and the polymer concentration of the resultant hydrogel. Hydrogels exhibited compressive moduli ranging from 1.8 ± 0.4 kPa to 172.7 ± 36 kPa with compressive strain at failure from 37.5 ± 0.9% to 61.2 ± 1.1%, and hydration by mass ranging from 18.7 ± 0.5% to 114.1 ± 1.3%. PGMma hydrogels also showed a broad range of degradation rates and were cell-adhesive, enabling the spreading of adherent cells. Overall, this work introduces a class of cell-adhesive, mechanically tunable and biodegradable glucose-based hydrogels that may be useful for various tissue engineering and cell culture applications.

1. Introduction

Synthetic biodegradable polymers are of great interest for various biomedical applications such as drug delivery and tissue engineering [1]. For many biomedical applications, it is desirable to control the mechanical and biological properties of these materials [2]. Previously, various synthetic biodegradable polymers have been made to improve the properties of biomaterials for various applications [3–10]. However, these polymers are generally hydrophobic, greatly limiting the ability to encapsulate cells into the construct. Hydrogels, a class of biomaterials formed from hydrophilic polymers, are attractive for many reasons, such as their biocompatibility and the fact that they contain similar water content and mechanical properties as natural tissues [11–13]. In particular, hydrogels from photocrosslinkable polymers can be injected into the body, encapsulate cells uniformly, and enable temporal and spatial control in the fabrication of complex structures [11,12,14–16].

Over the years, a number of synthetic hydrogels have been developed for biomedical applications. Poly(2-hydroxyethyl methacrylate) (PHEMA), poly(N-isopropylacrylamide) (PNIPAAm), poly (vinyl alcohol) (PVA) and their derivatives are vinyl monomer based synthetic polymers that have been studied for applications such as contact lenses, drug delivery, and tissue engineering [17–23]. However, these hydrogels are non-degradable and their vinyl monomers and crosslinking molecules may be toxic [11]. Poly(ethylene glycol) (PEG) is one of the most studied hydrophilic biomaterials and has been approved by the FDA for certain applications. While PEG hydrogels are inert and exhibit low toxicity, they are not biodegradable. To render PEG biodegradable, several methods have been developed, such as co-polymerization of PEG with biodegradable poly(α-hydroxy esters), such as poly(lactic acid) (PLA) and poly(glycolic acid) (PGA), or with peptides that are enzymatically degradable [24–27]. Recently, a new hydrophilic biodegradable polymer, poly(xylitol citrate)methacrylate (PXCma), was synthesized from non-toxic starting monomers: xylitol and citric acid [9]. While PEG-based hydrogels include PEG macromers in their degradation products, PXCma hydrogels completely degrade into the original monomers, xylitol and citric acid, which are endogenous to the human metabolic system. However, despite its merits, PXCma was mechanically weak and not cell-adherent.

In this study, we synthesized a hydrophilic biodegradable polymer, designated poly(glucose malate)methacrylate (PGMma), which can form hydrogels that degrade into the starting monomers, glucose and malic acid. Glucose is a metabolic intermediate, which is commonly available, inexpensive and could potentially be used as an energy resource by cells when released through degradation of the polymer. Malic acid is non-toxic, an ingredient in many foods and its anion is an intermediate in the citric acid cycle [28]. The polymer form, poly(malic acid), has been demonstrated in various

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biomedical applications [29,30]. As in previous reports on using polycondensation reactions with multifunctional monomer(s) to synthesize biodegradable elastomers or hydrogels, we used two hydrophilic, multifunctional monomers, glucose and malic acid, to form a randomly branched, hydrophilic, and hydrolyzable polyester, poly(glucose malate) (PGM) by polycondensation [3,4,9,10]. After the polycondensation reaction, the remaining unreacted hydroxyl groups enabled further functionalization. To render the PGM photocrosslinkable, we functionalized the free hydroxyl groups of PGM with methacrylate groups by reacting PGM with methacrylic anhydride, as previously described for the methacrylation of hyaluronic acid [31,32]. Finally, we used the resulting photocrosslinkable PGMma to fabricate hydrogels through a light-initiated crosslinking process. We characterized the properties of the resulting hydrogel as a function of the stoichiometric ratio of the starting monomers, the degree of methacrylation, and polymer concentration. Furthermore, cell-adhesion tests showed that PGMma is cell-adhesive. Given its broad range of properties, PGMma may be useful for various tissue engineering applications or as a material in cell culture.

2. Materials and methods

2.1. Synthesis of PGMma polymers

All chemicals were obtained from Sigma–Aldrich. PGMma was synthesized as follows (Fig. 1). Two batches of PGMs with varying molar ratios of starting materials were synthesized. D-(+)-glucose and DL-malic acid were mixed in a round bottom flask with molar ratio of 1:1 for PGM1:1 and 1:2 for PGM1:2. They were heated and stirred under argon gas to 135 °C. Under these conditions, malic acid melted and dissolved the glucose in the mixture. After the glucose dissolved completely, vacuum was applied for 5 min and the resulting viscous intermediate material was cured at 90 °C for 2 days inside a vacuum oven. The resulting mixture was dissolved in distilled water, dialyzed by a membrane with molecular weight cutoff of 6–8 kD, and lyophilized. PGM macromers were methacrylated as previously described [32]. Briefly, methacrylic anhydride was reacted with PGM in distilled water on ice for 24 h. The pH of the solution was kept at 8 with 5 N NaOH. The solution was then dialyzed (MW cutoff 6–8 kDa) for 48 h, and lyophilized to yield PGMma. To modify the degree of methacrylation (DM), we added varying amounts of methacrylic anhydride (i.e. 1 ml, 2 ml, and 4 ml per 1 g of PGM).

2.2. Characterization of PGMma polymers

^{1}H NMR spectra of PGM and PGMma polymers were obtained in D_2O on a Varian 300 NMR spectrometer. The chemical composition of the polymers was calculated by the signal integrals of glucose, malic acid and methacrylate groups. The molar ratio of glucose and malic acid in the polymers were calculated using the peaks at 3.2–4.5 ppm from glucose compared with peaks at 2.7–3.2 ppm from malic acid. DM was calculated by the peaks at 1.8–2.0 ppm from methacrylate groups and the peaks from malic acid. DM was defined as the number of methacrylate groups divided by the number of free hydroxyl groups prior to the methacrylation reaction. Since one hydroxyl group is always removed whenever either a glucose or malic acid monomer becomes attached to the PGM polymer, regardless of the location, the total number of free hydroxyl groups in the resulting PGM polymer will not vary based on the degree of branching. Therefore, the number of hydroxyl groups was counted as the number of glucose monomers multiplied by 3 plus the number of malic acid monomers because, regardless of how the polymer is branched, the number of hydroxyl groups in the resulting PGM structure will be the same as the simplified case where each glucose monomer has three remaining hydroxyl groups and each malic acid monomer has one remaining hydroxyl group. FT-IR analysis was performed on a Bruker Alpha FT-IR spectrometer. Molecular weight distribution was determined by gel permeation chromatography (GPC, Viscotek TDAmax) using PEG standard, 0.05 M $NaNO_3$ aqueous solution as eluentia and a 3× Viscotek GPMWxL column with triple detection (refractive index, light scattering, and viscometer detector). By combining the data set obtained from three kinds of detectors, the absolute molecular weight was calculated with high accuracy independent of the degree of branching. Densities of polymers were measured with an Ultrapycnometer 1000 (Quantacrome Instruments).

2.3. Photopolymerization

PGMma polymer solutions were prepared by dissolving PGMma polymers at three different concentrations (10, 15, 20 wt.%) in phosphate buffered saline (PBS) containing 0.05 wt.% photoinitiator (Irgacure 2959). They were subsequently molded into disks (~8 mm diameter, ~1 mm thickness) and cured by exposure to light (320–500 nm, ~4 mW cm^{-2} for 10 min) (EXFO OmniCure S2000).

2.4. Hydrogel characterization

Sol content was determined by measuring the difference in mass of dried sample before (m_i) and after (m_f) immersion in distilled water with agitation for 1 h. It was calculated as:

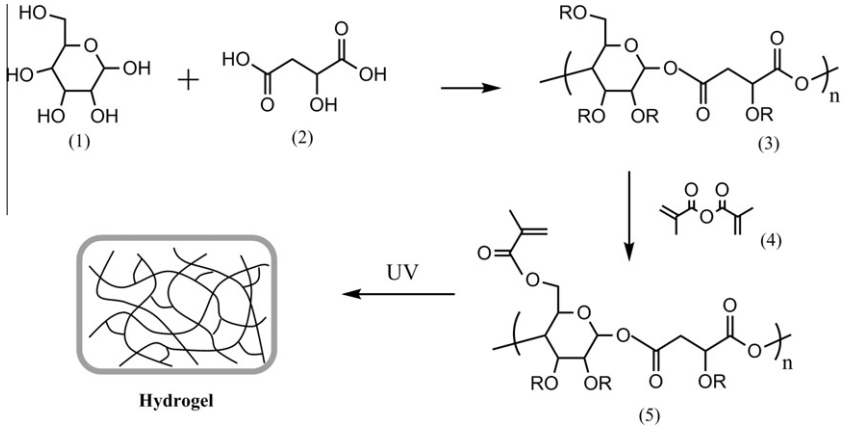

Fig. 1. General synthetic scheme of PGMma hydrogel. (1) Glucose, (2) malic acid, (3) PGM, (4) methacrylic anhydride, (5) PGMma. PGM and PGMma is a randomly branched polymer as R can be H, glucose, malic acid, or a polymer chain.

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