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Poly(ethylene glycol)-grafted poly(propylene fumarate) networks and parabolic dependence of MC3T3 cell behavior on the network composition

Lei Cai, Kan Wang, Shanfeng Wang*

Department of Materials Science and Engineering, The University of Tennessee, Knoxville, TN 37996, USA

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ABSTRACT

We present a method to modify poly(propylene fumarate) (PPF), an injectable biomaterial for bone-tissue-engineering applications, by photo-crosslinking it with methoxy poly(ethylene glycol) monoacrylate (mPEGA) at various mPEGA compositions of 0–30%. The bulk properties such as thermal and rheological properties of uncrosslinked mPEGA/PPF blends and the mechanical properties of photo-crosslinked mPEGA/PPF blends were also investigated and correlated with surface characteristics to elaborate on the modulation of mouse MC3T3 cell adhesion, spreading, proliferation and differentiation through controlled physicochemical properties. Unlike PPF crosslinked with PEG dimethacrylate, mPEGA chains tethered on the surface of crosslinked PPF did not influence the swelling ratio in water while increased surface hydrophilicity greatly. Meanwhile, surface frictional coefficient and the capability of adsorbing proteins from cell culture medium decreased continuously with increasing the mPEGA composition in mPEGA/PPF networks. Demonstrating cell repulsive effect at the mPEGA compositions higher than 7%, the modified surfaces improved MC3T3 cell attachment, proliferation and differentiation, which reached maxima at the mPEGA composition of 5–7%. Besides revealing that mPEGA pendant chains could enhance cell responses by increasing hydrophilicity when their fraction on the hydrophobic surface was small, the present study also offered a new method of improving the wettability and performance of the scaffolds made from PPF for bone repair.

1. Introduction

Poly(propylene fumarate) (PPF) is a promising candidate biomaterial for orthopedic applications because its crosslinked form has excellent biocompatibility, mechanical properties, osteoconductivity, and biodegradability [1–3]. PPF has unique handling characteristics as it can be injected together with diluents and crosslinked by ultraviolet (UV) light or thermal initiation to fill bone defects or fabricate three-dimensional (3D) porous scaffolds with controllable pore size, porosity, and geometry [4,5]. Various oligomers or small molecules have been incorporated with PPF through copolymerization or blending and then crosslinking to achieve biomaterials with a wide range of tunable bulk and surface properties [6–15]. These oligomers and small molecules include poly(ε-caprolactone) (PCL) diols [6], PCL fumarates (PCLFs) [7], polyethylene glycols (PEGs) [8–12], PEG dimethacrylates (PEGDMAs) [13], and diethyl fumarate (DEF) [14,15].

Hydrophobicity is one limitation for crosslinked PPF in its applications because the wettability of medical devices is critical for allowing surrounding body fluids to penetrate and supply nutrients to cells and tissue that grow inside. Photo-crosslinked PPF has a water contact angle of ~90° at room temperature [7]. In this study, we aim to modify and control the bulk and surface properties of crosslinked PPF with improved wettability which can be used to regulate cell responses. Surface graft reactions using PEG tethered chains or other hydrophilic polymers have been extensively applied to improve material hydrophilicity and wettability, rendering bio-interfacial properties such as protein and cell resistance and suppression of immunogenic activities, which are particularly crucial for blood compatibility [16–29]. As mentioned earlier, PPF network has been incorporated with PEG in the Mikos group and much attention was paid to hydrogel formed by the water-swellable PEG segments constrained by the PPF crosslinks [8–13]. Reduced platelet adhesion was further revealed on the hydrogels formed by PPF-*co*-PEG multi-block copolymers [12].

Instead of forming hydrogels, we presently photo-crosslink PPF with methoxy PEG monoacrylate (mPEGA) short chains and consequently achieve non-water-swellable PPF networks that is modified with PEG chains tethered on the surface and also inside.

* Corresponding author. Tel.: +1 865 974 7809; fax: +1 865 974 4115.
E-mail address: swang16@utk.edu (S. Wang).

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For mPEGA used here, we applied a new synthetic method by using potassium carbonate (K_2CO_3) as the proton scavenger in the condensation between methoxy PEG and acryloyl chloride. In contrast, mPEGA, PEGDMAs, and PEG diacrylates were previously synthesized via acrylation in the presence of triethylamine [30–33], which was recently reported by us to form colored, water-soluble cytotoxic complexes with unsaturated acyl chlorides [34–38]. In this study, mPEGA with a double bond at one end can be incorporated together as pendant chains with PPF network via free radical polymerization. By grafting mPEGA short chains, we are able to modulate the mechanical properties, improve the hydrophilicity and wettability, and lubricate the surface of photo-crosslinked PPF network, consequently regulate protein adsorption and cell behavior.

Besides developing injectable, crosslinkable, non-cytotoxic, and biodegradable polymeric systems with improved processability and controllable bulk and surface properties such as swelling, thermal, mechanical, rheological properties, topology, frictional coefficient, hydrophilicity, and the capability of adsorbing proteins, another goal of this study is to investigate cell-biomaterial interactions on crosslinked mPEGA/PPF blends with varied surface physicochemical properties. Surface chemistry, morphological features, and surface stiffness are three major categories of factors in determining cell-biomaterial interactions, the essential and fundamental task in developing biomaterials for tissue engineering and clinical applications [39–41]. In the present study, with different amounts of mPEGA chains grafted onto PPF networks, we are able to control material characteristics and allow them to work collectively on modulating mouse MC3T3 cell behavior that includes cell attachment, spreading, proliferation, and differentiation. An unusual parabolic dependence of cell responses on the composition of crosslinked mPEGA/PPF blends is to be discussed in terms of competition between different factors.

2. Materials and methods

2.1. Synthesis of methoxy poly(ethylene glycol) monoacrylate (mPEGA) and polypropylene fumarate (PPF)

Methoxy poly(ethylene glycol) (mPEG) with a nominal molecular weight of 350 g mol^{-1} was purchased from Sigma (St. Louis, MO). All other chemicals were purchased from Sigma unless noted otherwise. Methylene chloride was dried and distilled over calcium hydride (CaH_2) before the reaction. Acryloyl chloride was used as received. Ground K_2CO_3 was dried at 100 °C overnight and then cooled down in vacuum. As described in Scheme 1, mPEG, acryloyl chloride and K_2CO_3 were measured in a molar ratio of 1:3:3. mPEG was dissolved in methylene chloride (1:2 v/v) and placed in a 250 mL three-neck flask along with K_2CO_3 powder. The mixture was stirred with a magnetic stirrer, to which a solution of acryloyl chloride in methylene chloride (1:10 v/v) was added dropwise using a pressure-equalizing dropping funnel. The reaction was maintained at room temperature under nitrogen for 24 h. After reaction, the mixture was filtered to remove the solids composed of KCl, $KHCO_3$, and unreacted K_2CO_3. The filtrate was then rotary-evaporated and added dropwise to diethyl ether. The oil-like product was further dried in vacuum. Gel permeation chromatography (GPC) was carried out at room temperature using EcoSEC GPC system (Tosoh Bioscience LLC, Montgomeryville, PA) with a RI-8320 detector (Tosoh Bioscience LLC) to measure the molecular weights (weight-average M_w, and number-average M_n) and polydispersity M_w/M_n of mPEG and mPEGA. Tetrahydrofuran (THF) was used as the eluent at a flow rate of 0.35 mL/min and standard monodisperse polystyrenes (PStQuick, Tosoh Bioscience LLC) were used for calibration. PPF (M_n = 1820 g mol^{-1}, M_w = 3410 g mol^{-1}) was synthesized in our laboratory according to the procedure described in earlier reports [1–3,6]. Homogeneous mPEGA/PPF blends were prepared by dissolving mPEGA and PPF with mPEGA weight compositions (φ_{mPEGA}) of 5%, 10%, 20%, and 30% in methylene chloride and then fully dried.

2.2. Characterization of mPEGA/PPF blends

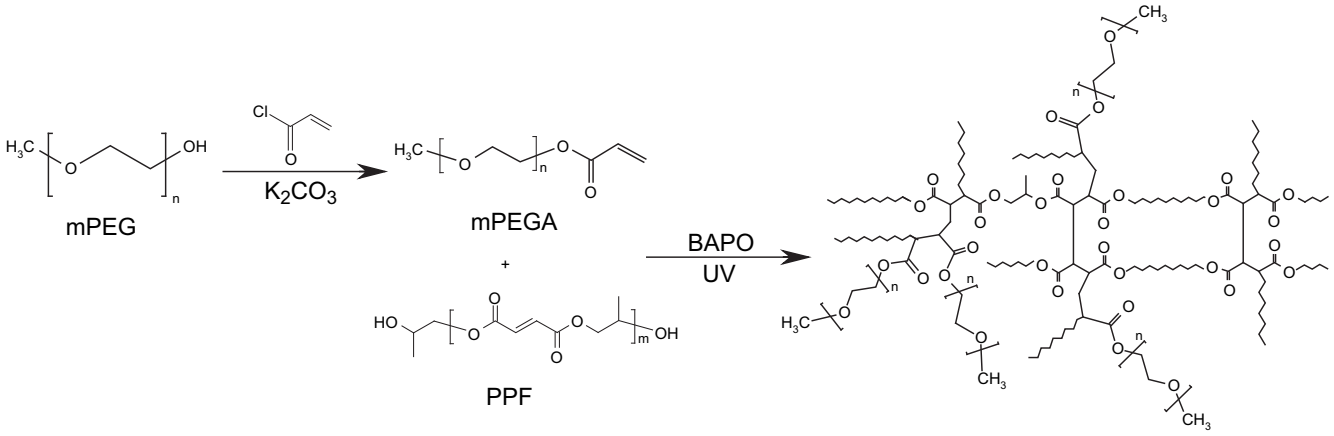
Fourier Transform Infrared (FTIR) spectra were obtained on a Perkin Elmer Spectrum Spotlight 300 spectrometer with a dedicated Diamond Attenuated Total Reflectance (DATR) accessory. ^1H Nuclear Magnetic Resonance (NMR) spectra were acquired on a Varian Mercury 300 spectrometer using $CDCl_3$ containing tetramethylsilane (TMS) as the solvent. Differential Scanning Calorimetry (DSC) measurements were performed on a Perkin Elmer Diamond differential scanning calorimeter in a nitrogen atmosphere. To keep the same thermal history, each sample was first heated from room temperature to 100 °C and cooled to −90 °C at 5 °C/min. Then a subsequent heating run was performed from −90 °C to 100 °C at 10 °C/min. Thermogravimetric analysis (TGA) was performed on a TA Q50 in flowing nitrogen at a heating rate of 20 °C/min. Zero-shear viscosities (η_0) of uncrosslinked mPEGA/PPF blends were measured from the Newtonian region at temperatures of 25, 37, 60, and 80 °C using a strain-controlled rheometer (RDS-2, Rheometric Scientific) in the frequency range of 0.1–100 rad/s. A 25 mm diameter parallel plate flow cell and a gap of ~0.5 mm were used.

2.3. Photo-crosslinking of mPEGA/PPF

Photo-crosslinking of mPEGA/PPF was initiated by the UV light (λ = 315–380 nm) generated from a Spectroline high-intensity long-wave UV lamp (SB-100P, Intensity: 4800 uw/cm^2) with the help of photoinitiator phenyl bis(2,4,6-trimethyl benzoyl) phosphine oxide (BAPO, Irgacure 819™, Ciba Specialty Chemicals, Tarrytown, NY). In crosslinking, 75 μL of BAPO/CH_2Cl_2 (300 mg/1.5 mL) solution was mixed with mPEGA/PPF blend solution in CH_2Cl_2 (1.5 g/500 μL). The mixture was then transferred into a mold consisting of two glass plates (2.1 mm, thickness) and a Teflon spacer (0.37 mm, thickness) or a silicone spacer (1.0 mm, thickness). The filled mold was placed under UV light for 20 min at a distance of ~7 cm from the lamp head. Crosslinked mPEGA/PPF sheets or disks were removed from the mold after cooled down to room temperature and then completely dried in a vacuum oven. Except for the measurements of swelling ratio and gel fraction, original crosslinked samples were soaked in acetone to remove the sol fraction and completely dried in vacuum for physical characterizations and cell studies.

2.4. Characterizations of mPEGA/PPF networks

FTIR, DSC, and TGA were performed on crosslinked mPEGA/PPFs as described in Section 2.2. The swelling ratios and gel fractions of mPEGA/PPF networks were determined by immersing two crosslinked mPEGA/PPF disks (~8 × ~1.0 mm,



Scheme 1. Synthesis and photo-crosslinking of mPEGA/PPF.