



Parabolic dependence of material properties and cell behavior on the composition of polymer networks via simultaneously controlling crosslinking density and crystallinity

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ABSTRACT

A systematic investigation was performed on regulating materials properties and cell behavior using hybrid networks composed of amorphous poly(propylene fumarate) (PPF) and three poly(ϵ -caprolactone) diacrylates (PCLDAs) with variance in crystallinity and melting temperature. Through controlling both crosslinking density and crystallinity in the photo-crosslinked PPF/PCLDA blends, mechanical properties could be tuned efficiently in a wide range. For PCLDA synthesized from a low-molecular weight PCL diol precursor with a low crystallinity and a low melting point, crosslinks could completely suppress crystalline domains over the composition range in the PPF/PCLDA networks. Consequently, tensile, shear, torsional, and compression moduli all increased with the composition of PPF or the crosslinking density continuously for amorphous PPF/PCLDA networks. For PCLDAs synthesized using two PCL diols with higher molecular weights, crystallinity remained for the PCLDA compositions between ~80% and 100%. Minimum moduli and tensile stress at break were found at the lowest required composition of PPF for suppressing crystallinity. Surface physicochemical properties and morphology of the crosslinked blend disks have been characterized and their capabilities of adsorbing proteins from cell culture medium have been determined. Using both mouse MC3T3-E1 cells and rat Schwann cell precursor line (Spl201) cells, cell responses to these polymer networks such as cell adhesion, spreading, and proliferation were found to be dramatically distinct on different polymer networks and demonstrated non-monotonic or parabolic dependence on the network composition, coincident with the composition dependence of the mechanical properties.

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1. Introduction

Cell–material interactions are essential to the tissue-engineering applications of biomaterials [1–3]. In the past several decades, pioneer scientists have achieved great understanding on the roles of the surface physicochemical characteristics of biomaterials in determining cell behavior [1–3]. These factors can be divided into three major categories: surface chemistry such as hydrophilicity, charge characteristics and density, surface morphology, and surface mechanical properties [1–3]. Surface physicochemical characteristics are correlated together to influence cell behavior. Since Pelham and Wang reported that surface stiffness of polymer substrate could play a critical role in determining cell phenotype and proliferation [4,5], many investigations have been performed on numerous polymeric biomaterials with varied

mechanical properties, particularly hydrogels coated with adhesive proteins [6–9]. Although the majority of all existing polymers are hydrophobic and many of them have been applied in clinical applications, less attention has been paid to how their surface stiffness influences cell behavior except a few hydrophobic polymer networks [10–15]. The lack of extensive investigations on this topic may be due to no appropriate, wide range of mechanical properties of hydrophobic polymer systems to demonstrate the dependence of cell behavior.

In this study, we used model blend systems composed of amorphous poly(propylene fumarate) (PPF) and three semi-crystalline poly(ϵ -caprolactone) diacrylates (PCLDAs), all of which were photo-crosslinkable and hydrophobic [15–23]. As demonstrated in Fig. 1, PPF is a bone–tissue-engineering biomaterial designed to be injectable and able to harden *in situ* in bone defects by thermal or UV initiation [16–23]. Having a high crosslinking density, crosslinked PPF is often stiff [12,16–23]. Used as another component in the present blends, PCLDAs (Fig. 1) were synthesized via a facile condensation method and employed as model polymers to

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demonstrate the role of crystallite-strengthened mechanical properties in enhancing cell attachment and proliferation [15]. Previously, one author and his colleagues have applied hybrid polymer networks composed of PPF and PCL fumarate (PCLF) to modulate material mechanical properties and consequently the cell responses to the networks using rat bone marrow stromal cells (BMSCs) and Schwann cell precursor line (SpL201) cells [12]. Meanwhile, a series of multi-block copolymer PPF-co-PCL consisting of PPF and PCL blocks with controllable lengths and compositions has been synthesized and crosslinked into 2D substrates and 3D scaffolds with tunable physicochemical properties for satisfying the needs in bone and peripheral nerve tissue repair [24,25]. In these two studies [12,25], the composition range for forming crystalline networks at 37 °C was either absent or limited. Consequently, only monotonous composition dependence was found for the thermal and mechanical properties of crosslinked PPF/PCLF blends and PPF-co-PCL copolymers [12,25]. Our goal in this study is to investigate how crystalline structure and crosslinking density can be applied to modulate both bulk and surface properties of polymer networks collectively and then to regulate cell behavior on these networks.

As crystallinity increases with the molecular weight of PCL diol precursor, PCLDAs or PCLFs prepared from these PCL diols also demonstrate different crystallinities and melting points [13–15,26–28]. When the nominal molecular weight of PCL diol is as low as 530 g mol⁻¹, the crystallinity of PCLDA or PCLF diminishes at room temperature after crosslinking [12–15,27,28]. For another two nominal molecular weights of PCL diols, 1250 and 2000 g mol⁻¹, crosslinked PCLDAs or PCLFs are still crystalline at room temperature or even body temperature [12–15,27,28]. As indicated by the chemical structures in Fig. 1, the number and density of crosslinkable segments in one PPF chain are much higher than in PCLDA. Consequently, the mesh size of polymer network can be modulated by crosslinking PPF/PCLDA blends with different compositions. Because crosslinks can suppress the crystallinity of polymer networks [27,29,30], crystallinity will work with crosslinking density in modulating the mechanical properties of PPF/PCLDA1250 or 2000 networks in a parabolic means instead of showing monotonic composition dependence for PPF/PCLDA530 networks. Such non-monotonic dependence of material properties on polymer composition is well known in poly(lactic-co-glycolic acid) (PLGA) copolymers with different compositions of poly(lactic acid) (PLA) and poly(glycolic acid) (PGA) because of varied crystallinity [31]. Cell behavior also demonstrates non-monotonic dependence on substrate composition in numerous systems although materials properties such as wettability, surface compliance, and adhesive-ligand density can vary progressively with composition [1,3,19,32–35].

Tissue engineering requires non-cytotoxic and biodegradable polymeric materials with biocompatibility, feasibility of fabrication, and controllability of mechanical properties to satisfy clinical needs ranging from hard-tissue replacement to soft tissue replacement. Meanwhile, diverse applications also require polymer materials to possess different surface physicochemical properties to induce distinct cell/tissue responses. Therefore, the goal of this study is not only to modulate materials properties using photo-crosslinkable

polymer systems but also to investigate how these controllable material characteristics can be used to regulate cell behavior. Among three crosslinked PCLDAs, PCLDA2000 with the highest crystallinity and melting point was found to favor cell attachment and proliferation most [15]. Our hypothesis was that cell behavior should not be monotonic with the network composition as the modulated mechanical properties would demonstrate a minimum when the crosslinking density was just sufficient to suppress the crystallinity. As different applications require different cell attachment, proliferation, and differentiation, it is of importance to demonstrate how this non-monotonic or parabolic dependence of both materials properties and cell behavior on polymer composition can be achieved via controlling crystallinity and crosslinking density simultaneously.

In this paper, first we present the materials properties including bulk and surface characteristics of uncrosslinked and crosslinked PPF/PCLDA blends as well as the pure components and the correlation between them. Then we demonstrate the potentials of the materials in hard and soft tissue replacements exemplified by bone and nerve regeneration, using both mouse pre-osteoblastic MC3T3-E1 cells and rat SpL201 cells to evaluate cell toxicity, attachment, spreading, and proliferation. Finally, we emphasize on parabolic dependence of cell behavior on the composition of PPF/PCLDA networks.

2. Materials and methods

2.1. Materials

PPF and PCLDAs were synthesized using the methods described previously [15–19,24]. Briefly, PPF with a weight-average molecular weight (M_w) of 3410 g/mol and a number-average molecular weight (M_n) of 1820 g/mol was synthesized using a two-step polycondensation between diethyl fumarate and 1,2-propane diol in the presence of crosslinking inhibitor hydroquinone and catalyst ZnCl₂. Acrylation of PCL diols (Sigma–Aldrich, Milwaukee, WI) having different nominal molecular weights of 530, 1250, and 2000 g/mol was performed with acryloyl chloride in the presence of potassium carbonate [15,36]. The resulted PCLDA530, 1250, and 2000 had M_n of 1120, 2990, 3510 g mol⁻¹ and M_w of 1390, 4150, 5150 g mol⁻¹, respectively [15]. All other chemicals were purchased from Aldrich unless otherwise noted. PPF/PCLDA blends were prepared by dissolving PPF and PCLDA at different ratios in a co-solvent methylene chloride and then dried completely in a vacuum oven for physical characterizations.

2.2. Photo-crosslinking

Photo-initiator, phenyl bis(2,4,6-trimethyl benzoyl) phosphine oxide (BAPO, IRGACURE 819™) used in this study was a gift from Ciba Specialty Chemicals (Tarrytown, NY). Before crosslinking, pre-dissolved solution of 1.5 g PPF/PCLDA in 500 μL CH₂Cl₂ was mixed with 75 μL of BAPO/CH₂Cl₂ (300 mg/1.5 mL) solution. Homogeneous PPF/PCLDA/BAPO/CH₂Cl₂ mixture was transferred into a mold consisting of two glass plates (2.1 mm, thickness) and a Teflon spacer (0.37 mm, thickness) or a Silicon spacer (1.0 mm, thickness). The filled mold was placed under a high-intensity long-wave UV lamp (Spectrolite, SB-100P, Intensity: 4800 uw/cm², Wavelength: 315–380 nm) for 20 min with a distance of ~7 cm from the lamp head. Crosslinked PPF/PCLDA sheets or disks were removed from the mold after cooled down to room temperature and then dried in a vacuum oven. The swelling ratio and gel fraction of crosslinked PPF/PCLDA samples were measured using the methods described previously [12,15]. For other physical characterizations and cell studies, crosslinked samples were soaked in acetone for two days and dried completely in vacuum.

2.3. Characterization of bulk properties

Fourier transform infra-red (FTIR) spectra were obtained on a Perkin Elmer Spectrum Spotlight 300 spectrometer with Diamond Attenuated Total Reflectance

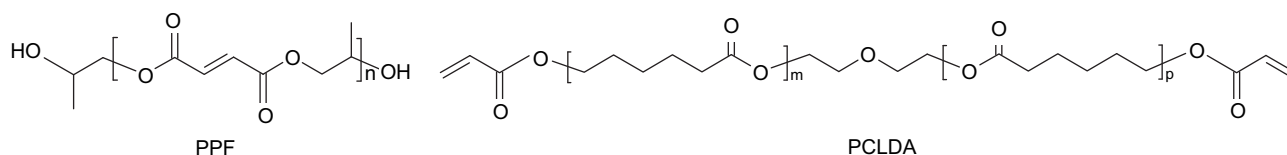


Fig. 1. Chemical structures of PPF and PCLDA.

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