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Biomimetic L-aspartic acid-derived functional poly(ester amide)s for vascular tissue engineering

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ABSTRACT

Functionalization of polymeric biomaterials permits the conjugation of cell signaling molecules capable of directing cell function. In this study, L-phenylalanine and L-aspartic acid were used to synthesize poly(ester amide)s (PEAs) with pendant carboxylic acid groups through an interfacial polycondensation approach. Human coronary artery smooth muscle cell (HCASMC) attachment, spreading and proliferation was observed on all PEA films. Vinculin expression at the cell periphery suggested that HCASMCs formed focal adhesions on the functional PEAs, while the absence of smooth muscle α-actin (SMαA) expression implied the cells adopted a proliferative phenotype. The PEAs were also electrospun to yield nanoscale three-dimensional (3-D) scaffolds with average fiber diameters ranging from 130 to 294 nm. Immunoblotting studies suggested a potential increase in SMαA and calponin expression from HCASMCs cultured on 3-D fibrous scaffolds when compared to 2-D films. X-ray photoelectron spectroscopy and immunofluorescence demonstrated the conjugation of transforming growth factor-β1 to the surface of the functional PEA through the pendant carboxylic acid groups. Taken together, this study demonstrates that PEAs containing aspartic acid are viable biomaterials for further investigation in vascular tissue engineering.

1. Introduction

Successful tissue engineering strategies rely on the design of appropriate biomaterials that mimic the natural extracellular matrix (ECM). Cells seeded on a degradable three-dimensional (3-D) scaffold can either be directly implanted in a host or matured in a bioreactor prior to implantation. As part of the biomaterials toolbox, the use of functionalized materials that can modulate cell function is attractive [1,2]. Degradable, functional polymers that permit the immobilization of growth factors or other signaling molecules may facilitate the development of new biomimetic biomaterials. Functional polymers with well-defined surface chemistry can be achieved through the introduction of trifunctional monomers during polymerization. Although synthetic monomers have been used in these co-polymerizations, significant research has focused on the incorporation of functional α-amino acids, yielding a variety of pendant functional groups [3]. The co-polymerization of functional α-amino acids provides polymers with the combined properties of synthetic polymers and naturally occurring polypeptides. Poly(ester amide)s (PEAs), a class of biodegradable polymers consisting of ester and amide linkages along the polymer backbone, can be synthesized from naturally occurring α-amino acids [4,5]. PEAs derived from α-amino acids have been investigated for drug delivery [6,7], non-viral gene delivery [8], stimuli-induced degradation [9], coatings for drug-eluting stents [10] and vascular tissue engineering [11–15].

The most appropriate choices to introduce functional amino acid side chains into PEAs for conjugating biomolecules are L-lysine, L-glutamic acid and L-aspartic acid. Our group has previously reported the incorporation of L-lysine into PEAs [12,16], to which model compounds *N*-acetyl-L-valine and 2-[2-(2-methoxyethoxy)ethoxy]acetic acid were conjugated [17], while also promoting vascular smooth muscle cell attachment, proliferation and focal adhesion formation [12,13]. The introduction of L-aspartic is complementary in that it can allow for the conjugation of N-terminated biomolecules, and will present a negative charge to cells owing to the pendant carboxylate at neutral pH, in comparison with the cationically charged pendant amine groups in the L-lysine functional PEA. However, data on the synthesis of suitable L-aspartic acid-containing PEA biomaterials is limited. For instance,

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water-soluble polydepsipeptides incorporating aspartic acid were synthesized by ring-opening polymerization of cyclodepsipeptides and tin 2-ethylhexanoate catalyst at elevated temperatures, which produced low molecular weight polymers with little practical utility [18]. More recently, Li and co-workers [19] have synthesized hyperbranched PEAs from α-amino acids including L-aspartic acid at elevated temperatures in the presence of catalyst after converting the functional groups to a methyl ester, limiting the availability of the pendant functionality. We have previously reported the incorporation of L-aspartic acid into PEAs comprising amino acids, dicarboxylic acids and diols through a homogeneous polycondensation [16]. However, the solution polymerization approach provided relatively low molecular weight polymers not suitable for biomaterials. It should be reiterated that polymeric biomaterials designed for tissue engineering applications must have sufficiently high molecular weight to be processed into films and scaffolds to have the necessary initial mechanical and dimensional stability for use in a cell culture environment.

Although there are several strategies to fabricate or regenerate vascular tissues, scaffold-guided tissue engineering remains the most frequently encountered approach [20]. Thus far, PEAs containing α-amino acids have been demonstrated to support vascular smooth muscle cell (VSMC) attachment [14,15], proliferation and focal adhesions [12,13]. In vascular tissue engineering, transforming growth factor-β1 (TGF-β1) is a target biomolecule as it has been reported to modulate smooth muscle cell proliferation, differentiation and up-regulation of the quiescent contractile phenotype [21–23]. The ability to deliver TGF-β1 easily and locally from a biodegradable PEA scaffold could aid in vascular tissue regeneration. The immobilization of TGF-β1 within a degradable scaffold may also promote prolonged signaling beneficial in elastogenesis and adoption of quiescent smooth muscle cells following controlled release of the cytokine. As the functional PEAs containing L-aspartic acid are anticipated to support VSMC adhesion and proliferation, TGF-β1 may further promote ECM production [24] and the contractile VSMC phenotype [25]. In view of the above, a synergistic effect in the development of a tissue-engineered vascular graft was sought.

Several key developments in the application of PEAs for vascular tissue engineering are herein described. PEAs containing L-aspartic acid were synthesized, characterized and evaluated through the in vitro culture of human coronary artery smooth muscle cells (HCASMCs). Nanoscale electrospun fibrous mats were prepared from PEAs and the effect of PEA fibrous topography and exogenous TGF-β1 on contractile phenotype marker protein expression of smooth muscle α-actin (SMαA) and calponin by HCASMCs were investigated. Finally, TGF-β1 was successfully conjugated onto the surface of functional PEAs containing L-aspartic acid.

2. Materials and methods

2.1. Materials

Di-*p*-toluenesulfonic acid salt monomers **1** and **2** and bis-L-aspartic acid-*β*-(*t*-butyl ester) diester **3** were prepared as previously reported (Scheme 1) [12,16,26]. Solvents were purchased from Caledon Labs (Georgetown, ON). All other chemicals were purchased from Sigma–Aldrich (Oakville, ON). Unless noted otherwise, all chemicals were used as received. Dichloromethane (DCM) was distilled from CaH_2 and dried over molecular sieves 4A. Flash chromatography was performed using silica gel 60 with a particle size range of 40–63 μm (SiliCycle Inc, Quebec City, QC). Dialysis was performed against *N*,*N*-dimethylformamide (DMF) with Spectra/Por 6 dialysis tubing (Spectrum Laboratories, Inc., Rancho Dominguez, CA), with a molecular weight cutoff of 25 kDa.

2.2. Methods

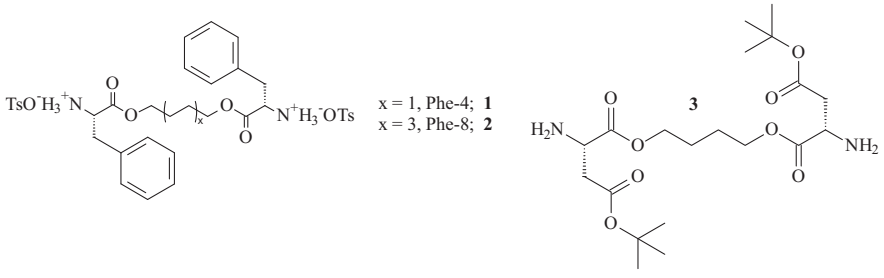
^{1}H (400 MHz) nuclear magnetic resonance (NMR) spectra were obtained on a Varian Inova 400 spectrometer (Varian Canada Inc., Mississauga, ON). Chemical shifts are reported in parts per million (ppm) and are calibrated against residual solvent signals of chloroform ($CDCl_3$, δ 7.27 ppm) or dimethyl sulfoxide ($DMSO\text{-}d_6$, δ 2.50 ppm). All coupling constants (*J*) are reported in Hertz (Hz). Fourier transform infrared (FTIR) spectra were obtained using a Bruker Tensor 27 (Bruker Corporation, Milton, ON) from KBr disks. Gel permeation chromatography (GPC) data were obtained using a Waters 2695 Separations Module equipped with a Waters 2414 Refractive Index Detector (Waters Limited, Mississauga, ON) and two PLgel 5 μm mixed-D (300 mm × 7.5 mm) columns connected in series (Varian Canada Inc., Mississauga, ON). Samples (5 mg ml^{-1}) dissolved in the eluent, which consisted of 10 mM LiBr and 1% (v/v) NEt_3 in DMF at 85 °C were injected (100 μl) at a flow rate of 1 ml min^{-1} and calibrated against polystyrene standards. Molecular weights are reported in kg mol^{-1}. Thermogravimetric analyses were performed on a SDT Q600 (TA Instruments–Waters LLC, New Castle, DE) under dry nitrogen at a heating rate of 20 °C min^{-1} up to 600 °C. Differential scanning calorimetry was performed on a DSC Q20 (TA Instruments–Waters LLC, New Castle, DE) at a heating rate of 10 °C min^{-1} from −50 to 200 °C. All sample masses ranged from 2 to 5 mg, and glass transition temperatures (T_gs) were obtained from the second heating cycle.

2.3. PEA nomenclature

The polymers are labeled by the number of methylene groups contributed by the diacid, the three-letter amino acid designation, and finally by the number of methylene groups in the diol—e.g. 8-Phe-8. Asp(O-*t*-Bu) represents the incorporation of the aspartic acid monomer **3** as a co-monomer at a mole ratio of 10% relative to the di-*p*-toluenesulfonic acid salt monomer **1** or **2**—e.g. 8-Phe-8-Asp(O-*t*-Bu)-4. The 4 represents the number of methylene groups in the butanediol moiety of **3**.

2.4. General procedure for interfacial polymerization of the functional poly(ester amide)s

The di-*p*-toluenesulfonic acid salt monomer **1** or **2** (0.9 equiv.) and sodium carbonate (2.0 equiv.) were dissolved in distilled water



Scheme 1. Structures of monomers 1–3.

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