Functionalized Poly(ester amide)s as Vascular Tissue Engineering Scaffolds

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Introduction:

The design of cardiovascular biomaterials focuses on biomimetic properties that are capable of eliciting specific cellular responses and directing tissue formation. In vascular tissue engineering, the ability to direct smooth muscle cells (SMCs) to acquire the quiescent, contractile phenotype following infiltration into a three-dimensional (3D) biodegradable scaffold and ensuing secretion of the extracellular matrix (ECM) remains a key challenge.[1]

Poly(ester amide)s (PEAs) derived from α-amino acids, diols, and diacids are promising materials due to their tunability and potential for either hydrolytic or enzymatic degradation.[2] The incorporation of amino acids with functional side chains, such as L-aspartic acid, would permit the conjugation of a desired biomolecule. The immobilization of transforming growth factor β1 (TGF β1) to these scaffolds may initially improve extracellular matrix protein secretion including elastin[3-5] whose absence has plagued the development of many tissue engineered blood vessels.[6] TGF β1 has also been shown to upregulate contractile phenotype markers in a biostable 3D scaffold model *in vitro*.[5] Therefore, the syntheses of these biodegradable functional PEAs and their subsequent evaluation as potential vascular scaffold materials were the primary objectives of this study.

Materials and Methods:

To synthesize these aspartic acid containing PEAs, selectively protected L-aspartic acid (Z-Asp(O*t*Bu)-OH) was coupled via carbodiimide chemistry to 1,4-butanediol followed by its hydrogenation to produce the functional monomer.[7] Additional monomers based on L-phenylalanine were synthesized via the acid catalyzed condensation of these amino acids with aliphatic diols: 1,4-butanediol and 1,8-octanediol.[2,8] The functional PEAs were obtained through the interfacial polymerization of sebacoyl chloride with monomers from each of the first two steps. Finally, deprotection of the *t*-butyl protecting groups with trifluoroacetic acid (TFA) yielded the pendant carboxylic acid groups.

Three-dimensional fibrous PEA mats were achieved by electrospinning various concentrations of PEA solutions (5-12 wt%) in co-solvent mixtures of chloroform ($CHCl_3$):*N*,*N*-dimethylformamide (DMF) (9:1). The fibers were collected on aluminum foil on either a static collector or on a rotating mandrel (1000 rpm).

Conjugation of TGF β1 to the surface of the pendant carboxylic acid groups of the electrospun fibrous mat was again achieved through carbodiimide chemistry (*N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride / *N*-hydroxysulfosuccinimide (EDC.HCl/sulfo-NHS)) and confirmed using X-ray photoelectron spectroscopy (XPS).

The viability of the PEAs as potential vascular scaffolds was assessed through the culture of human coronary artery smooth muscle cells (HCASMCs) on the surface of 3D scaffolds and compared to 2D controls. Cell morphology was examined with confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM).

For smooth muscle cell phenotype marker protein analysis via Western blot, protein lysates obtained from a radioimmunoprecipitation assay (RIPA) buffer supplemented with phenylmethylsulfonyl fluoride (PMSF) and a protease inhibitor mixture were quantified with Pierce 660 nm Protein Assay. The protein lysates were then separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, 10%) and subsequently transferred to a nitrocellulose blotting membrane. Following blocking with 5% non-fat dried milk, the membranes were incubated overnight at 4°C with either mouse monoclonal anti-α-actin, (1:250 dilution), rabbit polyclonal calponin 1/2/3 antibody (1:250 dilution) or mouse monoclonal anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 1:1000 dilution). Membranes were then incubated for 1 hour in a horseradish peroxidase (HRP)-conjugated secondary antibody (1:5000 dilution) followed by enhanced chemiluminescent detection.

Results and Discussion:

The structures of the aspartic acid containing PEAs were confirmed throughout the synthetic approach with nuclear magnetic resonance (NMR) spectroscopy and characterized via gel permeation chromatography (GPC), Fourier transform infrared (FTIR) spectroscopy, differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). Both non-functional and aspartic acid containing PEAs could be electrospun to yield fibrous mats with average fiber diameters ranging from 100 to 500 nm, which were polymer, molecular weight and concentration dependent.

HCASMC viability on these newly synthesized PEAs was confirmed with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays. The aspartic acid containing PEAs supported HCASMC proliferation as well or better than their corresponding non-functional counterparts. CLSM and SEM images showed that the HCASMCs were well-spread on the surface of the fibers up to 4 days culture. Preliminary Western blot data suggest that α-actin was upregulated in the non-functional 3D fibrous mats with their corresponding 2D films.

The aspartic acid containing PEAs were successfully synthesized and electrospun alongside the non-functional PEAs yielding nanoscale fibrous mats, which supported HCASMC attachment and spreading further suggesting their potential use as vascular biomaterials.

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Acknowledgements:

The authors would like to acknowledge the financial support from the Natural Sciences and Engineering Research Council of Canada (NSERC).