Controlled delivery of angiogenic factors from biodegradable amino acid-based fibers for therapeutic angiogenesis

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Introduction: Notwithstanding the rapid advances in treatment options, cardiovascular diseases are the leading cause of death in developed countries (Roger et al. 2012). In 2008, ischemic heart disease accounted for 7.3 million deaths worldwide (Gaziano et al. 2010). Many ischemic heart disease patients are ineligible for standard revascularization techniques such as angioplasty or bypass surgery (Williams et al. 2010). In these patients, the challenge to improve blood flow to the ischemic heart has led to extensive research and innovative approaches in the field of vascular regenerative medicine including therapeutic angiogenesis; which is the administration of growth factors (GFs) to induce new vessel formation. Angiogenic growth factors were commonly delivered either by bolus injection or infusion into systemic circulation or the tissues of interest, but the short half-life of these proteins usually resulted in their low local availability and diminished efficacy (Cao and Mooney 2007). One approach to overcome such limitations is the sustained delivery of growth factors at the desired site from electrospun fibers (Said et al. 2013). We hypothesize that poly(ester amide) (PEA) electrospun fibers can act as a controlled delivery system for GFs promoting therapeutic angiogenesis. Our goal is to optimize the electrospinning parameters of GFs-loaded PEA fibers and characterize the fabricated fibers in terms of morphological properties and *in vitro* scaffold degradation. Also, the *in vitro* GFs release kinetics were studied together with ensuring the bioactivity of the released GF.

Materials and Methods: Biodegradable electrospun PEAs fibers were fabricated, and loaded with a model protein, bovine serum albumin (BSA) using blend and emulsion electrospinning techniques, before the actual fibroblast growth factor-9 (FGF9) loading. Morphological analysis was carried out using scanning electron microscopy (SEM). In-vitro scaffold degradation of PEA fibers was examined in phosphate buffer saline (PBS; pH 7.4) at 37 ºC for a period up to 4 weeks, qualitatively using SEM and quantitatively by % mass loss determination method and molecular weight analysis using gel permeation chromatography. Moreover, *in vitro* scaffold degradation was carried out in a conditioned smooth muscle cells culture media (SMCM) at 37 ºC and examined qualitatively using SEM. The *in vitro* release kinetics of BSA and FGF9 from loaded PEA fibers was studied in PBS (pH 7.4) at 37 ºC over a period of 28 days. At predetermined time intervals, samples were assayed for the released BSA and FGF9 using BCA and ELISA kits respectively.

Results: Scanning electron micrographs of PEA electrospun fibers revealed non-woven mats of uniform fiber diameter distribution (200-500 nm), displaying high surface area-to-volume ratio and

considerable porosity. The effect of PBS (pH 7.4) at 37 °C on scaffolds degradation was minimal over the 4-week study period in comparison with drastic changes in case of the conditioned SMCM. Quantitative analysis showed 21% mass loss over the 28-day study period together with initial decrease in the molecular weight (MW) in week 1, followed by no change in MW over the following 3 weeks in PBS (pH 7.4) at 37 °C. BSA and FGF9-loaded PEA fibers exhibited controlled-release profile over 28 days with limited initial burst effect and 90% BSA liberation for the blend electrsopun BSA-loaded PEA fibers and 30% FGF9 liberation for the blend electrsopun FGF9-loaded PEA fibers. For emulsion electrospun fibers, the release profile showed decreased BSA and FGF9 release rates, reaching 37% and 15% respectively at day 28.

Discussion: Defect-free fibers, of mean fiber diameter ~250 nm, and uniform fiber diameter distribution were produced using a binary solvent system of chloroform ($CHCl_3$) and dimethyl sulfoxide (DMSO) (9:1). The use of a binary solvent system of $CHCl_3$/DMSO (9:1) instead of 100% $CHCl_3$ resulted in getting rid of the beads, which could be attributed to the high dielectric constant of DMSO (ε=46.6) contributing to the increased conductivity of the polymer solution. In-vitro scaffold degradation in PBS (pH 7.4) at 37 °C showed that scaffolds preserved their fibrous structure over the 4-week study period, with minimal degradation when compared to the drastic changes that took place in the degradation study in SMCM with loss of fibrous structure starting from week 2. In case of degradation in PBS (pH 7.4), the decrease in mass coupled with constant MW may indicate that the PEA scaffolds degradation in PBS (pH 7.4) is dominated by surface erosion. BSA and FGF9 release was sustained over the 28-day study with a limited burst effect. Absence of extensive burst release pointed out BSA/FGF9 compatibility with the polymer solution while the sustained release pattern confirmed adequate structural integrity of the fibers throughout the study. The reduced release rate in case of emulsion electrospun fibers was predictable based on the notion that the protein is encapsulated in the aqueous vesicles, which require the degradation of the polymeric matrix, followed by subsequent diffusion of the protein from the vesicles. These data support the premise of growth factor(s) sustained release from PEA electrospun fibers for their potential application in therapeutic angiogenesis.

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