**Controlled Delivery of Fibroblast Growth Factor-9 from Protein-analog Fibers for Therapeutic Angiogenesis**

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**Statement of Purpose:** Cardiovascular diseases are the leading cause of death in developed countries[1]. In 2008, ischemic heart disease accounted for 7.3 million deaths worldwide[2]. Many ischemic heart disease patients are ineligible for standard revascularization techniques such as angioplasty or bypass surgery[3]. In these patients, the challenge to improve blood flow to the ischemic heart has led to extensive research 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 are commonly delivered either by bolus injection or infusion into the systemic circulation or the tissues of interest, but the short half-life of these GFs usually resulted in their low local availability and diminished efficacy at the site of interest[4]. One approach to overcome such limitation is the sustained delivery of growth factors at the desired site from electrospun fibers[5]. We hypothesize that protein-analog poly(ester amide) (PEA) electrospun fibers can act as a controlled delivery system for fibroblast growth factor-9 (FGF9) intended for therapeutic angiogenesis applications. Our goal is to optimize the electrospinning parameters of FGF9-loaded PEA fibers and characterize the fabricated fibers in terms of morphological properties and *in vitro* scaffold degradation. Moreover, the *in vitro* release kinetics were studied together with the bioactivity of the released FGF9.

**Methods:** Biodegradable electrospun PEA fibers were fabricated and loaded with FGF9 using blend and emulsion electrospinning techniques. 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 four weeks, qualitatively using SEM and quantitatively by percentage mass loss determination method and molecular weight analysis using gel permeation chromatography. Moreover, *in vitro* degradation was carried out in a conditioned smooth muscle cells culture media (CSmGM) at 37 ºC and examined qualitatively using SEM. The *in vitro* release kinetics of FGF9 from loaded PEA fibers was studied in PBS (pH 7.4) at 37 ºC over a period of 28 days using ELISA kit. The bioactivity of the released FGF9 was tested using an MTT assay. The *in vitro* biocompatibility of the FGF9-loaded and unloaded electrospun fibers was tested using NIH-3T3 fibroblasts.

**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 four-week study period. Quantitative analysis showed 21% mass loss over the 28-day study period together with no change in molecular weight in PBS (pH 7.4) at 37 °C. FGF9-loaded PEA fibers exhibited controlled-release profile over the four-week study with limited initial burst effect and FGF9 liberation of 30% and 15% at day 28 for the blend and emulsion electrsopun FGF9-loaded PEA fibers, respectively. The MTT assay showed insignificant difference ($p < 0.05$) in fibroblast metabolic activity between the released and control FGF9, which indicate that FGF9 released from the fibers, prepared either by blend or emulsion techniques, was bioactive up to 28 days. Both FGF9-loaded and unloaded electrospun fibers were found to support the proliferation of fibroblasts up to five days even in serum-depleted conditions.

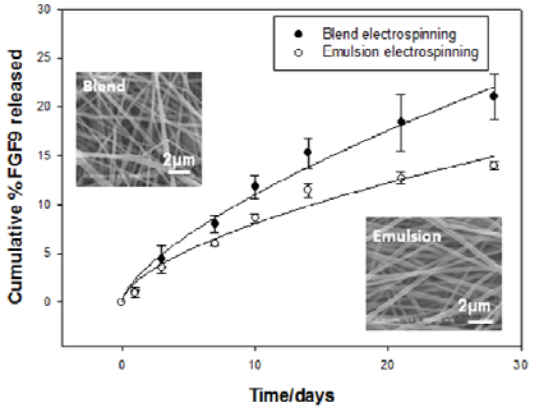


**Figure 1.** *In vitro* release of FGF9-loaded PEA fibers using blend and emulsion electrospinning techniques in PBS (pH=7.4) at 37 ºC.

**Conclusions:** Beads-free PEA fibers, of mean fiber diameter ~250 nm, and uniform fiber diameter distribution were produced using a binary solvent system of chloroform and dimethyl sulfoxide (9:1). *In vitro* scaffold degradation in PBS (pH 7.4) and the biorelevant medium at 37 °C showed that scaffolds preserved their fibrous structure over the four-week study period. In the case of degradation in PBS (pH 7.4), the decrease in mass coupled with constant molecular weight may indicate that the PEA scaffolds degradation in PBS (pH 7.4) is dominated by surface erosion. FGF9 release was sustained over the 28-day study with a limited burst effect and preserved FGF9 bioactivity. Moreover, the FGF9-loaded PEA elecrospun fibers were found to maintain the growth and proliferation of NIH-3T3 fibroblasts for up to five days. These data support the premise of growth factor(s) controlled delivery from protein-analog PEA electrospun fibers and their potential application in therapeutic angiogenesis and regenerative medicine.

**References:**

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