Incorporation of Alginate Microparticles Encapsulating Basic-Fibroblast Growth Factor in Elastomeric Micro-Fibrous Scaffolds to Encourage Cell Infiltration and Proliferation

Hirokazu Sakaguchi [1, 2], Sang-Ho Ye[2] and William R. Wagner[2]

[1] Toray Industries Inc., Tokyo, JAPAN, [2] McGowan Institute for Regenerative Medicine, Departments of Bioengineering & Surgery University of Pittsburgh, Pittsburgh, PA, USA

Statement of Purpose: Electrospinning of elastomers enables the creation the micro-fibrous scaffolds with mechanics that can be tuned by both polymer selection and processing conditions [1]. To encourage cell infiltration, a variety of techniques have been employed to generate large pores or low fiber densities [2]. Bioactivity has been built into such scaffolds with controlled release directly from fibers or from particulate components [3]. The objective of this work was to design an elastomeric scaffold that would provide both controlled release of an angiogenic growth factor and also utilize the particulates carrying the growth factor to serve as a means to create regions void of fibers for subsequent cellular infiltration. To achieve the above features, biodegradable and elastomeric poly(ester urethane)urea (PEUU) fibers were electrospun with co-deposition of alginate microparticles (Alg-mp) with the latter used to carry growth factor and to interrupt the fiber network.

Methods: Alg-mp were prepared by an emulsion method. sodium alginate aqueous solution (2 wt%) was mixed with a range of concentrations of basic fibroblast growth factor (b-FGF, 5-30 ng/mL) and bovine serum albumin (BSA) (weight ratio of BSA/b-FGF=100). The above solutions were added dropwise into 2 wt% dioctyl sulfosuccinate sodium salt (AOT)/ dichloromethane solution with stirring. After vortexing the solution for 1 min, 5 wt% $CaCl_2$ solution was added dropwise as a crosslinker and the mixed solutions were stirred for 1 h. Isopropanol was added to harden Alg-mp, and Alg-mp were collected after 5 cycles of centrifugation and washing with deionized water.

The resultant Alg-mp were observed by SEM and confocal laser scanning microscopy (CLSM). For CLSM observation, fluorescein isothiocyanate labeled b-FGF was used. Alg-mp were immersed in PBS at 37°C for the indicated time and the released amount of b-FGF was determined by ELISA. PEUU was synthesized as previously reported [4] and dissolved in hexafluoro isopropanol (HFIP). Alg-mp encapsulating b-FGF were suspended and dropped onto the collector during electrospinning of PEUU fibers. A voltage of +8 kV was applied to the PEUU/HFIP solution.

Results: Alg-mp were well-dispersed with a diameter range of 2 - 20 μm (**Figures 1a** and **b**). By CLSM imaging, Alg-mp encapsulated b-FGF was confirmed (**Figure 1b**). As shown in **Figure 1c**, an initial burst release of b-FGF was observed and release continued over a one week period. Initially, the Alg-mp suspension solution was co-electrosprayed with applied voltage, however, most Alg-mp were collapsed because of their low mechanical strength and pores of PEUU electrospun fibrous scaffold were clogged with them. To prevent Alg-mp collapse, the applied voltage for Alg-mp was gradually decreased. Ultimately, the Alg-mp suspension solution was deposited in the absence of voltage during the PEUU electrospinning, and resulted in Alg-mp remaining spherical and distinct within the scaffold (**Figure 1d**).

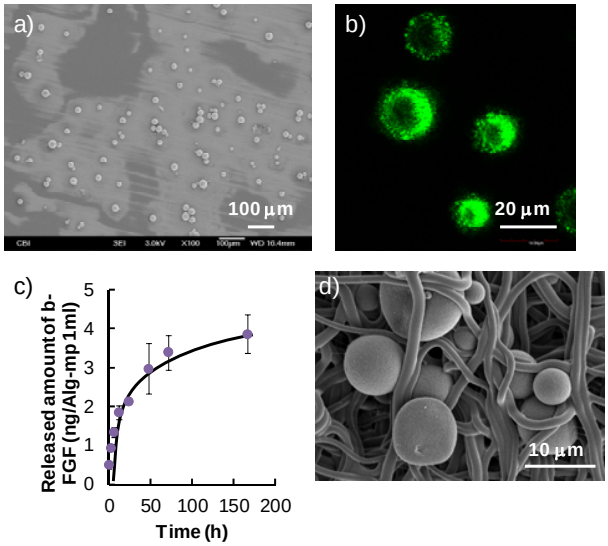

Figure 1 (a) SEM and (b) CLSM images of Alg-mp encapsulating b-FGF. (c) Release profile for b-FGF from Alg-mp. (d) SEM image of PEUU electrospun scaffold with co-deposited Alg-mp encapsulating b-FGF.

Conclusions: A composite elastomeric scaffold based on PEUU fibers and Alg-mp encapsulating b-FGF was generated in a manner that preserved the space holding property of the microparticles. The release of b-FGF from the Alg-mp was shown to continue over a 1 wk period in vitro. Previous studies have shown how the incorporation of such microparticles can alter the mechanical behavior of such scaffolds [5] and cell infiltration studies are ongoing. Such scaffolds could be designed for several applications, including cardiovascular tissue replacement.

References:

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