Spatial control of drug delivery in multilayered poly(vinyl alcohol) scaffold for tissue regeneration

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Statement of Purpose:
Multilayered scaffolds exhibit optimal mechanical properties and functionality for complex tissue regeneration. One major challenge for the application of multilayered scaffolds is the spatial control of drug release. To address this challenge, a poly (vinyl alcohol) (PVA) scaffold consisting of three layers was fabricated, with two layers containing fluorescent salt (FITC) encapsulated alginate-graft-poly(ethylene glycol) (AA-g-PEG) microspheres. AA-g-PEG microspheres exhibit mechanical integrity, a neutral charge, and can be further chemically modified. In this preliminary study, we examine microsphere containment and FITC release within the PVA scaffold. Our long term goal is to develop the multilayered scaffold for tissue regeneration and wound healing.

Methods:
Microsphere Fabrication: AA-g-PEG microspheres consisted of an ionically-crosslinked alginate-PEG copolymer.[1, 2] Alginate (MW=170 Kg/mol) was first mixed with N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride and N-hydroxysulfosuccinimide sodium salt for 30 min and then a 4% PEG (MW=2000 g/mol) solution was added; the ratio of alginate to PEG was 10:1. After reacting for 12 h at room temperature, the polymer solution was dialyzed against DI water for 4 days (MWCO=3.5Kg/mol); dialysis solution was changed every 12 h. The polymer solution was lyophilized and re-suspended in phosphate buffered saline (PBS) solution adjusted to pH=7.4. To make FITC-encapsulated AA-g-PEG microspheres, 0.1% (w/w) of FITC was added to a 1.0% (w/v) AA-g-PEG solution. The polymer solution was then slowly added to the vortex of 5% (v/v) Span 80 mineral oil solution and homogenized at 1200 rpm for 5 min. 400 µl of 30% (v/v) Tween 80 aqueous solution was then mixed with the reaction system and homogenized for another 5 min. While continuing mixing, 5 ml of 1M $CaCl_2$ solution was added to the emulsion and stirred for 30 min. Finally, 3 ml of 2-propanoal was dispersed in the reaction system to wash out the unreacted reactants and remove mineral oil residue. The AA-g-PEG microspheres were washed 2 additional times with 2-propanoal and 3 times with DI water.
Multilayered Scaffold Fabrication: Porous multilayered scaffolds were fabricated by freeze-thaw method using 3% (w/v) PVA (MW=145 Kg/mol) solution. For the bottom layer, the PVA solution was frozen at -20°C and subsequently thawed at room temperature, this was performed 3 times. After the last thaw, a high concentration of AA-g-PEG microspheres dispersed in PVA solution was added to form the middle layer of the scaffold and the freeze-thaw process was repeated an additional 3 times. Then for the top layer, a low concentration of AA-g-PEG microspheres dispersed in PVA solution was added. The complex system underwent another 3 freeze-thaw cycles before imaging under an inverted fluorescence microscope.

Results:
The AA-g-PEG microspheres were successfully formed as verified by scanning electron microscopy (image not shown) with a nominal diameter of 1µm. AA-g-PEG microspheres were not only retained but spatially controlled within a multilayered PVA scaffold (**Figure 1**). A FITC gradient was achieved for the different layers of the scaffold. The top layer was the layer with less AA-g-PEG microspheres; the middle layer was the layer with more AA-g-PEG microspheres; the bottom layer was pure PVA scaffold layer. The high concentration of AA-g-PEG microspheres in the middle layer did not appear to migrate into the bottom layer.

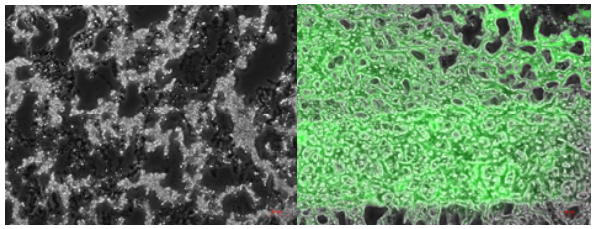

Figure 1. The bright-field micrograph (left image) for PVA scaffold top layer with AA-g-PEG microspheres; the bright white dots are FITC-encapsulated microspheres. Multilayered PVA scaffold (right image) showing spatial control of FITC-encapsulated microspheres of varying concentrations (top = low concentration, middle = high concentration, bottom = no microspheres).

Conclusions:
The study demonstrates the efficacy of using a multilayered PVA scaffold for the spatial control of drug delivery through the incorporation of drug-encapsulated microspheres. Future experiments will include encapsulating various growth factors in AA-g-PEG microspheres and measuring the spatial and temporal release in multilayered PVA scaffolds. Cytotoxicity experiments are currently underway using primary human mesenchymal stem cells; differentiation experiments will be performed in the near future.

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Reference: [1] Hrynyk M *et al.* Biomacromolecules 2012;13:1478-85. [2] Meng X-W *et al.* Langmuir 2011;27:14401-7.