Multifunctional Alginate Scaffolds for Spinal Cord Repair

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Statement of Purpose: Spinal cord injury (SCI) is a devastating condition with no available treatment that affects millions of people. In this work, we propose bioengineered scaffold technology as a viable SCI therapy. An ideal scaffold should consist of a high lumen volume of linear channels, a chemically inert material that is stiff to guide nerves yet soft to be biocompatible, and is capable of delivering growth factor(s). Previously it has been shown that highly ordered templated agarose scaffolds could linearly guide axons by cellular delivery of nerve growth factors such as brain derived neurotrophic factor (BDNF)[1,2]. To make this scaffolding technology acellular, we have reported acellular hydrogen-bonded layer-by-layer (LbL) protein release from agarose hydrogel over 30 days[3]. Although LbL is a viable technique for providing protein release, the scaffold must be fabricated from a degradable hydrogel if this technology is to be considered viable for SCI therapy (agarose does not degrade). Alginate is a hydrogel that ionically crosslinks in the presence of divalent cations and degrades by their gradual loss, releasing benign byproducts. Alginate, being a hydrogel, is expected to have a highly porous structure comparable to agarose, which may allow incorporation of the LbL process. By utilizing alginate and integrating LbL a degradable scaffold with acellular sustained protein release for nerve repair is proposed.

Methods: Alginate with high guluronate residues (Protanal LF 10/60) was supplied by FMC Corp, NovaMatrix (NJ). Similar alginate but with low-endotoxicity levels (Pronova UP-MVG) suited for *in vitro* and *in vivo* studies was purchased from the same supplier. 3w% alginate in water was crosslinked with 100mM $CaCl_2$. A templating technology is adapted to prepare low-endotoxin alginate scaffolds[4]. LbL is deposited onto the surface of alginate by sequential dipping of polyacrylic acid, polyethylene glycol and lysozyme – a suitable and inexpensive analog for BDNF – in acidic conditions. During the rise in pH to physiological conditions, the LbL dissociates, exhibiting controlled release of lysozyme. Bicinchoninic acid (BCA) protein assays determined the concentrations of lysozyme released from LbL-functionalized alginate samples in 1xDPBS solution as well as 100mM $CaCl_2$ solution corresponding to degrading versus non-degrading alginate, respectively. To study the pore morphology, alginate was supercritically dried by successive washes with ethanol followed by exchange with liquid CO_2 and subsequent supercritical extraction. Scanning electron microscopy (SEM) and nitrogen adsorption were used to measure and characterize hydrogel pore size distribution and surface area.

Results: Alginate scaffolds with hexagonal packing and highly linear channels were prepared. Alginate was compatible with the established templating technology[4] but with modifications to the casting and etching processes. LbL protein release shows a sustained daily protein release reaching a total of 2-2.5mg per mL of hydrogel over more than 45 days (Fig. 2). Nitrogen adsorption on supercritically dried 3w% alginate reports a total surface area of 424.4m^2/g: a significant contribution to the large dose response seen by the LbL method. Furthermore, the highly porous structure of this hydrogel can be visualized by SEM (Fig. 3).

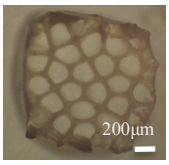

Fig. 1. Alginate scaffold with hexagonal packing

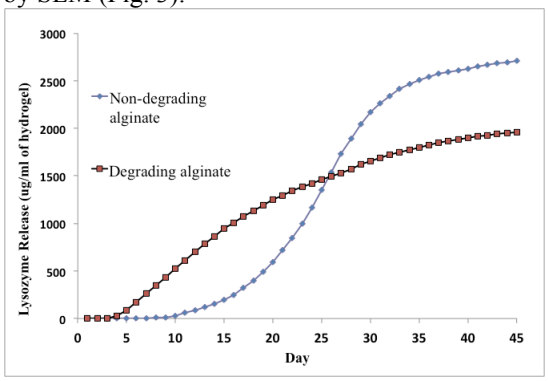

Fig. 2. Sustained protein release from LbL coated alginate

Conclusions: Through the use of our unique templating technology[4], we were able to prepare degradable alginate scaffolds with strictly linear microchannels. Moreover, SEM and nitrogen adsorption results show that alginate has a high surface area favorable for substantial and sustained protein release through H-bonded LbL assembly and disassembly. The LbL release profile from alginate shows a remarkable total protein release of 2-2.5mg per mL of hydrogel for over 45 days, suggesting a viable technique for controlled delivery of proteins such as BDNF. This work is being translated to measuring the *in vivo* efficacy of LbL-coated alginate scaffolds in rodent SCI models.

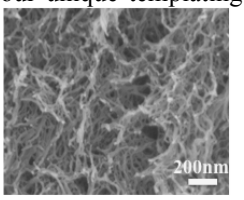

Fig. 3. SEM image of supercritically dried alginate

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