Novel Rapidly-Gelling Injectable Chitosan Sponge to Promote Oligodendrocyte Progenitor Cells' Differentiation

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Statement of Purpose: One of the pathophysiological outcomes of spinal cord injuries (SCI) is demyelination. Demyelination occurs after oligodendrocyte death due to necrosis or apoptosis, leaving behind intact axons that cannot propagate action potentials due to loss of myelin [1]. Endogenous Oligodendrocyte Progenitor Cells (OPCs) migrate to the site of injury and remyelinate denuded axons. However, remyelination is incomplete and abnormal. One of the reasons for that is the failure of OPCs to fully differentiate into mature oligodendrocytes at the site of injury due to the hostile inflammatory environment [1]. Therefore, there is a need to improve remyelination post-SCI to allow functional recovery of axons that could be lost otherwise. We developed a rapidly-gelling injectable chitosan sponge crosslinked using Guanosine 5' diphosphate (GDP) to specifically promote OPCs' differentiation. The anionic phosphate groups of GDP crosslink the cationic amine groups of chitosan, and the guanosine part of GDP promotes OPC survival, attachment and differentiation [1].

Methods: Chitosan with a molecular weight of 2000-3000 cp and degree of Deacetylation >90% was purchased from MP Biomedicals, and GDP from Sigma Aldrich. Four chitosan formulations were designated acronyms as C(X)PH(Y), where 'X' and 'Y' represent the chitosan concentration and solution pH, respectively, giving the following formulations: C3PH5, C3PH6, C6PH5 and C6PH6. Each chitosan concentration was prepared in a 0.01M HCl solution and the pH was increased using a 1M sodium bicarbonate solution. Each chitosan formulation (1.7 ml) was then supplemented with 0.3 ml of a GDP solution (final GDP concentration of 34 mM) through rapid injection, instantaneously producing a GDP-crosslinked chitosan sponge. The sponge was then characterized using Scanning Electron Microscopy, Infrared Spectroscopy, X-ray diffraction, Impedance Spectroscopy, and mechanical indentation. The water retention was also assessed along with an initial cell viability experiment using 3T3 fibroblasts. OPC attachment and differentiation were assessed after OPC-culture on the sponges for 5 days followed by immunostaining with the antibodies A2B5, a marker for OPC and anti-GalC for oligodendrocytes. Confocal microscopy was used to image 3D sections of the sponge and a cell count was performed to quantify differentiated cells compared to total cell numbers.

Results: All chitosan sponges were shown to form in less than 1.6 seconds after mixing the chitosan and GDP solutions (as measured by an Impedance Analyzer). SEM images revealed a highly porous sponge with excellent pore interconnectivity. FTIR confirmed incorporation of GDP within the chitosan sponge. XRD demonstrated a reduction in crystallinity of the sponges as compared to the chitosan powder. Sponges retained water up to ten times their weight and were shown to possess an elasticity modulus in the range of 0.4 – 0.8 MPa, which is close to that of the spinal cord [2]. Biocompatibility studies with fibroblasts demonstrated excellent attachment and proliferation on all four sponges for up to 7 days. OPCs attached to all sponges, while significantly more cells were present in C6PH5 as compared to C3PH5 ($P<0.05$). OPCs were also shown to infiltrate into the sponges and to differentiate best in C3PH6 (Figure 1). Other non-stained cells on the sponges could be confidently assumed to be astrocytes, since OPCs have the potential to differentiate into astrocytes or oligodendrocytes.

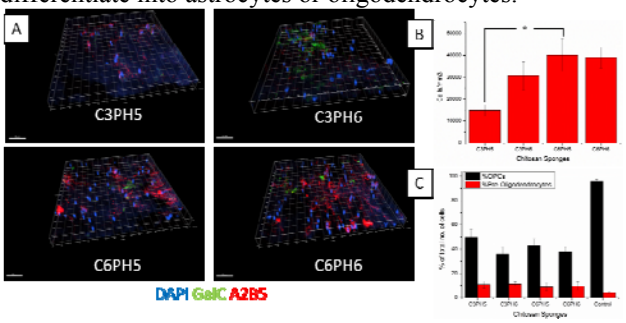

Figure 1. A: 3D compilations of Z-sections showing OPCs and oligodendrocytes on the sponges after 5d of culture; B: Total cell numbers normalized to volume in each sponge; C: Number of OPCs and oligodendrocytes in each of the sponges, control being OPCs cultured on glass slides (n = 3)

Conclusions: We have developed a biomaterial to specifically target remyelination post-SCI. A thorough physicochemical characterization of the sponges demonstrated that they were also excellent candidates for many other tissue regenerative applications. Particularly, the use of GDP as an anionic crosslinker of chitosan which is a cationic polymer, allowed not only for rapid gel formation (t_{gel} < 1.6 sec), which is faster than most injectable gels found in the literature, but also its guanosine group revealed to induce OPC attachment and differentiation. This was in contrast with poor OPC adhesion on uncrosslinked chitosan films. More interestingly, OPC differentiation was demonstrated on the sponge, even though the culture media was supplemented with bFGF and PDGF to keep OPCs from differentiating. Future work will investigate the incorporation of NT-3 into the sponge to promote more OPC differentiation. In addition, the sponges will be injected *in-vivo* in a rat SCI model to assess functional recovery post-SCI.

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References:

[1] Mekhail, M. Prog Neurobiol. 2012; 96: 322-339.
[2] Ichihara K. J Neurotrauma. 2001; 18: 361-367