A Rapidly-gelling Injectable Chitosan Sponge to Enhance Remyelination Post-Spinal Cord Injuries

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Introduction:

Demyelination is one of the pathophysiological outcomes of spinal cord injuries (SCI) that occurs early in the injury and continues to take place for more than three weeks [1]. It occurs when oligodendrocytes (myelinating cells in the central nervous system) undergo necrosis and apoptosis due to the injury, thus causing myelin loss from intact axons [1]. Remyelination takes place by endogenous Oligodendrocyte Progenitor Cells (OPCs); however, the myelin produced is abnormal. The incomplete remyelination process has been attributed to the inability of OPCs to fully differentiate into myelinating oligodendrocytes and to the presence of inhibiting factors at the site of injury [2]. Therefore, there is a need to enhance remyelination post-SCI to preserve demyelinated axons and to provide functional neuronal recovery.

We developed a rapidly-gelling, injectable chitosan sponge that is crosslinked with Guanosine 5'-diphosphate (GDP). The rapid gelation occurs due to the electrostatic interactions between cationic amine groups in chitosan and anionic phosphate groups in GDP. Although GDP has never been tested as an anionic crosslinker for chitosan, we explored its potential since it includes guanosine, a molecule that has been shown to induce remyelination post-SCI [1]. We hypothesized, that GDP-crosslinked injectable chitosan sponges could promote OPCs' attachment and differentiation in-vitro. The objectives of this study where to: (1) optimize the GDP-crosslinked chitosan sponges, (2) study their biocompatibility on cultured OPCs, and (3) investigate OPC differentiation on sponges.

Materials and Methods:

Materials: Chitosan (MW = 2000-3000 cp and degree of deacetylation >90%) was purchased from MP Biomedicals and GDP from Sigma Aldrich.

***Chitosan Sponge Fabrication*:** Four chitosan formulations were designated acronyms 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. Chitosan was dissolved in a 0.01M HCl solution and the pH was adjusted to 5 and 6 using a 1M sodium bicarbonate solution. Each chitosan formulation (1.7 ml) was then supplemented with 0.3 ml of a GDP solution (34 mM) through rapid injection, instantaneously producing a GDP-crosslinked chitosan sponge.

Characterization: The sponges were dried using critical point drying, coated with Pd-Au and imaged using scanning electron microscopy (SEM). The rate of gelation was measured using impedance spectroscopy, while the moduli of elasticity were measured using a 1 mm sphere indenter and a rate of deformation of 5 µm/s.

Cell Experiments: OPCs were obtained from the forebrains of newborn Sprague Dawley rats. OPC isolation, purification and culturing were prepared as described previously [3]. Sponges were sterilized under UV for 1 hour and washed thoroughly with serum free media (SFM). OPCs at a concentration of 1×10^6 cells/ml (or 1000 cells/mm^3) were seeded on the sponges. After 1 day the media was removed, and SFM supplemented with PDGF and bFGF was added to the cultures. The OPCs were maintained for another 4 days before fixation with 4% paraformaldehyde and staining for

A2B5 (OPC marker), GalC (Oligodendrocyte marker) and DAPI (Nucleus stain). Confocal microscopy was then used to image up to 50 µm sections of the sponge.

Results:

All chitosan sponges formed in less than 1.6 seconds after mixing the chitosan and GDP solutions. The crosslinking was confirmed using FTIR. SEM images revealed sponges that were composed of fused polymeric aggregates with an average size of 140 ± 20 nm (Fig. 1A). The sponges were found to be highly porous and had excellent pore-interconnectivity; however, the pores were heterogeneous in size. The moduli of elasticity for C3PH5, C3PH6, C6PH5 and C6PH6 were 0.789±0.07, 0.432±0.05, 0.867±0.09, and 0.606±0.09 respectively (Fig. 1B). These results demonstrated that a lower pH produced sponges with a higher modulus of elasticity. This was attributed to the increased crosslinking at pH 5, due to the presence of more charged amine groups on the chitosan. OPCs cultured on the sponges adhered to the surface, migrated into the sponges and on average 15% of the total numbers of cells differentiated into mature oligodendrocytes as opposed to 4% in control groups (Fig. 1C).

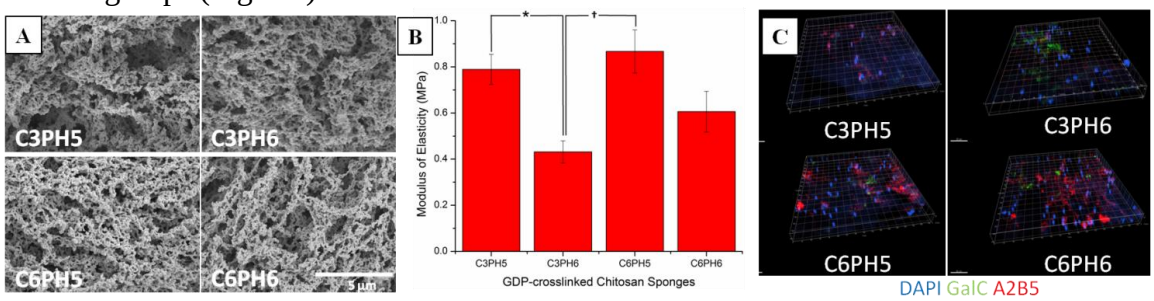

Figure 1. A: SEM images of the different sponge formulations; B: The moduli of elasticity of the different sponges; C: 3D confocal images of OPCs cultured on sponges, acquired using compiled Z-stack images

Discussion:

A novel injectable chitosan sponge that undergoes very rapid gelation (t_{gel} < 1.6 sec) has been developed. Moreover, the use of GDP as a crosslinking molecule has not been previously explored, and is another novel aspect of this research project. The rapid gelation ensures the localization of the sponge at the site of injection and prevents flow during gelation. This is essential in both tissue regeneration and drug delivery applications. The porosity and interconnected porous structure of the sponges allow for cellular migration as demonstrated in the 3D confocal image shown in Fig 3C. Measurement of the moduli of elasticity of the sponges revealed soft biomaterials that have a moduli close to that of the human spinal cord [4]. Mimicking the in-vivo microenvironment is essential for promoting OPC growth and differentiation, especially since they are mechano-sensitive. Initial results using the sponges demonstrated OPC attachment and migration into the sponge. In addition, OPC differentiation occurred, with most differentiation in C3PH6 samples. The unique properties of the sponge in conjunction with a growth factor delivery system that is currently being implemented will result in a promising therapeutic intervention for enhancing remyelination post-SCI.

References:

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