Study of GDNF Release From Chitosan Scaffolds

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Statement of purpose: It is known that severely damaged peripheral nerves cannot repair themselves to their pre-injury level without assistance. Typically for larger nerve gaps due to severance, an autologous nerve graft is used to repair injured nerves. This method has several disadvantages such as loss of function at donor site and requires multiple surgeries. Major steps have been taken in the field of bioengineering to develop a biomaterial which can provide nerves with a regenerative environment and guide them in proper direction. These biomaterials can act as a bridge between nerve gaps generated during injury and release factors required for nerve regeneration. It is known that chitosan can act as a noble biomaterial as it is biocompatible and fabricated as nerve guides. But still, growth factor addition needs to be optimized for full functional recovery. In this preliminary research, we evaluated two chitosan scaffolds fabrication procedures and its effect on the release of glial cell derived neurotrophic factor (GDNF) and laminin.

Materials and Methods: We first prepared three solutions made from 1.5% chitosan (80%DDA). Chitosan(C) alone as control, Chitosan with the addition of 25µg laminin (CL) and Chitosan with the addition of 25µg laminin and 36µg GDNF (CLG). We used two different protocols to see the effect of pore size on the release of growth factors. Group I-(Gel)-incubated the scaffold solution in ammonium over night to gel then froze it in liquid nitrogen. Group II- (Liquid N_2)-directly froze the solution in liquid nitrogen. Both groups were lyophilized over night. Scaffolds were then cut into 10mm X 5mm pieces. These pieces were neutralized and SEM was conducted to determine starting pore size. The scaffolds were arranged in six 24 well plates according to their group and class. 0.5 ml PBS was added and plates were placed on shaker (400 rounds/min.). This assembly was kept in incubator at 37^0C. PBS solution from each well was removed at appropriate time periods and frozen until tested. Scaffolds were removed at eight different time slots over the period of 5 days. We determined the protein concentration using BCA™ protein assay kit on all acquired samples. Imunohistochemistry against laminin and GDNF were also evaluated. Histological assessment was examined using the Image Pro Plus software to assess optical density of the stained scaffolds.

Results: SEM results displayed that scaffolds directly frozen in liquid N_2 had larger pore size (average 60µm) compared to the gel scaffolds (average 3µm). GDNF release was determined to be significantly less at specific time points when comparing the two fabrication techniques. Release through group I scaffolds (gel) was much less than group II scaffolds (Liquid N2). Group I release was minimal within the 5 day period as compared to group II which showed consistent release for the first 18 hours, then a large release was noticed at the 24 hour slot. Immunohistochemistry assessment confirmed the protein release results and displayed an even release of bioactive factors off the scaffolds.

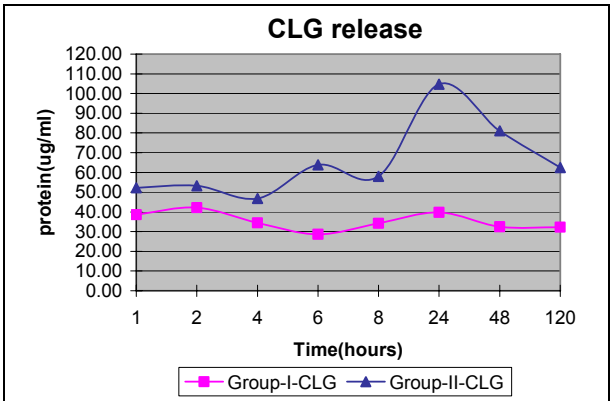

Figure: Comparison of the CLG release through scaffold with two different fabrication techniques

Conclusion: From this study we can conclude that the technique of freezing scaffolds directly in liquid nitrogen helps the controlled release of the GDNF. We believe these results are the direct result of the differing pore sizes created during the fabrication process. Since it is known that a specific cascade of growth factors will be needed .for nerve regeneration, this study begins to evaluate the time period of release.