Spiral Structured Nanofibrous Scaffolds for Neural Tissue Engineering

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Statement of Purpose: Nerve injury affects 2.8% of trauma patients in the United States [1]. The current gold standard in treating such injuries is surgical reconnection or autologous graft [2]. Several tissue-engineering strategies [2,3] have been studied and employed to create synthetic grafts. However, the traditional tubular nerve grafts can only provide contact guidance for axons along the channel walls, which apparently is not adequate for promoting sufficient axonal regeneration. Here, we create a novel spiral structured, nanofibrous tubular scaffold. The rationale behind using spiral architecture is to increase the surface area available for cell attachment and for media flow into the scaffold. The fibrous structure on the surface would help to further augment the surface area and provide an analog to extra cellular matrix (ECM). In addition, a rotating bioreactor dynamic culture system was adopted to fasten Schwann cell proliferation on the scaffolds and to potentially reduce the time needed to produce a large number of cells that is essential for creating an efficient tissue engineered nerve graft.

Methods: Poly (lactide-co-glycotide) (PLGA) microspheres were made using oil in water emulsion technique. The microspheres were sieved; spheres in the range of 100-200 µm were used for making the scaffolds. Salt was used as a porogen to enhance the porosity of the scaffolds. Sintered microsphere sheets along with salt were made by standard sintering procedures [4,5] and these sheets were cut to form thin strips approximately 5 cm in length and 5 mm in width. They were then rolled to obtain spiral tubular structures. As a modification to the above standard scaffolds, PLGA fibers were electrospun onto both sides of the scaffolds before rolling to create the nanofibrous spiral architecture. The salt was leached off by continuous washing in water. As a control scaffolds that had no salt and fibers was used. Optical and scanning electron microscopy images were taken to characterize the morphology of the scaffolds. The porosity and the mechanical properties of the scaffolds were also characterized.

Rat Schwann cells (cell line from ATCC), were cultured on the scaffolds under dynamic conditions in a rotating bioreactor with a rotation speed of 30 RPM. Complete DMEM supplemented with 10% bovine serum and 1% penicillin streptomycin was used. The cell attachment and proliferation data was analyzed using MTS.

Results/Discussion: The scaffolds had a uniform spiral structure with even gaps and wall thickness as shown in Fig 1. They also had a well spread fibrous surface with interconnected pores.

Figure 1: Scaffold design showing the spiral architecture. B) Sintered microspheres showing good pore interconnectivity and pore size. C) SEM image of a fibrous surface covering the sintered microspheres.

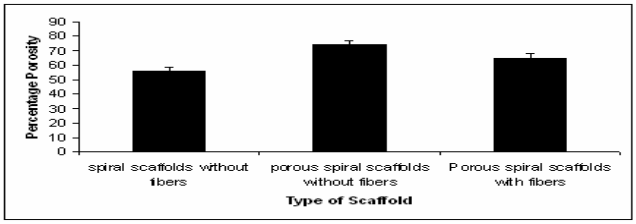

Figure 2: Porosity values of scaffolds

As shown in Fig. 2, porosity values increased with the addition of salt into the scaffolds and the addition of fibers to the surface of the scaffolds slightly reduced the porosity.

The spiral scaffolds without the salt or the fibers showed good mechanical properties (Fig. 3) and the addition of salt to the scaffolds reduced the ultimate stress. The mechanical properties also increased with the addition of fibers to the porous PLGA scaffolds. The natural acellular sciatic nerve has a maximum failure stress of about 1 MPa [6] and all of scaffolds in this study had better strength than the sciatic nerve.

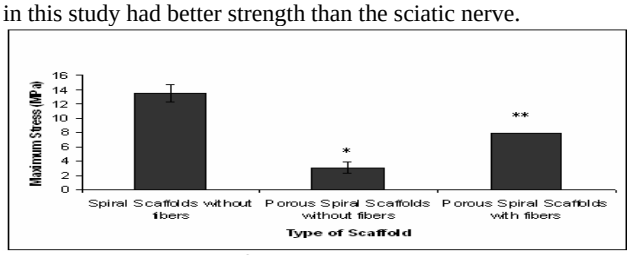

Figure 3: Maximum Stress of plain PLGA spiral structure as compared to scaffolds with salt and salt and fibers. (*) Porous spiral scaffolds without fibers showed a significant difference ($p<0.05$) than the spiral scaffolds without salts and (**) indicates that the porous scaffolds with salt and fiber had a significant difference when compared to porous scaffolds with salt and without fibers.

MTS data on day 1 and 8 showed improved cell attachment and cell proliferation on the scaffolds with higher porosities. Also, the addition of fibers on the surface of scaffolds further enhanced cell attachment and proliferation (Fig. 4).

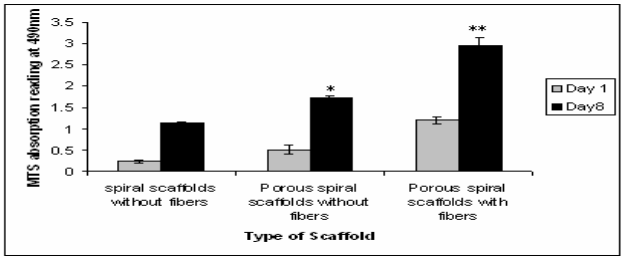

Figure 4: MTS data showing improved cell attachment and proliferation on spiral scaffolds with higher porosity and a fibrous surface. (*) shows that the porous spiral scaffolds had a significant difference and (**) shows that there was a significant difference between the porous scaffolds with fibers when compared to scaffolds without fibers.

Conclusions: The results indicate that the spiral structured nanofibrous scaffolds meet the requirements for mechanical properties for nerve tissue engineering, and the porous fibrous structure helps to improve the attachment and proliferation of Schwann cells cultured on the scaffolds in dynamic rotating biorectors. The tissue-engineered scaffolds have the potential to be used as nerve grafts for bridging nerve gaps.

References:

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