Development of axially-aligned scaffolds for optimization of neural regeneration

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Statement of Purpose: Developing a clinically-useful, off-the shelf graft for peripheral nerve injury remains a challenge. Autologous grafts are the clinical standard, though their use is often limited by availability of acceptable tissue or by the extent of the injury. Decellularized allogeneic grafts are a potential alternative as they provide a physical context for regeneration. However, the channels or conduits that result from decellularized grafts are the result of architecture created during development and growth but are not necessarily optimal for regenerative processes. Fabricated biomaterial scaffolds offer an appealing alternative because they can be designed for off-the shelf use and can be constructed with physical cues that are designed to optimize regenerative processes following injury. The objective of this work is to fabricate a biomaterial-based scaffold with axially-aligned conduits of defined cross-sectional diameter. The scaffolds resulting from the fabrication process provide a mechanism to assess conduit dimensions optimal for regenerative processes and are a step toward an off-the-shelf biomaterial solution to peripheral nerve repair.

Methods: 100,000 Da poly(methyl methacrylate) (pMMA) fibers were extruded on a piston extrusion system at the NC State College of Textiles. Fiber diameter was varied based on the extrusion temperature and rate of uptake on collection godets. Diameters of resulting pMMA fibers were assessed by light microscopy and the fibers were subsequently used as templates for scaffold fabrication. These pMMA fiber templates were packed into polytetrafluoroethylene (PTFE) molds and back-filled with a fibrinogen solution, which was then polymerized to fibrin with a thrombin solution. pMMA fibers were selectively dissolved from the system by successive washes with acetone to yield hollow conduits within the scaffold. By utilization of template fibers of different diameters, the conduit dimension of the resulting scaffolds could be controlled to allow assessment of the role of conduit dimensions on cellular infiltration.

Diameters of the resultant conduits in the scaffold and the axial alignment of the conduits were assessed by scanning electron microscopy (SEM). Young's modulus and the maximum strain were determined by tensile testing with an Instron 5400 mechanical tester and a strain rate of 10mm/min. Cellular infiltration into scaffolds has been assessed by incubating scaffolds with whole day 7 embryonic chicken dorsal root ganglia (DRG) for defined periods of time and staining for DAPI.

Results: Figure 1 shows diameters for the templates and resulting conduits based on four different fiber templates.

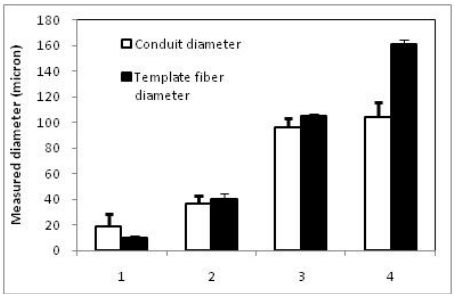

Figure 1. Diameters of fiber templates and resulting scaffold conduits.

Conduit diameters generally follow the diameters of the templating fibers. Figure 2 shows SEM images of a representative scaffold formed by the templating approach. The conduits are closely packed in a manner similar to the appearance of decellularized nerve scaffolds (2A). Further, the aligned conduits run the length of the scaffold (2B). Images such as those in Figure 2 were utilized to determine conduit alignment within the scaffolds. Figure 3 shows that scaffolds are highly aligned over five template diameters studied. Mechanical testing demonstrated a Young's modulus independent of conduit diameters and of the same magnitude as literature values for native tissue (data not shown). Initial studies have shown that embryonic chick DRG attach to the scaffold with cells infiltrating into the scaffold and extending processes on the scaffolds (data not shown).

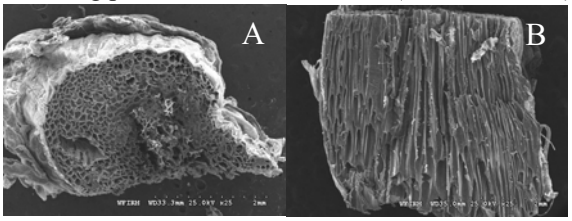

Figure 2. SEM images of scaffolds with 105μm fiber templates indicating tight-packed nature of conduits (A) and axial alignment (B).

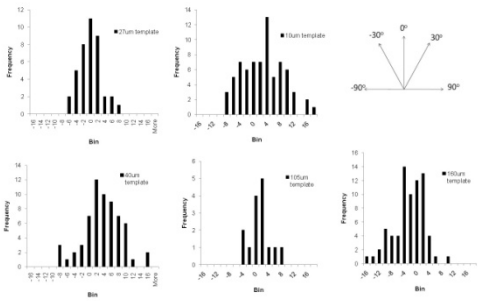

Figure 3. Histograms demonstrating axial alignment of conduits in scaffolds for five different template diameters.

Conclusions: Biomaterial scaffolds with conduits of well-defined diameters have been successfully fabricated. The resulting scaffolds are suitable for determination of optimal conduit dimensions for neural regeneration processes and as a potential alternative to autografts or decellularized allogeneic grafts.