Peripheral Nerve Regeneration through Structurally Modified Acellular Scaffolds in a Rabbit Model

Paulina Sierpinski, Jacquie Burnell, James Yoo, Anthony Atala, Mark Van Dyke[2]
Wake Forest Institute for Regenerative Medicine, Wake Forest University School of Medicine
Medical Center Boulevard, Winston Salem, NC 27157

Statement of Purpose: Acellular nerve grafts have been shown to be effective in promoting regeneration over short distances. However, these acellular scaffolds are known to possess a dense architecture that may serve as a barrier to infiltrating regenerative cells and outgrowing axons. We hypothesize that the use of a graft with a more porous architecture may lead to improved functional recovery, even over long distances. The objectives of this study were to determine (1) if chemical processing techniques could be used to increase the porosity of a scaffold and (2) if such a scaffold could be used to regenerate long segmental nerve defects *in vivo.*

Materials and Methods: Sciatic nerves were harvested from New Zealand White rabbit cadaveric donors. The tibial nerves were isolated, placed in deionized (DI) water and treated with 0.5% (v/v) Triton X-100. Decellularized nerve segments were assessed histologically for cellular content. Residual DNA in acellular grafts was quantified (QIAGEN DNeasy). Acellular grafts were treated with peracetic acid (PA) at 1%, 5% and 10% (w/v) concentrations to increase porosity. Nerve segments were lyophilized and analyzed by SEM. To determine the ability of decellularized and chemically etched acellular grafts to regenerate large nerve defects *in vivo,* the tibial nerves of rabbits (n=20) were transected and used to repair a 5cm tibial nerve defect. Animals were randomized into: 1) Acellular graft, 2) Acellular graft pre-treated with 1% PA, 3) Acellular graft pre-treated with 5% PA or 4) Autograft. Following 6 months of regeneration, rabbits were sacrificed and nerve tissues harvested for examination by histology. Sections were stained with H&E to analyze the regenerated nerve structure and Masson's trichrome to assess scarring of the implanted grafts. NF-200, S100b and toluidine blue staining was also performed to analyze neurite outgrowth, myelination and degree of axonal regeneration.

Results: Acellular nerve grafts were prepared by removal of cellular components from rabbit sciatic nerves using a detergent at basic pH. The insoluble extracellular matrix (ECM) of processed nerves was left behind, whereas the cellular components were removed. Histological analysis by H&E and DAPI showed no evidence of intact cellular structures in decellularized grafts in comparison to native nerve. Quantification of residual DNA showed a significant decrease in cellular components following decellularization. Analysis of scaffolds by SEM showed a network of aligned collagen fibrils in all groups. Grafts chemically etched with increasing concentrations of PA were significantly more porous than non-oxidized grafts (Fig 1). The endoneurial tubes which surround individual axons remained intact following processing but appeared to be cleared of dense cellular debris.

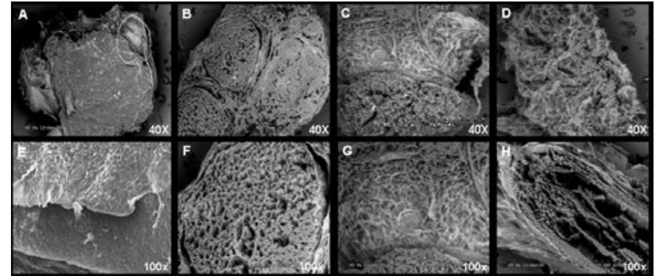

Figure 1. SEM images of decelluarized and untreated nerves (A&E) with a dense physical architecture. Treatment with 1% PA (B&F), 5% PA (C&G) and 10% (D&H) resulted in grafts with increased porosity.

There was no observable loss of mechanical integrity in the 1% and 5% PA treated scaffolds, however the 10% PA treated grafts were more brittle and collapsed, and were therefore not selected for implantation. Implanted grafts retrieved at 6 months appeared intact with few apparent neuromas at sites of anastomosis. Grossly, there was no evidence of persistent inflammation. In all groups, axons sprouted from the proximal stump and reached the distal portions of the nerve grafts. The density of regenerated nerve fibers was highest in the autograft group in comparison to all other treatment groups. However, chemically oxidized grafts had a higher density of regenerating fibers with improved alignment and more myelinating Schwann cells than grafts without this modification. Furthermore, the size and density of regenerating axons was higher in PA treated grafts and comparable to autograft.

Conclusions: Acellular grafts have previously been shown to support nerve regeneration over small defects without evidence of immunogenic rejection. However, many studies have reported difficulties with homogenous cell seeding of such scaffolds using both static and dynamic techniques. Due to the dense architecture of these grafts, cells are unable to penetrate the full depth of the scaffold and require long remodeling times *in vivo*, sometimes remodeling incompletely with excessive scar tissue formation. This study demonstrates that an oxidant such as peracetic acid can be used to modify the scaffold microarchitecture prior to seeding and/or implantation *in vivo.* Furthermore, a chemically etched scaffold appears to support neurite outgrowth *in vivo* better than acellular scaffolds without this modification.