Peripheral Nerve Regeneration Using a Keratin Gel-Based Scaffold: Long-Term Functional and Histological Outcomes in a Mouse Model

Peter J. Apel1,2, Jeffrey Garrett1, Paulina Sierpinski1, Jianjun Ma1, Jeffrey Hick1, Thomas L. Smith1,2, L. A. Komansky, Mark E. Van Dyke2
1Orthopaedic Surgery, Wake Forest University, Winston-Salem, NC; 2Institute for Regenerative Medicine, Wake Forest University, Winston-Salem, NC

Introduction: Nerve gaps/defects are common in various clinical situations, such as trauma and tumor ablation. It has been estimated that there are 18 million extremity injuries in the United States each year that result in a substantial number of peripheral nerve injuries. Repair techniques are limited by injury severity, donor tissue availability, and patient factors such as age and comorbidities. Currently, no biomaterial is available that improves functional recovery over the gold-standard, sensory nerve autografts. The purpose of this study was to evaluate the effect a keratin gel-based scaffold for peripheral nerve regeneration compared to sensory nerve autografts and empty nerve guidance conduits.

Materials and Methods: DESIGN-Forty five CD1 mice were randomized into three experimental groups: 1) empty conduit, 2) sensory nerve autograft, and 3) keratin gel-filled conduit. SCAFFOLD PREPARATION-Human hair was treated with an oxidizing agent, followed by extraction with a base. The extracts were dialyzed, concentrated, neutralized, freeze-dried and ground into a fine powder. Hydrogels were prepared by addition of 85% PBS. The hydrogels were sterilized with gamma irradiation prior to use. SURGICAL PROCEDURE-The tibial nerve was exposed and transected. Silicone tubing (762 μm internal diameter, 7 mm in length) was interposed between the severed nerve ends and secured with 10-0 nylon. The hydrogel scaffold was injected into the conduit with a needle. Autografts were fashioned in a cable manner from the ipsilateral sural nerve and secured with 11-0 nylon. FUNCTIONAL OUTCOME MEASURES-The primary outcome measure was compound motor action potential (CMAP) of the neuromuscular unit. Other outcome measures included muscle force generation and muscle mass. For CMAP, the nerve was exposed and directly stimulated proximal to the repair site while the depolarization was recorded over the gastrocnemius. HISTOMORPHOMETRY-The regenerated nerve was fixed, embedded and 1μm sections from the midpoint of the nerve were obtained. ImageJ software was used to measure nerve diameter, area, axon number and axon diameter. STATISTICS-Repaired nerves were compared to their contralateral controls using a paired Student’s t-test. Two-factor analysis of variance (ANOVA) was used to compare between groups and time points. α was set at 0.05.

Results: FUNCTIONAL OUTCOMES-At 6 weeks, the keratin group showed significantly improved conduction delay and amplitude recovery (p<0.05) compared to both autografts and empty conduits. At 3 and 6 months, the keratin group showed significantly improved conduction delay and amplitude (p<0.05) compared to empty conduits, but were not different from autografts. Similar trends were seen with other parameters. HISTOMORPHOMETRY-At 6 weeks, the keratin-treated nerves had greater cross-sectional area (376,878 μm²±/−144,764) than both autografts (94,313 μm²±/−55,634) and empty conduits (19,280 μm²±/−4,335). The number of axons per nerve in the keratin group (3999±/−935) was also more than both autografts (870±/−77) and empty conduits (892±/−40). Keratin-treated nerves had a greater average axon diameter (3.49 μm±/−0.14) than nerves from empty conduits (1.94 μm±/−0.26). However, keratin-treated axons were on average smaller than those in autografts (4.08 μm±/−0.11).

Discussion: Keratin appears to accelerate nerve regeneration at early time points as evidenced by the improved recovery at 6 weeks, even when compared to sensory nerve autografts. Moreover, there was little difference in functional outcomes between the keratin and autograft groups at 6 months, while the empty conduit group had not similarly recovered. Keratin-treated animals scored consistently better than empty conduits and were no different than sensory nerve autografts by nearly all parameters. Histologically, keratin-treated nerves were better than empty tubes for all parameters and better than autografts for some. These results suggest that keratin acts as a provisional matrix, facilitating the early stages of nerve regeneration. While this study is preliminary in nature, these results are very encouraging as they suggest that keratin positively affects peripheral nerve regeneration and promotes neuromuscular recovery equivalent to the gold-standard, sensory nerve autografts.

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