PERIPHERAL NERVE REGENERATION THROUGH A NERVE CONDUIT USING A SELF-ASSEMBLED KERATIN HYDROGEL MATRIX IN AN ANIMAL MODEL

Jeffrey P. Garrett,1 Paulina Sierpinski,1 Jianjun Ma,1 Jacquie Burnett,1 Sang Jin Lee,1 Jeff Hick,2 Thomas L. Smith,1 L. Andrew Koman,2 Anthony Atala1 and Mark Van Dyke2

1The Wake Forest Institute for Regenerative Medicine, and 2the Department of Orthopaedic Surgery
Wake Forest University School of Medicine, Winston Salem, North Carolina
jgarrett@wfubmc.edu

Introduction:
Annually, over 18 million extremity injuries are reported in the US resulting in a substantial number of peripheral nerve injuries. Nerve defect management includes primary repair, nerve grafting, and nerve conduits. Clinically, nerve conduit use has been restricted to smaller defects because of limited functional recovery with larger nerve gaps. It is hypothesized that a tissue engineering approach employing a nerve conduit filled with an optimized scaffold will accelerate regeneration. Keratins extracted from human hair fiber, act as cell binding scaffolds and provide an alternative to other nerve conduit fillers (Figure 1).

Methods:
Swiss Webster mice were assigned to two groups. Each animal underwent transection of the left tibial nerve, 5mm above neural insertion into the gastrocnemius. A 7mm silastic conduit was secured using 10-0 microsuture, creating a 4 mm gap between the proximal and distal nerve ends. In Group I, a keratin hydrogel was injected into the 4 mm gap. The gap was left empty in Group II. After 6 weeks, the regenerating nerve and control nerve were exposed and evaluated using: 1) electrophysiology (amplitude and latency), 2) muscle force generation (twitch and tetanus), and 3) histological examination.

Results:
At 6 weeks, substantial axonal regeneration had occurred with visible axon fibers crossing the conduits in both groups (Figure 2).

In Group I (Keratin) the amplitude was 34% of the control (13.99mV vs. 40.65mV) with Group II (Empty) achieving only 11% (3.44mV vs. 30.24mV). The latency revealed an 18% conduction delay compared to control in Group I (1.3msec vs. 1.1msec) and a 77% delay in Group II (2.3msec vs. 1.3msec) (Figure 3).

Muscle force generation data was similar to electrophysiology data. Cross-sectional histology demonstrated regenerating myelinated axon fibers in both groups, with increased neovascularization in Group I.

Discussion and Conclusion:
These data suggest that a keratin matrix may facilitate nerve regeneration through a conduit. Certain keratin preparations have the ability to self-assemble into porous, fibrous morphologies acting as scaffolds for regenerating axons. Axon regeneration may be enhanced by the inherent growth factors present in the conduit matrix. This tissue engineering approach may allow use of nerve conduits in correcting larger nerve defects, with enhanced regeneration and return of function. In conclusion, keratin is a promising conduit filler with future clinical application.

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Figure 1. Keratose hydrogels spontaneously form fibrous architectures that are conducive to cell seeding and mimic the extracellular matrix. The homogeneous architecture, fibrous morphology, and high porosity is conducive to cell infiltration. Indigenous growth factors promote tissue regeneration.

Figure 2. Nerve regeneration 6 weeks following axotomy and repair. A 7 mm long silicone tube (internal diameter of 0.5 mm) was used to bridge a 4 mm nerve gap. The conduit was secured with 10-0 microsuture.

Figure 3. Electrophysiology readings of tibial nerve activity in uninjured controls (C) and following 6 weeks of regeneration in empty (A) and keratose filled (B) conduits.

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<thead>
<tr>
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<th>Latency (ms)</th>
<th>Amplitude (mV)</th>
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<tbody>
<tr>
<td>Empty conduit</td>
<td>2.27</td>
<td>3.41</td>
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<tr>
<td>Keratose conduit</td>
<td>1.33</td>
<td>14.00</td>
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<tr>
<td>Uninjured nerve</td>
<td>1.30</td>
<td>30.24</td>
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