Rapid Peripheral Nerve Regeneration Using Self-Assembled Human Hair Keratin Scaffolds in a Mouse Model

Paulina Sierpinski,[†] Jeffrey Garrett,[‡] Jianjun Ma,[‡] Jacquie Burnell,[†] Sang Jin Lee,[†] Tom Smith,[‡] Anthony Atala[†] and Mark Van Dyke[†]

[†]The Wake Forest Institute for Regenerative Medicine, and [‡]the Department of Orthopaedic Surgery
Wake Forest University School of Medicine, Winston Salem, NC 27157. mavandyk@wfubmc.edu

Statement of Purpose:

Over 18 million extremity injuries are recorded in the United States each year, resulting in a substantial number of peripheral nerve injuries.[1] Current treatments for peripheral nerve injury consist of surgical reconnection of the damaged nerve ends, the use of an autologous nerve graft, or the insertion of guidance conduits. Clinically, the use of nerve conduits has been restricted to smaller defects because of limited functional recovery with increased nerve gaps. We hypothesize that a tissue engineering approach that employs a nerve guidance conduit filled with an optimized keratin scaffold can accelerate regeneration beyond current clinical limits. Keratins extracted from human hair fibers are a novel group of biomaterials that act as a mimic of the extracellular matrix (ECM) by providing fibronectin-like binding domains.[2] Additionally, human hair has been identified as a "depo" of growth factors involved in normal follicle cycling, including nerve growth factor.[3] Recently, we have also discovered a remarkable ability for certain keratin preparations to self-assemble into porous, fibrous morphologies that are amenable to cell infiltration.[4] The specific aims of this study are to assess the ability of novel keratin hydrogel matrices to accelerate functional nerve regeneration *in vivo.*

Methods:

Human hair was obtained from a local barber shop and cut into small length fibers, washed, and degreased. Keratose fractions were prepared by treating hair fibers with peracetic acid, followed by extraction with aqueous tris base and deionized (DI) water. The extracts were combined and dialyzed against DI water. The dialyzate was concentrated, neutralized, lyophilized, and the resulting keratose solid ground into a fine powder. Hydrogels were prepared by re-hydrating the keratin powder. Micro-architecture of the self-assembled gels was assessed by scanning electron microscopy of lyophilized samples. Biocompatibility was established using *in vitro* proliferation and cell viability assays, as well as subcutaneous implantation in mice.

To determine the effects of keratin on nerve regeneration *in vivo*, a tibial nerve axotomy model was used. The tibial nerve of C57 mice was transected and a 4 mm nerve gap was created between the proximal and distal ends. Empty and keratose filled silicone tubes were used to bridge the 4 mm gap created by severing the nerve. After 6 weeks, the treated nerve and contralateral control were exposed and evaluated using electrophysiology (amplitude and latency) and histological examination.

Results/Discussion:

Microscopic examination of lyophilized hydrogels showed a fibrous and highly porous architecture. The fibrous architecture and porosity were homogeneous throughout the entire thickness of the gel. Excellent biocompatibility was demonstrated by viability and proliferation of stem cells exposed to culture media containing keratose. Retrieved SC implants showed vascular ingrowth with concomitant host cell integration.

In the peripheral nerve regeneration study, small axon fibers were visible across the conduit in both treatment groups at 6 weeks. However, only in the keratin treated group were blood vessels grossly visible within the conduit. Electrophysiology recordings demonstrated improved functional recovery in mice implanted with keratin filled tubes. In the keratin treated group, amplitude was 34% of the contralateral control (13.987 vs. 40.65) with the empty conduit group achieving only 11% (3.439 vs. 30.24). Latency was 85% of control in the keratin treated group (1.3 vs. 1.1) and 57% in the empty conduit group (2.3 vs. 1.3). Cross-sectional histology demonstrated myelinated axons in both groups. However, image analysis showed that keratin treated nerves had more myelinated fibers than empty conduits, demonstrating improved axon regeneration.

Conclusions:

The data generated in this study demonstrates that keratin hydrogels can serve as effective scaffolds for axonal regeneration and facilitate rapid, functional recovery of damaged peripheral nerve. We believe axon regeneration is enhanced by the unique structure of self-assembled keratin scaffolds as well as the inherent growth factors present within the matrix.

References

1) Praemer A, Furner S, Rice DP (1999) In: Musculoskeletal conditions in the United States pp 3-162. Park Ridge, IL.: American Academy of Orthopaedic Surgeons.

2) Tachibana A, Furuta Y, Takeshima H, Tanabe T, Yamauchi K. Fabrication of wool keratin sponge scaffolds for long-term cell cultivation. J Biotechnol. 2002;93(2):165-70

3) Stenn KS, Prouty M, and Seiberg. Molecules of the cycling hair follicle – a tabulated review. J Dermatology Sci 1994;7S:S109-24

4) Lee, SJ and Van Dyke ME. Tissue engineering scaffolds from self-assembled human hair keratins. Polym Prep 2005;46(1):112