Clinical Translation of a Keratin Biomaterial Hydrogel for Nerve Repair

Pace, L A; Hill P S; Garrett J; Ma J; Apel P J; Mannava S; Barnwell J; Smith B P; Li Z; Koman L A; Smith T L; VanDyke M E

Wake Forest Institute for Regenerative Medicine and Department of Orthopaedic Surgery
Wake Forest University School of Medicine, Winston-Salem, North Carolina
lapace@wakehealth.edu

SIGNIFICANCE:
This is the first preclinical study to translate a biomaterial conduit filler to clinical trial for peripheral nerve injury patients with the goal of providing better long-term functional recovery than any other available technology.

INTRODUCTION:
Large peripheral nerve defects can be surgically repaired by autograft or implantation of a nerve guidance conduit. Many investigators have published the finding that an appropriate conduit filler can modestly improve functional recovery in preclinical nerve injury models. However, none of these technologies have advanced to pivotal preclinical studies or clinical trials. Human hair keratins (HHK) are the main structural element of hair fibers, and we have developed a hydrogel conduit filler based on a HHK biomaterial. A mouse 4mm and rabbit 2cm tibial nerve injury model showed that HHK hydrogel in a conduit significantly improved nerve conduction latency and compound motor action potential (CMAP) amplitude of the gastrocnemius muscle over sural nerve autograft and saline-filled conduits at 6 weeks and 3 months, respectively. In order to obtain FDA approval for human clinical study of this technology, a non-human primate (NHP) nerve injury model is currently being evaluated.

METHODS:
Human hair was obtained from a commercial vendor and keratin proteins were oxidized, extracted, purified and lyophilized. The lyophilized powder was re-hydrated with sterile PBS to form a 15% weight/volume hydrogel. 10 female cynomolgus macaques underwent unilateral (n=4) or bilateral (n=6) transection and repair of a 1 cm median nerve defect with an HHK-filled or saline-filled conduit (n=8 HHK, n=8 saline). Baseline and monthly nerve conduction velocity (NCV) measurements are performed with a Sierra Wave electrodiagnostic system (Cadwell Laboratories, Kennewick, WA) and baseline and monthly dexterity testing is performed with a puzzle feeder and recorded for analysis. At 12 months, the nerves are tested by electrophysiology, harvested and fixed in 10% neutral buffered formalin, washed with PBS and post-fixed in 1% osmium tetroxide, dehydrated in increasing concentrations of ethanol and embedded using epoxy resin. Semi-thin sections (.5µm) are cut, stained with 1% toluidine blue and mounted on slides for analysis by light microscopy. The abductor pollicis brevis (APB) muscles are harvested, flash frozen, sectioned at 10µm, fixed in 4% paraformaldehyde and stained with Masson’s trichrome to visualize myofibers while 25µm sections are stained by immunofluorescence with antibodies for neurofilament light chain, vesicular acetylcholine transporter and alpha-bungarotoxin to label neuromuscular junctions (NMJ). Quantification of nerve area, axon diameter, myofiber width and NMJ density is performed using ImageJ software (National Institutes of Health, Bethesda, MD). All surgical procedures and post-surgical monitoring are in accordance with the Animal Care and Use Committee guidelines governing Wake Forest University and institutional review committee approval was obtained. Data processing was performed using Prism 4.0 software (GraphPad, San Diego, CA). Statistical significance was calculated with either one-way ANOVA with Bonferroni’s multiple comparisons post-test, student’s t test, or two-way repeated measures ANOVA with Bonferroni post-test with the level of significance set at p < .05.

RESULTS:
The unilateral HHK group showed an average of 76% recovery of the contralateral uninjured median nerve conduction velocity (NCV) and an average of 64% recovery of the APB CMAP amplitude at 12 months. In the bilateral animals, the HHK nerves (n=4) showed an average of 45% recovery of the baseline median NCV at 36 weeks while the saline group (n=3) showed 32% recovery. The visible return of pinch grasp function occurred in an average of 20 weeks (n=3) for both HHK and saline groups following surgery although the HHK group had significantly greater recovery of the baseline NCV (p < .01) at this time point. Analysis of nerve histomorphometry for the unilateral HHK nerves showed a unimodal distribution of axon diameter compared to a bimodal distribution for uninjured nerves. Preliminary analysis of NMJ density showed significantly lower density for the injured HHK APB muscles compared to uninjured controls (p < .01) and analysis of myofiber width (as a measure of muscle atrophy) showed significantly lower fiber width in HHK APB myofibers than uninjured controls (p < .001). The regenerated APB muscles had only 19.5% atrophy compared to uninjured controls at 12 months and the APB CMAP amplitude showed no significant difference (p > .05) between HHK APB muscles and uninjured controls.

DISCUSSION:
Based on these preclinical studies, the first-ever randomized, prospective, blinded human clinical trial of a keratin biomaterial has been approved for peripheral nerve regeneration. The clinical trial will compare the use of saline-filled conduits to those filled with HHK hydrogel in patients with nerve injuries to the lower arm and hand. Archibald et al. published the first study comparing autografts to nerve conduits in a NHP median nerve injury model and reported longitudinal findings showing that the two surgical techniques had similar outcomes for functional recovery. With this technology, we seek to enhance the nerve conduit and create a more effective alternative to autograft surgery.