Repair of a segmental peripheral nerve injury with an aligned-nanofiber conduit filled with a collagen & hyaluronan hydrogel in a rat model

Sonja Limburg, Sunil K. Joshi, Rebecca Landman, Jenny J. Jin, Qia Zhang, Hubert T. Kim, & Alfred C. Kuo
Department of Orthopaedic Surgery, San Francisco Veterans Affairs Medical Center & UC San Francisco

Introduction

Peripheral nerve injuries are a challenging and potentially debilitating clinical problem with an incidence of 1-4.2% in admitted trauma patients, leading to significant long-term morbidity despite more than 200,000 surgical repairs performed annually. Nerve regeneration with segmental tissue-loss requires grafting if a tensionless primary repair cannot be performed and the current gold standard for gap repair is sensory nerve autograft. However, sensory autografts are complicated by donor site morbidity, limited availability, size mismatches, and suboptimal results, particularly when used to repair motor nerves. As a result, clinical outcomes remain inferior to primary and to-end repair.

Current alternatives to autograft include processed nerve allograft and biodegradable nerve conduits. Potential benefits of nerve conduits include unlimited supply, size variability, shorter anesthesia time, and no donor site requirement. The US FDA has approved several nerve conduits made of collagen or synthetic materials. However, there is a lack of variability in the clinical outcomes on the available conduits, and their use is generally limited to repair of short gaps in small sensory nerves.

Current synthetic conduits lack important structural and biochemical cues that are present in autograft. These include physical alignment of fibers and the presence of neurotrophic factors as well as extracellular matrix (ECM) components normally provided by Schwann cells. Conduits with longitudinally aligned internal surfaces may mimic the longitudinal alignment of nerve autografts to direct nerve growth and promote Schwann cell maturation. In vitro and in vivo studies have identified collagen (C) and hyaluronan (H) as two constituents of neuronal ECM that improve neural sprouting after traumatic injury. Intramuscular delivery of nerve growth factor (NGF) within synthetic conduits has previously been shown to improve axonal regeneration of gaps in rat sciatic nerves by improving sensory neuron fiber density, myelination, and Schwann cell migration. We therefore hypothesized that delivery of NGF may further enhance the efficacy of a hydrogel-filled nanofiber nerve conduit for segmental gap repair in a rat model.

Methods

For 12 mm aligned nanofiberPoly/ECSCo- 
crosslinked (PLCL) conduits (~1 mg/ml) of 
collagen/hyaluronan hydrogel (C/H); and 
PLCL/4C+4H

A. Gastrocnemius Tetanic Force

B. Gastrocnemius Muscle Mass

C. Nerve Conduction Latency

D. Compound Muscle Action Potential

Figure 1. Evaluation of muscle and electrophysiological recovery at 12 weeks. (A) Proportion of gastrocnemius isometric tetanic force in the operated limbs compared to the contralateral side. No statistically significant differences were found among the groups. (B) Proportion of gastrocnemius muscle mass in the operated limbs compared to the contralateral side. Animals repaired with autograft had significantly (P<0.0001) greater muscle mass than those repaired with any conduit. (C) Nerve conduction latency. Conduction was significantly faster in control nerves compared to the four operative groups (P<0.0001). There were no statistically significant differences among the operated groups. (D) Compound Muscle Action Potential. The CMAP amplitudes of the operative legs were significantly smaller than the control legs for all groups (P=0.0001). There were no statistically significant differences among the operated groups. Means±SEM are shown. PLCL indicates animals repaired with empty conduit.

Figure 2. Histomorphometry results at 8mm at 12 weeks. There were significantly more axons in the autograft group compared to the PLCL group (P<0.0001). There was no significant difference in the axon number between the PLCL/AC-4H and the PLCL/4C+4H/NGF groups, which also did not differ significantly from the autograft and PLCL groups (P>0.05). There were no statistically significant differences in axon diameters among groups with autograft yielding the largest axons (P=0.0001 compared to the PLCL and PLCL/AC-4H groups; P=0.012 for the PLCL/AC-4H/NGF group). PLCL/AC-4H/NGF showed a significant difference versus PLCL (P=0.014) but not versus PLCL/AC-4H (P=0.06). The difference in average axon diameter between PLCL/AC-4H and PLCL did not reach significance (P=0.08).

Figure 3. Representative light microscopy images of sciatic nerve cross-sections at 8mm at 12 weeks stained with 2% Osmium Tetroxide. (A) Autograft (B) PLCL (C) PLCL/AC-4H (D) PLCL/AC-4H/NGF. Scalebar=100µm, magnification 20x.

Figure 4. Sensory Recovery via thermal hyperalgesia test measuring the difference in paw withdrawal time, from a 45°C water bath between operated and control limbs. At three weeks, the control limbs withdrew significantly faster than the operated limbs for all four groups (P<0.0001). At twelve weeks, the operated limbs withdrew significantly slower than the control limbs for the autograft and PLCL (empty conduit) groups (P<0.0001). The withdrawal times for the operated limbs in both hydrogel groups were statistically equivalent to the control limbs at 12 weeks. Means±SEM are shown.

Discussion

Even when there was no statistical difference among all groups for motor force, all three conduit groups remained inferior to autograft. Neither the addition of hyaluronan NGF had an effect on motor force in our study, which might be due to insufficient NGF concentration released from the conduit, but worth expected because we did not incorporate any growth factors to especially expedite motor function, e.g. glial cell-derived neurotrophic factor (GDNF).

The muscle mass ratio between operated and contralateral side ranged between 46-63%, which is consistent with other reports in the literature, and our findings for nerve conduction latency and amplitude are in accordance with our other muscle results as well. Sensory function in vivo was improved in both hydrogel filled conduit groups compared to autograft and empty PLCL conduit.

The clinical significance of axon number and diameter is unclear, as it was not associated with detectable functional differences in our model for the muscle force. Correlation between axon diameter and sensory function regain was negative for autograft with the biggest diameter, and questionable for the PLCL/AC-4H or without NGF, which completely regains sensory function after 12 weeks, while only having a medium size diameter.

Limitations to our study were that the transplantation of the hydrogel filled conduit in the segmental nerve gap, we did not quantitatively measure the amount of hydrogel and NGF inserted, or the length of time these remained in the conduit lumen.

In summary, in our study we addition of a novel composite AC-4H hyaluronan filled with or without NGF, inside an aligned nanofiber tubule nerve conduit appeared to increase sensory recovery over the gold standard autograft, with at least an equivalent motor outcome in the mixed sciatic nerve of the rat.

This could be particularly promising in the repair of segmental defects in small sensory nerves. But despite the promising results there are still more biological factors necessary to further enhance sensory and especially motor function.

Conclusion

Our optimized PLCL conduit provides a promising alternative to nerve autograft. In this study, we demonstrated enhanced sensory recovery, but no improvement of motor recovery.

Ultimately, we hope to design a conduit that can successfully replace autograft repair, avoiding the morbidity of donor nerve harvest while producing equivalent or better results than autograft for both, sensory, and motor function.

Future Directions

Despite the promising results with our aligned PLCL conduit and collagen/hyaluronan hydrogel setup, there are still more biological factors necessary to further enhance sensory and especially motor function.

- Cells as laminar fibers
- Larger animal models
- Longer timepoints
- Longer nerve gaps

Acknowledgements

The authors wish to express their gratitude to all other members of our group for their support and valuable input on this project.

We also like to thank the UCSF Department of Orthopaedic Surgery, the Department of Defense (ODD Grant 1560), NanoFem (Berkeley) and the San Francisco VA Medical Center.