**Purpose:** Advanced prosthetic limbs can be controlled directly via the peripheral nervous system, however, seamless control of both motor and sensory components remain a futuristic goal. The Osseointegrated Neural Interface (ONI) builds on the clinical treatment for amputation neuromas, neural interfacing and osseointegration to create a chronic, stable neural interface with direct transcutaneous connection to a prosthesis required for naturalistic prosthetic control. The objective of our current research is demonstrate the capacity of nerves transposed into bone for chronic bi-directional electrophysiology in rabbits, towards future prosthetic control.

**Methods:** Above knee amputation was performed in New Zealand white rabbits. Briefly, the sciatic nerve was isolated and severed above the trifurcation. The femur was amputated at the midpoint and the nerve passed through a corticotomy. The terminal end of the nerve was sutured into a bipolar cuff electrode, and pressed back into the medullary canal. A second bi-polar cuff electrode was secured proximal to the corticotomy in order to stimulate and record efferent and afferent signals between the proximal and distal electrodes respectively. Both electrodes were connected to independent printed circuit boards, which were intern secured to a stainless steel screw. The stainless steel screw served as both the osseointegrated and percutaneous portion of the ONI device. The muscle and skin were closed over the femur. Animals underwent electrophysiological recordings of compound nerve action potentials (CNAPs) at weeks 3, 5, 8 and 12 weeks under anesthesia, as well as terminal recordings of somatosensory evoked potentials (SSEPs) at week 12.

**Results:** Chronic bi-directional signaling was achieved via an ONI, as demonstrated by the ability to record both afferent and efferent CNAPs over 12 weeks. Furthermore, physiological function of amputated nerves transposed into bone improve over time, indicated by achieving higher peak amplitudes from lower stimulation over time. Finally, the ability to record SSEPs generated from stimulation of the nerve via the ONI demonstrates the ability to write sensory information to the central nervous system, required for true haptic feedback.

**Conclusion:** The ONI represents a viable model for directly interfacing peripheral nerves with advanced prosthesis to improved prosthetic control.

---

**A Novel Hydrogel-based Nanofiber Drug Delivery System For Sustained Release Of Igf-1 Nanoparticles To Improve Functional Outcomes After Nerve Repair**

**Karim A. Sarhane, MD, MSc, Chenhu Qiu, PhD candidate, Benjamin R. Slavin, MD candidate, Nicholas Hricz, BS, Marcos Iglesias, PhD, DVM, Nicholas von Guionneau, MBBS, Philip J. Hanwright, MD, Ruifa Mi, MD, PhD, Ahmet Höke, MD, PhD, Hai-Quan Mao, PhD, Sami H. Tuffaha, MD**

**Johns Hopkins University, Baltimore, MD, USA.**

**Purpose:** Despite extensive research efforts, no therapeutic agents are currently clinically indicated for the treatment of peripheral nerve injuries. Insulin-like growth factor 1 (IGF-1) is an ideal therapeutic candidate as it can accelerate axonal regeneration and also minimize the deleterious effects of prolonged denervation on muscle and Schwann cells. However, given its short half-life, a practical delivery system is needed to stabilize the protein and provide sustained release to target tissues. Using a novel encapsulation method, we demonstrated sustained release of bioactive IGF-1 from nanoparticles, in vitro, and improved nerve regeneration and functional recovery, in vivo. An optimized carrier system to maintain the nanoparticles at target tissue sites for the duration of drug release and avoid frequent re-dosing is now needed. We therefore developed a biocompatible nanofiber hydrogel composite that could be loaded with IGF-1 nanoparticles; fine-tuned its drug release kinetics in vitro and in vivo; and applied it in a chronic denervation median nerve model to assess its impact on functional recovery.

**Methods:** An injectable nanofiber-hydrogel composite system (made of PCL nanofibers covalently bonded to hyaluronic acid) was developed by electrospinning. Its 3-D structure was formulated to mimic that of fat extracellular matrix (ECM). The release kinetics of this delivery system were then optimized in vitro and in vivo (using ELISA and immunofluorescent staining) to achieve controlled release of IGF-1 at therapeutic levels (~10 times EC50) for a prolonged period. Finally, using a chronic median nerve denervation model, we tested the effects of this modality on axonal regeneration, Schwann cell senescence, muscle atrophy and muscle force.

**Results:** The level of synthetic mimicry between our drug delivery system and ECM fat was noted to confer high levels of biocompatibility as evidenced by a minimal inflammatory
response 25 days post injection. The composite system polarized the invading macrophages into an anti-inflammatory and pro-regenerative M2 phenotype. The release kinetics of IGF-1 from the nanofiber system were superior to other carriers (fibrin glue and saline), both in vitro and in vivo. When injected into the target muscle and deposited at the nerve coaptation site in a chronic median nerve denervation model, functional outcomes were improved (although not reaching a customary level of statistical significance).

Conclusion: We introduce a novel drug delivery system in which IGF-1 nanoparticles are combined with a nanofiber hydrogel carrier to provide sustained local concentrations of bioactive IGF-1 within target nerve and muscle. This therapeutic approach has the potential to improve functional outcomes via enhanced axonal regeneration and maintenance of denervated muscle and Schwann cells. IGF-1 and the polymer components of the engineered delivery system are currently used in FDA-approved formulations and devices, which will facilitate clearance of regulatory hurdles.

115

Unlike A Conditioning Crush Lesion, Conditioning Electrical Stimulation Promotes Functional Sensory And Motor Nerve Regeneration In A Non-inflammatory Manner

Christine A. Webber, PhD, Jenna-Lynn Senger, PhD, MD, Leah Acton, Susanne Lingrell, K. Ming Chan, MD

University of Alberta, Edmonton, AB, Canada.

Purpose: Prior to a nerve repair, conditioning electrical stimulation (CES) promotes regeneration associated gene (RAG) expression at the neuronal cell bodies and promotes functional nerve regeneration as well as, or beyond that of a conditioning crush lesion (CCL) (Senger et al., 2017; 2019). Forty years of research has established that the inflammatory response evoked by the CCL injury prior to the cut and coaptation is essential to the conditioning response. It is not known how CES promotes nerve regeneration. We propose that CES promotes functional nerve regeneration in a non-injurious, and non-inflammatory manner.

Methods: Sprague Dawley rats were equally divided into three cohorts (n=10/cohort): i) CES, ii) CCL (positive control) and iv) sham-ES (negative control). CES, CCL and sham-ES conditioning were delivered one week prior to nerve cut/coaptation. The conditioning site and the corresponding dorsal root ganglion neurons were harvest at 1 day, 3 day and 14 days post-conditioning (thus the 14 day cohort was the only one to receive cut/coaptation). Immunocytochemistry for macrophages and neurofilament to investigate inflammation and Wallerian degeneration, respectively was performed and this data was corroborated by qRT-PCR analysis. Immunocytochemistry and qRT-PCR analysis were performed on the neuronal cell bodies to study the RAG expression following conditioning. In a separate experiment, CCR2-/- (macrophage depleted transgenic mice) and wild type C57/Bl mice (n=6/cohort) were conditioned with CES, CCL or no-ES. Previous studies have shown that CCL does not evoke a conditioning effect in the CCR2-/- mice indicating the inflammatory response is necessary for this effect. These experiments were designed to determine if macrophages are necessary for CES to promote nerve regeneration.

Results: Rats treated with CCL alone at all time points evoked an inflammatory response. Unlike CES and sham-ES, there was significant macrophage infiltration at 1 and 3 days in the CCL animals, whereas both CES and CCL demonstrated accelerate nerve regeneration at 14 days post-conditioning. Furthermore, although CCL induced Wallerian degeneration distal to the conditioning site, CES and sham-ES did not cause degeneration of the distal axons. At the neuronal cell bodies, qRT-PCR and immunocytochemistry demonstrated there was an increase in inflammatory (IL-6) and injury markers (ATF3) at 24 hours post-injury in the CCL cohort only whereas the RAG upregulation (pCREB, GAP43, BDNF) of CES and CCL was similar at 3 days. These data suggest that CES and CCL may upregulate RAGs through independent but converging pathways. In the mouse study, the CES treated CCR2-/- (macrophage depleted) mice both showed the same upregulation of pCREB, GAP-43 and BDNF as their corresponding C57/Bl wildtype mice, suggesting CES does not require the immune response to evoke the conditioning effect.

Conclusion: CES significantly improves regeneration and reinnervation beyond that attainable with current clinical standards. It is a clinically feasible method of improving outcomes for patients with peripheral nerve injury as it does not evoke an inflammatory response or Wallerian degeneration. Further investigation is required to delineate the molecular and cellular mechanisms associated with the ability of CES to promote regeneration without evoking an immune response.