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Prophylactic Regenerative Peripheral Nerve Interfaces for the Mitigation of Neuroma Pain and Phantom Limb Pain

Carrie A. Kubiak, MD, Stephen W. P. Kemp, PhD, Paul S. Cederna, MD, Theodore A. Kung, MD

University of Michigan, Ann Arbor, MI

PURPOSE: Regenerative Peripheral Nerve Interfaces (RPNIs) can be used to treat symptomatic end neuromas that develop after major limb amputation. Symptomatic neuromas occur in approximately 30–40% of individuals after limb loss and phantom limb pain affects 70–95% of these patients. We investigate the potential of prophylactic RPNIs to prevent neuroma formation and to mitigate the experience of phantom limb pain. Furthermore, we examine the potential complications resulting from the addition of prophylactic RPNIs to major limb amputation surgery.

METHODS: RPNIs were performed during the time of amputation by implanting transected peripheral nerves into free muscle grafts harvested from the amputated limb. Patients who underwent major limb amputation with simultaneous prophylactic RPNI implantation were identified. A retrospective chart review was performed to ascertain patient demographics, indication for amputation, level of amputation, characteristics of postamputation pain, perioperative pain management strategies, and postoperative complications. During follow up, all patients were evaluated specifically for symptomatic neuromas, residual limb pain, and phantom limb pain through history and physical examination.

RESULTS: RPNIs were prophylactically implanted in 28 patients who underwent 29 major limb amputations. The mean patient age was 46 years (range 13–79) and mean follow up was 89 days (range 6–273). The most common indication for amputation was infection (n=14, 48%) followed by trauma (n=8, 28%). Below knee amputations comprised the majority of subjects (n=23, 79%). Major postoperative complications were defined as events that resulted in admission or surgery; one patient was admitted for postoperative nausea and vomiting, but this was unrelated to the RPNI surgery. Minor complications included delayed wound healing (28%) and surgical site infection managed on an outpatient basis (14%). The incidence of overall complications after prophylactic RPNIs (32%) was not higher than that of traditional limb amputation procedures (20–70%). Zero of the twenty nine patients (0%) had any clinical evidence of symptomatic neuroma postoperatively. Only 8 patients (28.6%) reported symptoms of phantom limb pain at any point during their postoperative course.

CONCLUSIONS: Prophylactic RPNIs in major limb amputees resulted in a considerably lower incidence of both symptomatic neuromas and phantom limb pain as compared to published rates in the literature. Additionally, prophylactic RPNIs did not contribute to increased morbidity compared to standard amputation techniques. These findings suggest that prevention of peripheral nerve pain following major limb amputation may diminish the central pain mechanisms that lead to phantom limb pain. This pilot study supports prospective comparative investigation of using RPNIs to significantly reduce postamputation pain.

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Macrophage Recruitment and Activation to Skeletal Muscle after Acute Nerve Injury

Katherine B. Santosa, MD, Albina Jablonka-Shariff, PhD, Anja G. Fuchs, PhD, Alexandra M. Keane, B.A., Isaiah R. Turbull, MD, PhD, Alison K. Snyder-Warwick, MD

Washington University, Saint Louis, MO

PURPOSE: The skeletal muscle has a unique immunologic profile that is particularly dynamic following muscle injury. Immune cells such as macrophages, which are normally few in number during physiologic conditions, are recruited to the muscle following injury to assist in inflammatory and reparative processes. In response to their muscle microenvironment, macrophages are able to change their phenotypes and functions during these events. Although macrophage recruitment and activation have been demonstrated in direct muscle injury models, this dynamic process has not been described in muscle after nerve injury. The goal of this study was to determine if acute nerve injury resulted in the recruitment of macrophages to the distal muscle target, and to characterize the phenotype of these activated macrophages.

METHODS: We utilized two strategies to determine if macrophages are recruited to muscle after sciatic nerve
 transection without repair. First, we evaluated the extensor digitorum longus (EDL) muscles of 15 adult wildtype C57BL/6 mice (n=3 per time point) at days 1, 3, 5, 7, and 14 after sciatic nerve injury. The uninjured EDL muscles served as the experimental controls. These muscles were harvested for immunostaining with CD68 (monocytes/macrophages) and DAPI (nuclear) staining. Next, using the same injury and mouse model, flow cytometry was utilized to evaluate total cells present in EDL muscle after sciatic nerve injury. Animals were sacrificed at days 1 and 5 after nerve injury, and all muscles of the hindlimb innervated by the sciatic nerve were harvested from the right injured and left uninjured legs. Cells were analyzed following muscle digestion.

RESULTS: At all timepoints after nerve injury, there were significantly more CD68+ cells recruited to denervated EDL muscles than to uninjured controls in our immunocytochemistry analysis. On flow cytometry, there was a higher number of CD45+ hematopoietic cells isolated from denervated muscle than uninjured controls. Moreover, data demonstrate significantly more Ly6C^-F480^- monocytes and CD206^-MerTK^- macrophages recruited to the muscle following acute nerve injury. At postoperative day 5, CD206^-MerTK^- macrophages had decreased CD11c expression, suggesting activation of these immune cells.

CONCLUSIONS: Our studies demonstrate the novel finding that acute nerve injury induces macrophage recruitment to the distal target muscle. Moreover, recruited macrophages have an altered phenotype, which may suggest a functional transformation of these important inflammatory and regenerative immune cells. Further studies are ongoing to determine the functional impact of this macrophage phenotypic change on reinnervation of the muscle following acute nerve injury. Knowledge of this process may provide new therapeutic targets to improve functional recovery following nerve injury.

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Nanofiber-Hydrogel Composite with Human Adipose-Derived Stem Cells to Enable Soft Tissue Regeneration

Brian H. Cho, MD1,2, Xiaowei Li, PhD2,3, Sashank Reddy, MD, PhD1, Russell Martin, PhD2,3, Michelle Seu, BA1,2, Gurjot Walia, BS1, Hai-Quan Mao, PhD2,3, Justin M. Sacks, MD, MBA, FACS1

1Department of Plastic and Reconstructive Surgery, Johns Hopkins School of Medicine, Baltimore, MD, 2Translational Tissue Engineering Center, Johns Hopkins School of Medicine, Baltimore, MD, 3Department of Materials Science & Engineering, Whitman School of Engineering, Johns Hopkins University, Baltimore, MD

PURPOSE: Develop a mechanically-tunable nanofiber-hydrogel composite to promote vascular ingrowth, survival, and migration of transplanted human adipose-derived stem cells (hASCs) for soft-tissue regeneration. This composite material directly addresses the limited utility of current soft-tissue repair paradigms, including fat grafting and dermal fillers, which are limited to small-volume defects and transient-volume restoration, respectively.

METHODS: We developed a unique composite scaffold by interfacially bonding biodegradable poly(caprolactone) fibers with hyaluronic acid hydrogel, forming an integrated structure resembling the architecture and mechanical properties of adipose tissue. We optimized our composite for ability to promote hASC migration and vascularization in vitro. Using the optimized composite as a carrier, we subcutaneously delivered hASCs into rats to assess the effect of composite-mediated delivery on survival, adipose differentiation, and host-tissue integration of the transplanted cells.

RESULTS: Human ASCs migrating the longest distance within the composite compared to soft and medium hydrogel controls (203 vs. 122, and 0 μm; P<0.05). Within the soft hydrogel control and the composite, cultured ASCs exhibited vascular morphogenesis and organized to form multicellular tubular structures with branches and open luminal spaces. As shown in, composite exhibited the highest network density (total length of interconnected branches divided by total area, 16.4 vs. 12.4, and 2.1 mm/mm²). In the rat model, we observed a significantly higher density of RECA-1+ endothelial cells within our composite compared with controls. Additionally, composite-mediated hASC delivery yielded the highest degree of cell survival, spreading, and differentiation.

CONCLUSION: Our composite scaffold promotes angiogenesis and enables delivery of hASCs and tissue regeneration for treatment of soft tissue defects. This composite scaffold has the potential for wide application to improve soft-tissue restoration in the clinical setting.