Oral Presentation Abstracts

1. Sitting Is a PAIN in the Ischia
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Purpose: The ischial tuberosity, referred to as the “sit bone” by patients with pain when sitting, is surrounded by critical nerves. Depending upon how a person places weight on the ischial tuberosity, symptoms can vary from pudendal nerve symptoms, to sciatica, or to posterior femoral cutaneous nerve (PFCN) symptoms. This research relates, for the first time, the involvement of the PFCN, including its perineal and inferior cluneal branches to the symptom of “Pain in the ISCHIA”. The outcome of resecting this nerve is presented.

Methods & Materials: From 2010 through August of 2013, 14 patients had surgery on the PFCN. Twelve were women. Mean age of the 14 patients was 54.6 years (range 30 to 74 years). Each patient had pain with sitting and symptoms involving the buttock, posterior thigh and perineum. Patients spent much of the day standing or lying down. Mean symptom duration was 63.5 months (range 12 to 180 months). Mechanism of injury was a hamstring tear in 7 of the 14, usually related to an athletic event or a fall. Previous misdiagnosis: Nine had transgluteal pudendal nerve decompression, two had pyrimiformis excision and sciatic neurolysis. Each of the 14 patients in this series had a resection of the PFCN through an incision in the gluteal crease.

Results: Of the 14 patients, at a mean of 19.4 months post-operatively, there were 6 excellent, 5 good, 2 fair, and 1 poor result: 79% good to excellent results (pain relief, improvement in sitting). Two fair results were in the first patients, the only two in whom the entire PFCN was not removed. There were no surgical complications.

Conclusion: Ischial pain can be due to injury to PFCN, a previously unreported etiology. Good to excellent results can be expected in 79% of carefully selected patients.
Introduction: Surgical treatment for chronic headaches has demonstrated impressive and consistent efficacy in a number of clinical studies. The occipital region is one primary target for this surgical approach, yet the majority of published studies on this particular topic focus on the greater occipital nerve. The lesser occipital nerve (LON) may also be an important surgical target, yet is often only described in passing and is usually resected.

Methods: The aim of the current study was to define the anatomy and compression topography of the lesser occipital nerve. Eight fresh frozen cadaver heads were dissected to identify the LON. The emergence of the LON was measured in relation to established surgical landmarks: the occipital protuberance (OP), the midline and the nuchal line. 15 LONs were identified in eight dissected heads. Three potential compression zones have been identified clinically based on the senior author’s (ZP) experience: 1) the zone of emergence of the LON from deep to the sternocleidomastoid (SCM) muscle, 2) the ascent of the LON along the posterior border of the SCM and 3) the point at which the LON crosses the nuchal line. All three zones were examined and measured in this study.

Results: The emergence of the LON from deep to the SCM occurred on average 7.8 cm caudal to the OP and 6.4 cm from the midline. The midpoint of ascent of the LON along the posterior SCM border occurred on average 5.5 cm caudal to the OP and 6.2 cm from the midline. At least one consistent branch point below the nuchal line was noted in all specimens. This first branch point occurred on average 3.8 cm caudal to the OP and 5.9 cm from the midline. The medial-most LON branch crossed the nuchal line an average of 5.4 cm lateral to the midline and the lateral-most LON branch crossed the nuchal line an average of 6.5 cm lateral to the midline.

Conclusions: The compression topography of the LON consists of three distinct compression zones. The findings of this cadaver study corroborate the clinical experience of the senior author and can help the surgeon identify the LON at its proximal compression point as it emerges from deep to the SCM. While the more distal compression topography and branching pattern can be quite variable, these results may aid the surgeon in safely dissecting and preserving the LON during surgical treatment of chronic headache patients.
3. Comparison of Effectiveness Of Different Surgical Treatments for Meralgia Paresthetica: Results of a Prospective Observational Study and Protocol for a Randomized Controlled Trial
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**Introduction:** Various surgical procedures can be applied in the treatment of meralgia paresthetica, including neurolysis and neurectomy of the lateral femoral cutaneous nerve. To date, no randomized controlled trial has been reported on the effectiveness of these procedures. In this study we present the results of a prospective observational study and the protocol for a double blind randomized controlled trial, both aimed at comparing the outcome for neurolysis and neurectomy.

**Methods and analysis:** All patients that were surgically treated for persistent symptoms of idiopathic meralgia paresthetica were included. The decision for the type of treatment (neurolysis or neurectomy) was left to the patient after informed consent. Primary outcome was measured using the Likert scale (ranging from 1-7), which was obtained 6 weeks after the surgery. Successful pain reduction was defined as Likert 1 or 2. Secondary outcome measures were the Numeric Rating Scale (NRS, rating pain from 0-10) and Bothersomeness Index (BSI, rating the degree the patient is bothered from 0-6). In addition, after neurectomy the BSI for numbness was obtained (also rated from 0-6).

**Results:** Between August 2012 and April 2014 a total of 22 patients were operated: in 8 patients the neurolysis procedure was performed and in 14 patients the neurectomy procedure (one bilateral case). Successful pain reduction was observed more frequently after neurectomy (93.3%) than after neurolysis (37.5%, $P < 0.05$). Secondary outcome scores (NRS and BSI for pain) were also better after neurectomy, although not significantly (respectively $P = 0.07$ and 0.05). Paired analysis of the scores before and after the surgery showed an improvement in both the NRS and BSI after the neurectomy procedure (both $P < 0.001$), while scores were not significantly different before and after the neurolysis procedure (respectively $P = 0.25$ and 0.125). Interestingly, scores for the BSI for numbness after the neurectomy procedure were low (mean 1.4, SD +/- 1.0, range 0-3).

**Discussion:** This prospective observational study confirms results from our previous retrospective study which showed better results after the neurectomy procedure [1]. In addition, the results show that the impact of numbness after the neurectomy is low. Currently, we are undertaking a randomized controlled trial to investigate the difference in a double blind fashion. The same outcome measures presented in this study are thereby used.

4. Traction Neurectomy for the Treatment of Painful Residual Limb Neuromas in Lower Extremity Amputees
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Background: Traction neurectomy is a surgical treatment for symptomatic neuroma of the residual limb that entails neuroma excision, with high transection of the nerve while on traction. The nerve stump is thereby proximally relocated into an area more protected by muscle and soft tissue, where it is less likely to become irritated and symptomatic. This technique is favored by many surgeons for its simplicity, and the favorable outcomes documented in the limited available literature. Our group has performed this technique regularly in lower extremity amputees, and in this series we document our experience and outcomes.

Materials and Methods: We retrospectively reviewed all above and below knee amputees with a diagnosis of symptomatic neuroma of the residual limb, treated with traction neurectomy by a single surgeon at a high-volume trauma center between 2006 and 2014. The primary outcome was the presence or absence of neuroma-type pain at the time of last follow-up, and the secondary outcome was performance of revisional surgery for persistent or recurrent symptomatic neuroma(s) of the treated nerve(s). Demographic and clinical variables were investigated as potential predictors for persistent/recurrent disease. Results: 67 patients (108 nerves) met study inclusion criteria. 29 patients were excluded for inadequate follow-up (<10 months) leaving 38 patients (63 nerves) comprising our study group. There were no significant differences between the included and excluded groups with regards to age, gender, amputation level, reason for amputation, prior surgical therapy for symptomatic neuroma, time since amputation, or concomitant surgical procedures. 35/38 (92%) patients experienced initial symptomatic relief, but 16/38 patients (42%) had recurrent or persistent neuroma-type pain at a mean follow-up of 36 months (range, 11-91 months), and 8/38 (21%) underwent subsequent surgical treatment. The mean time to recurrence was 5.3 months (range 0-81 months). Of the demographic and clinical features listed above, only male gender was found to be a statistically significant predictor of persistent/recurrent neuroma-type pain.

Conclusions: In our cohort of lower extremity amputees with symptomatic neuroma of the residual limb, we found a high rate of persistent or recurrent neuroma pain. This is starkly in contrast to other similar series reporting recurrence at or approaching 0%. Most recurrences were early, and male gender was identified as a risk factor for persistent/recurrent symptoms. We conclude that traction neurectomy is not an optimal treatment, and the efforts of many groups who continue to seek better surgical and non-surgical treatments for this problem are justified.
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Object: The goals of this study were to retrospectively observe and evaluate the outcome of pain reduction and long length, acellular, processed cadaver allograft use in the surgical repair of sciatic nerve injuries of patients injured in recent military conflicts. Traumatic injury to the sciatic nerve sustained in military conflict tends to be severe with protean consequences. This injury is often associated with widespread soft tissue and bone injuries, significant neurologic impairment, severe neuritic pain, and a prolonged recovery time. There is limited data that describes the treatment of these significant and devastating nerve injuries.

Methods: We retrospectively reviewed the surgical records of 5137 combat related extremity injuries at three institutions between June 2007 and June 2013 to identify those patients with combat-related sciatic nerve injury without amputation of the affected side. Patients included in this study underwent a thorough chart review including pain assessments, radiographs, surgical reports, and intraoperative photographs to determine severity of injury, and the timing from injury to surgery, to assess outcome.

Results: Thirteen patients were identified as having combat related sciatic nerve injuries, all patients were male, mean age was 28 years. The mechanisms of injury were 9 gunshot wounds (69%), 2 rocket propelled grenade (RPG) blasts (15%) and 2 improvised explosive device (IED) blasts (15%). Three patients (23%) with dense sensory-motor loss were found to have a neuroma in continuity, and required only neurolysis. Eight, patients (53%) with nerve transections and neuroma formation had long length (5-7 cm) cadaver allograft grafts placed, one patient had a sural nerve autograft (5 cm), and 1 patient underwent end to end direct nerve repair. In comparing those patients who had early versus standard timing of nerve surgery, there was no difference in the amount of damaged nerve resected and both groups had equivalent reductions of pain and narcotic use at 6 weeks and 6 months postoperatively. There were no complications due to graft infection or rejection in either group.

Conclusions: Traditional teaching is to delay sciatic nerve injury repair for at least six months to provide the damaged nerve a period of self-recovery and to allow structural damage to the injured nerve to fully declare itself. Our experience demonstrates that combat related sciatic nerve injuries can be reliably operated on 21 to 30 days post injury, with great benefit toward reduction of severe neuritic pain.
6. Neurectomies and Neurolysis for the Treatment Of Chronic Postoperative Pain
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**Introduction:** Open and laparoscopic trunk surgeries are very commonly performed procedures in the US – there were approximately 920,000 hernia repairs and 500,000 laparoscopic cholecystectomies performed in 2006. The incidence of chronic pain after such surgeries has been reported to be between 1% and 20%. As such, there is a large population of patients suffering from chronic postoperative neurogenic pain. One possible treatment for such patients is selective surgical neurectomies and less commonly selective neurolysis.

**Methods:** All patients of the senior author (SMR) who underwent neurectomies or neurolysis for complaints of chronic postoperative pain following trunk or groin surgeries were identified. Based on history and physical exam, patients underwent selective neurectomies including intercostal, ilioinguinal, iliohypogastric, genitofemoral or lateral femoral cutaneous nerves. Patients were administered a phone survey based on the Pain Disability Index to assess their pre- and post-operative pain level and quality of life.

**Results:** 57 patients (32 male, 25 female) who underwent neurectomies were identified. Mean age was 49 years. All patients underwent preoperative nerve block by the senior author or the referring physician, and had either a complete or significant response. 46 patients completed the survey (7 declined, 2 could not be contacted after three attempts, 2 were deceased). Median follow-up was 2.8 years (0.5-5.7 years). Average pain level was reported to be 9.0 preoperatively and 3.5 postoperatively on a 0-10 Likert scale. Quality of life impairment improved from 8.2 preoperatively to 3.4 postoperatively. A subset of patients (n=12) had minimal improvement, reporting a pain decrease from 9.2 to 7.9, and a quality of life improvement from 9.2 to 7.6.

**Conclusions:** Neurectomies or less commonly neurolysis can be effective means of relieving chronic postoperative pain caused by trunk or groin surgeries, with excellent improvements in pain and quality of life scores. There is a subset of patients who respond to nerve blocks but have minimal improvement after surgery.
7. The Surgical Treatment of Chronic Headaches: Lessons Learned From the First 100 Cases
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Introduction: The surgical treatment of chronic headaches has been well established in the plastic surgical literature as a viable and successful method of addressing a very common and difficult clinical problem. This treatment modality is not only very effective, but especially useful in those cases refractory to medical management.

Methods and Results: Prospectively collected data on my first 100 surgical headache cases were analyzed. Migraine Headache Index, an established and reliable gauge of headache severity was used to measure outcomes. A successful result was defined as at least a 50% reduction in the frequency, duration, and/or severity of headache symptoms in keeping with results reported in previously published literature. Cases included frontal, temporal, and occipital procedures. 87% of patients experienced at least a 50% reduction in the frequency, duration, and/or severity of their headache symptoms. 40% experienced complete elimination of their severe headaches. Migraine Headache Index scores were also significantly improved post-operatively (143 vs 35, P<0.0001). Mean follow-up was 16.6 months.

Conclusions: Successful outcomes can be achieved with surgical treatment of chronic, severe headaches, but it is important to note that incorporating this modality into one’s practice can be challenging. There can be significant resistance in the medical community to this relatively novel treatment modality and the patients themselves can be demanding, much like any chronic pain population. Several practical suggestions as to how to deal with these barriers and challenges are proposed.
8. Modified Management of Sensory Nerve Neuroma of the Foot When Implantation into Muscle Is Not Available; Proximal Nerve Stump Capping with Blind-Ended Degradable Nerve Protector
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Introduction: Reconstruction of sensory nerve injuries is critical to prevent painful neuroma formation. Following trauma or surgery to the foot, injured cutaneous nerves are exposed to a number of influences that can lead to unfavorable outcomes. Poor outcomes can be attributed to scar formation within the nerve as well as tethering of the nerve to surrounding tissue. While the events are multi-factorial, a common basis for neuroma formation is disorganized growth of axon cylinders into proliferating granulation tissue. This has been shown to be preventable by an epineural sleeve, implantation into muscle fibers, or neural coaption to adjacent nerve fascicles. Isolation of a nerve from its surrounding environment may prevent scar ingrowth, minimize potential for nerve entrapment, and reduce the impact of aberrantly regenerating nerve fibers. We present an alternate technique for terminal nerve protection to provide an environment resistant to neuroma formation.

Materials and Methods: Seventeen patients underwent exploration for painful neuromas of the foot following trauma or previous surgery. All patients reported a defined focal point of maximal pain in a specific region and all were found to have a surgically identifiable cutaneous neuroma (n=17). Identified nerves were the intermediate or medial dorsal cutaneous nerve (n=8), medial plantar nerve (n=6) or distal sural nerve (n=3). All patients underwent excision of the neuroma, proximal neuroplasty to avoid the area of trauma, and capping of the terminal nerve with a blind-ended degradable nerve protector. Patients were followed for a minimum of nine months (range 9-16 months).

Results: Standardized pain questionnaires were collected on each patient at the time of most recent follow-up. The mean pre-operative pain score on a 0-10 scale for all patients was 6.8±1.4/10, and all patients reported typical nerve entrapment symptoms (Burning, Stabbing, Tingling) on selected descriptive word associations. Mean post-operative pain scores were all reduced and the average reduction was 4.7±0.7 points. All patients reported an immediate reduction and no patients have reported recurrence of the symptoms (n=17, mean pain score 1.7±0.5 points).

Conclusions: Prevention of post-operative neuroma is a major focus for peripheral nerve specialists. Engineered nerve protectors are designed to provide an interface between the nerve and the surrounding tissue, mechanically resist compression from surrounding tissue and exclude scar tissue ingrowth. In selected patients with no expendable or available muscle for implantation, this represents a safe and reasonable option for protection of the distal nerve and prevention of neuroma recurrence or further nerve injury.
9. Transient Receptor Potential Vanilloid-1 Channel Blockade in the Peripheral Nerve Terminal by Resiniferatoxin for the Treatment of Chronic Pain
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\textbf{Background:} Expression of TRPV-1 channels in peripheral nerve terminals is responsible for transducing thermal and chemical nociception. Various systemic chemical antagonists of the TRPV-1 receptor have been trialed for the treatment of chronic pain; however, they lead to increased basal body temperature and fevers. We have previously shown that TRPV-1 activity and pain signaling is reduced in the presence of botulinum toxin; however, adverse effects of botulinum toxin include local muscle paralysis. Recent emergence of resiniferatoxin (RTX) has shown to activate TRPV-1 in an irreversible manner and lead to ablation at the nerve terminal. With its low side-effect profile, we hypothesize that RTX may be used to target TRPV-1 nociceptive pathways in the peripheral nerve to treat chronic pain conditions such as complex regional pain syndrome, neuromas, and arthritis.

\textbf{Methods:} Increasing concentrations of RTX were injected into the plantar surface of the hind paw of wild-type mice. Capsaicin-induced nocifensive behavior was recorded. Response latencies to thermal stimulation in RTX-injected and control mice and were measured with a radiant heat apparatus. Dorsal root ganglion and plantar skin of the hind paw were stained for TRPV-1 for immunohistochemistry analysis and confocal imaging. Experiments were repeated with TRPV-1 knockout mice. Statistical significance was defined as $p<0.05$.

\textbf{Results:} Dose-dependent loss of capsaicin-induced nocifensive behavior was observed on day two following RTX administration. Capsaicin-induced nocifensive behavior gradually recovered to its basal level over a period of 63 days. Enhanced response latency to radiant heat was observed two days following RTX treatment. Response latency decreased gradually over a two-month period. Control mice injected with saline showed no change in response latency when subjected to the radiant heat test. TRPV-1 knockout mice showed moderate decrease in response latency as compared to RTX-treated mice. Immunohistochemistry analysis demonstrated no staining for TRPV-1 in paw skin after two days from RTX injection; however, TRPV-1 nerve fibers demonstrated regeneration over a two-month period. There was no change in the number of dorsal root ganglion TRPV-1 expressing cell bodies or the intensity of staining of these cell bodies.

\textbf{Conclusions:} This research investigates the effects of RTX on nociceptive targets in the peripheral nerve that mediate and sensitize pain signaling. In this study we have determined the role of TRPV-1 nerve terminal expression. RTX administration temporarily ablates TRPV-1 expressing peripheral nerve fibers without affecting their regeneration capacity. This provides novel therapeutic perspectives for treating chronic pain pathologies involving the peripheral nervous system.
10. A Novel Immunomodulatory Approach using a Selectively Permeable Nanofiber Wrap to Enhance Nerve Regeneration
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Purpose: Despite great advances in microsurgery, functional outcomes following nerve repair remain suboptimal. Scar formation at the repair site is recognized as a major impediment to regenerating axons. In this regard, an inert barrier around the coaptation site that prevents inflammatory cells infiltration while still allowing the diffusion of nutrients and nerve growth factors holds great potential in promoting nerve regeneration and functional return. In this study, we examined the efficacy of a novel semi-permeable nanofiber construct, prepared from FDA approved biomaterials, to be used as a wrap around the repair site to promote nerve regeneration and functional recovery.

Methods: Nerve wraps comprised of nonwoven electrospun poly (ε-caprolactone) nanofibers with pores smaller than 10 µm were synthesized (Fig. 1a). They were wrapped around the repair site in a sciatic transection/repair model in Thy-1 GFP rats. At 5 weeks, their neuro-protective and neuro-regenerative potentials were assessed. At 16 weeks, functional recovery was evaluated.

Results: At 5 weeks, the nanofiber wraps resulted in significantly decreased collagen deposition and inflammation/macrophage invasion at the repair site (Fig. 1b). The total number of myelinated axons was significantly increased (Fig. 1d), and there was a trend towards a higher number of regenerated dorsal root ganglion sensory neurons. Mechanistically, these outcomes were correlated to an up-regulation of the anti-inflammatory cytokine (IL-10) and down-regulation of the pro-inflammatory cytokine (TNF-α) (Fig. 1e). In addition, at 16 weeks, the nerve wrap group showed enhanced functional recovery as demonstrated by electrophysiology (Fig. 1f), gait analysis, neuromuscular junction re-innervation (Fig. 1g), and gastrocnemius muscle weight and histology.

Conclusions: Our results demonstrate favorable outcomes of a novel semi-permeable and clinically translatable nanofiber nerve wrap in protecting the coaptation site and enhancing axonal regeneration through scar-free nerve repair, resulting in optimal functional recovery.
11. Advanced Regenerative Electrodes Enable Functional Electrical Stimulation of Peripheral Motor and Sensory Axons
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Introduction: Neuroprosthetic technologies offer one of the most promising approaches to restoring native sensorimotor function following neurologic injury. Development of electrodes capable of facilitating chronic high-specificity nerve stimulation and recording may enable long-lasting restoration of motor function, hand/arm control, as well as improvements in sensory feedback and proprioception. Regenerative sieve electrodes represent a novel approach to achieving such an advanced interface with peripheral nerve tissue, but have yet to be proven as a singular interface to both motor and sensory nerve fibers. The present study aimed to examine the ability of chronically implanted regenerative sieve electrodes to functionally interface both motor and sensory axons in mammalian mixed nerve.

Materials & Methods: Custom-designed sieve electrodes were fabricated out of polyimide and gold using sacrificial photolithography. Regenerative sieve electrodes were then microsurgically implanted in the sciatic nerve of male Lewis rats using a dorsolateral gluteal muscle splitting incision. Post-operatively, functional nerve regeneration through implanted devices was assessed via nerve conduction studies and evoked muscle force measurement. Nerve interfacing was assessed in situ by stimulating regenerated nerve tissue via implanted sieve electrodes while simultaneously recording force production in distal musculature and local field potentials in sensory cortex. Regenerated nerve segments were terminally explanted and fixed for morphological or histomorphometric evaluation.

Results: Micro-surgical implantation of sieve electrodes in the sciatic nerve of healthy male rats for 1, 2, and 3 months demonstrated robust axonal regeneration through implanted devices. Chronically implanted sieve electrodes demonstrated recruitment of integrated nerve tissue at stimulus amplitudes as low as 10-20 µA, and highly selective activation of distal musculature (99.7% SI for EDL, 99.5% SI for TA, and 99.9% SI for Gastrocnemius) and thereby motor axons. Implanted sieve electrodes additionally demonstrated successful recruitment of sensory nerve fibers and induction of neural activity in somatosensory cortex (S1). Mapping of S1 utilizing silicon microelectrode arrays may further elucidate the selectivity of sensory fiber activation facilitated by chronically-implanted sieve electrodes.

Conclusions: The present study confirms the ability of sieve electrodes to facilitate a selective, stable interface with both motor and sensory nerve fibers. These findings suggest that regenerative sieve electrodes may be able to provide a stable, bi-directional, interface ideal for end translational use in advanced neuroprothetic systems.
A Novel Use for Partial Skeletal Muscle Grafts in Regenerative Peripheral Nerve Interfaces for Prosthetic Control
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Objective: Nonvascularized partial skeletal muscle grafts are notorious for their limited force-generating capacity and tendency to degenerate in the absence of reinnervation. Accompanied by peripheral nerve implantation, however, partial muscle grafts survive and transmit detectable electromyographic (EMG) signals capable of prosthetic control. Our study investigated partial muscle graft survival in the construction of regenerative peripheral nerve interfaces (RPNIs) and further characterized their electrophysiological properties across various muscle donor sites.

Methods: Twenty adult male F344 rats were assigned to 1 of 5 groups based on muscle graft type used for RPNI construction: 1) control-whole extensor digitorum longus; 2) partial biceps femoris; 3) partial rectus femoris; 4) partial lateral gastrocnemius; and 5) partial vastus medialis. Each graft of approximately 140-mg was transferred to the thigh, wrapped in small intestinal submucosa for tissue isolation, and implanted with the transected common peroneal nerve. After 4 months of RPNI recovery, in situ EMG and force testing were performed (Figure 1).

Results: Twelve of 16 partial muscle RPNIs demonstrated detectable EMG function at 4 months. Significant differences between control (n=4) and functional partial muscle RPNIs (n=12) included average mass (118±42 mg vs. 66±25 mg), EMG peak-to-peak amplitude (6.7±2.3 mV vs. 1.5±1.6 mV), and maximum tetanic force (729±666 mN vs. 175±154 mN) (Figure 2). RPNI mass was the overriding significant predictor of EMG peak-to-peak amplitude (p<0.01). After adjusting for RPNI mass, donor muscle selection showed no correlation with partial muscle EMG signal strength.

Conclusions: Partial muscle graft RPNIs transmit detectable EMG signals with a 75% success rate at 4 months. This proof of concept underscores the potential to develop and refine partial muscle graft-based interfaces to harness peripheral nerve signals for high-fidelity prosthetic control.

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Figure 1. Example of a regenerative peripheral nerve interface (RPNI) at 4 months, constructed with a partial lateral gastrocnemius muscle graft implanted with the common peroneal nerve in the thigh. Bipolar needle electromyography (EMG) was performed using direct stimulation of the common peroneal nerve. The tibial nerve was transected just prior to testing in order to minimize signal interference.

Figure 2. Comparison of control versus partial muscle RPNI mass, EMG peak-to-peak amplitude, and peak tetanic force at 4 months. Values for partial muscle RPNI groups are expressed as percentages of control group values, with n=4 per group. All values for partial muscle RPNI groups were significantly lower than their control group counterparts (*, p<0.05). EDL, extensor digitorum longus; BF, biceps femoris; GLH, gastrocnemius, lateral head; RF, rectus femoris; VM, vastus medialis.
13. Impact of Age on Outcomes in Peripheral Nerve Repair with Processed Nerve Allograft

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Introduction: Many factors that are outside of the surgeon's control impact the level of functional recovery following peripheral nerve repair. These factors include age of the patient, mechanism of injury, location of injury, and demographical background. Recent meta-analyses have reported that average functional recovery rates for adults can decrease by 50% in patient over 50, as compared to those under 30. The cause of these decreases are not well characterized, however age related changes in regenerative potential and decreased cortical plasticity are thought to play a role. To determine the impact of patient age on the outcomes from the use of processed nerve allograft, the RANGER registry database was queried and results stratified by subject age.

Materials & Methods: IRB approval was obtained and standardized data reports were used to collect utilization, safety and functional outcomes. Follow-up evaluations included 2-point discrimination, Monofilament testing, range of motion, electromyography, force testing, MRCC scores and safety assessments. Covariate analysis were performed to further characterize the sub-groups. Meaningful recovery was defined by the MRCC scale at S3/M3 or greater for sensory and motor function in all groups. Complex mechanisms of injury included avulsions, amputations, blasts and high energy injuries.

Results: Sufficient outcomes data was reported for 109 subjects with 151 nerve repairs. The subjects were placed into three age groups: 18-29, 30-49, and 50 years of age or older. Overall meaningful recovery rates were 78%, 81% and 91% respectively. Table 1 provides the breakdown of the demographics of each group. The groups were evenly distributed by gap length, injured nerve and time to repair. The 18-29 group contained 62% more complex injuries than the 50+ group. The 18-29 group contained a majority of the manual labor/construction workers and twice as many military injuries. There were no reported nerve related adverse events.

Conclusion: In this population, patient age did not have a significant impact on functional outcomes. The 50+ year old population did show trends toward a lower incidence of complex traumas and high energy injuries, as compared to the 18-29 group. Processed nerve allografts performed well regardless of age, and can be considered as part of the treatment algorithm independent of patient age. The RANGER® registry is in open enrollment and will continue to collect clinical data to analyze outcomes from nerve repairs.
14. Differential Abilities Of Acutely And Chronically Nerve Derived Schwann Cells And Skin Derived Schwann Cells To Support Axonal Regeneration And Remyelination

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Schwann cells (SCs) play a key role in supporting axonal regeneration and remyelination following a peripheral nerve injury. It is well known that outcomes following delayed nerve repair are poorer. Data suggests that in the chronically denervated nerve, SCs progressively lose their capacity to support axonal regeneration and may be less robust for remyelination. We hypothesized that recapitulating the early denervation phenotype of SCs in chronic denervation may restore remyelination and regeneration support capacity. In this study, we compared SCs from adult rodent sciatic nerve with acute and chronic denervation, adult rodent skin derived precursor SCs (SKP-SCs), and nerve derived SCs from E16 embryonic nerve. SCs re-express key pro-myelinating transcription factors (Oct-6 and Krox-20) following acute (day 5) nerve injury, but lose this phenotype with chronic denervation (day 56) both in vivo and in cultured nerve SCs in vitro. We found that SKP-SCs express Oct-6 and Krox-20, in vitro, to similar levels as the ones from acutely denervated nerve and significantly greater than ones from chronically denervated nerve. We next tested and compared the various SCs for myelination both in vitro and in vivo and neurite outgrowth assay (DRG-SCs co-culture) in vitro. Additionally we compared SKP-SCs and SCs for cellular proliferation, cytokine releasing capacity and immune modulation by macrophage (M2 type) activation. Adult SKP-SCs were comparable to acutely denervated nerve SCs or embryonic nerve SCs in terms of proliferation, survival in injured nerve, in vitro and in vivo myelination, in vitro neurite outgrowth and immune modulation in injured nerve. Chronically denervated SCs were significantly poorer in all these capabilities. From this study we conclude that: 1) temporal delay following injury results in important phenotypic changes in distal Schwann cells within the nerve and 2) adult SKP-SCs can be used as an alternate therapy to modulate immune response, restore myelination and promote axonal regeneration, in injured peripheral nerve, making these cells a favorable source of autologous cell transplantation.
15. Early Axonal Area Measurement Predicts Early Nerve Regeneration Outcomes
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Background: Study of peripheral nerve injury and regeneration in laboratory animals can be time consuming and expensive. This study determines if it is possible to reduce time and cost for a peripheral nerve regeneration study.

Purpose: To determine if nerve axonal area (NXA) or nerve fiber counting (NFC) correlates with Compound Muscle Action Potential (CMAP) recovery which predicts functional muscular recovery in the early stage of nerve regeneration.

Methods: Two experiments were performed in this study. Experiment 1: 12 rats were divided into two groups of 6 each. Groups: (1) Sham control group: a crush injury of the sciatic nerve with no treatment and (2) Calcitonin pump group: a crush injury of the sciatic nerve with a calcitonin micro-osmotic pump. These rats were evaluated at 4 weeks of recovery with the following assessments: 1. CMAP taking readings from the extensor digitorum longus (EDL); 2. NXA measurement; 3. NFC

Results: NXA correlated with CMAP for both treatment groups; NFC did not correlate with CMAP for both groups.

Experiment 2: 16 rats were divided into two groups of 8 each. Groups: (1) Sham control group: a transection injury of the sciatic nerve with no treatment and (2) Calcitonin pump group: a transection injury of the sciatic nerve with a calcitonin micro-osmotic pump. These were evaluated at 12 weeks of recovery with the following assessments: 1. CMAP; 2. Tetanic Muscle Force (TMF); 3. NFC

Results: NFC correlated with CMAP and TMF. The outcomes of this study validated the results in experiment 1.

Conclusion: 1. NFC is not a reliable method for predicting muscular recovery in the early stages. NXA is a dependable assessment for muscular recovery in the early stages of nerve regeneration. 2. Using nerve axon area measurement can predict later electrophysiological and functional recovery. 3. Using NXA with CMAP measurement for nerve injury, repair, and treatment in the animal study can save cost and time.

All research on these experiments were completed at the Medical College of Wisconsin.
16. Thin-film Wireless Implants Facilitate Therapeutic and Diagnostic Electrical Stimulation of Peripheral Nerve Tissue Following Injury
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Introduction: Brief electrical stimulation has previously been shown to improve axonal regeneration and functional recovery following peripheral nerve injury. Unfortunately, intraoperative methods of applying electrical stimulation to injured peripheral nerves are time consuming, cumbersome, and incompatible with existing clinical work flow. The present study highlights the design and evaluation of a novel system of implantable, thin-film wireless receiver capable of non-invasively and serially stimulating peripheral nerve tissue for therapeutic and diagnostic applications. In vivo implementation in a rodent model demonstrated the ability of wireless implants to deliver therapeutic stimulation to injured sciatic nerve and track differing time-courses of recovery following nerve crush and nerve transaction/repair. The present study provides preliminary evidence of the translational potential of the present system of wireless implantable devices.

Materials and Methods: Flexible thin-film wireless receivers were manufactured via sacrificial photolithography by Red Rock Laboratories (St. Louis, MO). Fabricated receivers were subcutaneously implanted into thirty three male Lewis rats and proximally attached to the sciatic nerve prior to injury. In Phase I, animals underwent either nerve crush injury (n=5), nerve crush injury with electrical stimulation (n=5), or sham surgery (n=5). Electrical stimulation consistent with prior studies was delivered intraoperatively per the wireless implant at the time of surgery (freq. = 20 Hz, duration = 1 hr.). In Phase II, animals underwent either nerve crush injury (n=6), transection / repair (n=6), or sham surgery (n=6). Post-operatively, all animals underwent weekly non-invasive functional assessment utilizing thin-film implants. Three months post-operatively all animals underwent terminal functional assessment and histomorphometric evaluation of explanted nerve tissue.

Results and Discussion: Thin-film receivers were successfully implanted in male Lewis rats, facilitating wireless serial stimulation of interfaced sciatic nerves. Animals receiving therapeutic electrical stimulation in Phase I demonstrated improved functional recovery compared to control animals 1-4 weeks post-operatively. Animals receiving wireless receivers in Phase II demonstrated varying time courses of functional recovery following nerve crush and nerve transaction / repair. Implanted devices successfully facilitated serial assessment of nerve and muscle function post-operatively, as measured via EMG, evoked muscle force measurement, and evoked joint torque. Resulting data demonstrated the unique diagnostic function of the wireless implants and the varied time-courses of functional recovery following peripheral nerve injury.

Conclusions: The present study suggests that thin-film wireless implants may serve as a unique clinical tool in the future treatment and characterization of peripheral nerve injuries.
17. The Monoclonal Antibody Herceptin Enhances Peripheral Nerve Regeneration through a Potential New Pathway involving Erbb2 and the Epidermal Growth Factor Receptor (ErB1)

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Introduction: Peripheral nerve regeneration is profoundly limited by aspects of chronic denervation that are known to involve attenuation of neurotrophic factors such as neuregulin and its endogenous receptor ErbB2. The neuregulin/ErbB2 signaling axis has been implicated in Schwann cell proliferation and remyelination of neurons following peripheral nerve injury. However, the specific impact of attenuated neuregulin/ErbB2 expression over the period of chronic denervation is unknown. In this study we selectively inhibited the receptor for neuregulin, ErbB2, with the high affinity monoclonal antibody Herceptin to examine its effect on nerve regeneration in a rat model.

Materials & Methods: Herceptin or placebo was administered to female Sprague-Dawley rats recovering after common peroneal nerve transection and repair. Nerve repair was performed immediately or after 4-months of chronic denervation. Axons from regenerating motoneurons were labeled with retrograde dye 1, 2 or 4 weeks following injury and counted in the ventral horn of the spinal cord. Histomorphometry was also performed 10 mm distal to the repair site after 4 weeks. Protein analysis and immunohistochemistry evaluated levels of ErbB2, Akt, BrdU and activated EGFR within the regenerating nerve.

Results: Herceptin administration increased the rate of motoneuron regeneration by 3x compared to saline treated animals after the first week, whereas the extent of regeneration was nearly complete in both groups by the end of the second week. In addition, the total number of myelinated fibers growing distally beyond the repair site was significantly increased in rats receiving Herceptin (2488 ± 154) compared to rats that received saline (1896 ± 251) (p < 0.05) four weeks after repair. When delayed repair was performed after a 4-month period of chronic denervation, Herceptin increased the number of acutely, but not chronically, axotomized motoneurons after two weeks. Interestingly, Western blot analysis revealed no change in ErbB2 activation. However, immunofluorescent imaging revealed decreased levels of activated EGFR on regenerating neurons, a factor known to be inhibitory to axon regeneration.

Conclusions: ErbB2 receptor blockade with Herceptin enhances nerve regeneration following acute and delayed nerve repair independent of neuregulin signaling. The mechanism proposed to explain these effects involves Herceptin’s prevention of an inhibitory association between ErbB2 and EGFR, a potential new pathway that regulates nerve regeneration. These findings also raise the exciting possibility of using therapeutic monoclonal antibody therapy to improve outcomes following surgical repair of nerve injuries.
18. Schwann Cell Senescence: A Key Role in Reducing Axon Regeneration
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Introduction: Nerve injuries repaired with long nerve grafts (autograft or processed/acellular nerve allograft) are associated with poor axonal regeneration and the accumulation of senescent Schwann cells (SenSCs). Senescent cells are commonly characterized by altered gene expression and protein secretion. We hypothesize that SenSCs have gene expression changes that are deleterious to regeneration. SenSCs impede axonal growth and are thus, a direct contributor to limited regeneration in nerve grafts. Using SenSC culture, we assessed gene expression changes in a neuroregulatory specific array and measured neuronal neurite extension in co-culture with SenSCs. In an in vivo model, we measured axonal regeneration through a conduit filled with normal or senescent SCs.

Materials and Methods: SCs from rat sciatic nerves were treated with aphidicolin (damages DNA) to induce senescence. A custom PCR array of 91 neuroregulatory genes (axonal inhibitors and growth factors) was used to screen for gene expression changes compared to normal SCs. To assess the effect on neurite outgrowth, rat dorsal root ganglia (DRG) neurons were co-cultured with varying densities of SCs and SenSCs and maximal neurite length was measured over time. In vivo, a 5mm conduit was implanted into the transected sciatic nerve of rats containing normal or SenSCs. After 4 weeks, axonal regeneration was measured by retrograde labeling of motoneurons regenerating axons distal to the conduit and histomorphometric analysis of the mid-conduit and distal nerve.

Results: Gene expression was significantly upregulated in 19 genes and downregulated in 17. The greatest increases in expression changes occurred with Notch ligands, semaphorins, and cell surface proteins associated with inhibition of nerve regeneration. Co-culture in the presence of SenSCs showed a consistent significant 21-28% decrease in neurite extension from DRG neurons. In vivo studies showed reduced axon regeneration in the mid-conduit in the presence of SenSCs compared to normal SCs. However, the difference diminished at the distal nerve. Retrograde labeling showed no difference in the number of motoneurons regenerating axons.

Conclusions: Neurite outgrowth in SenSC co-culture and midgraft histomorphometry showed reduced axon growth when the axons are directly in contact with SenSCs. As the number of motoneurons regenerating axons was not affected, these results suggest SenSCs reduce axonal sprouting. Upregulation of Notch ligands and cell surface proteins support mechanistic pathways for this reduction in sprouting. This model suggests targeting the changes resultant from SenSCs could improve axonal regeneration in long nerve grafts.
19. Modelling the Effect of Monopolar Electrical Stimulation on Axonal Activation within a Sensory Regenerative Peripheral Nerve Interface
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Introduction: Current state-of-the-art bio-engineered prosthetic limbs have the potential to restore cutaneous sensation via direct nerve stimulation; however, the mechanical mismatch between the rigid alloplastic electrodes and the soft biologic nerve negatively affects long term nerve health and signal quality. Our laboratory has developed an experimental sensory regenerative peripheral nerve interface (sRPNI) by implanting a residual sensory nerve into a freely grafted piece of muscle. Sensory axons in the sRPNI can then be indirectly stimulated, avoiding trauma induced by direct stimulation. sRPNI have been viable in rats for four months, with axonal sprouting throughout the muscle and no apparent neuroma formation. Our goals were to model the effect of sRPNI stimulation on axonal activation and quantify the percent neural activation as a function of stimulation location and intensity.

Methods: A finite element model of the sRPNI was developed using COMSOL (a finite element solver). Muscle (ellipsoidal, 335 mm³, conductivity: longitudinal = 0.5, transverse = 0.1 S/m) of the sRPNI was virtually placed in a saline tank (0.9%, 37°C, 2 S/m). Monopolar currents (0.05-1 mA) were injected at throughout the sRPNI. The calculated voltage field for each stimulus was used to compute the internal currents of the axons within MATLAB. These currents were compared to the threshold for sural axon activation previously evaluated in NEURON (a modelling software for neural tissues) and percent axonal activation was calculated.

Results: At stimulation locations within the sRPNI, percent axonal activation was calculated for increasing stimulation intensities (Figure 1). Stimulating at the surface of the sRPNI and the center of the RPNI resulted in 10% and 80% activation at 1 mA, respectively. Threshold axonal activation is observed as low as 250 µA at the surface and 50 µA in the center.

Conclusions: We developed a finite element model of a sRPNI that demonstrates the feasibility of activating restricted populations of axons, thereby permitting the transmission of differential sensations via a prosthesis to its user. The more central the stimulation, the greater the percent axonal activation. This model will help guide the development of our sRPNI stimulation algorithms to restore the sense of touch to prosthetic users, improving not only their function, but also their quality of life.
20. Effects of Growth Hormone Therapy on Axonal Regeneration, Muscle Atrophy, Schwann Cell Proliferation and End-Organ Reinnervation Following Nerve Injury and Repair

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Plastic and Reconstructive Surgery, Johns Hopkins Medical Institutions, Baltimore, MD

**Purpose:** Poor outcomes following delayed and proximal nerve repairs result from a prolonged period of latency prior to reinnervation of distal targets. Denervated muscle undergoes atrophic changes involving permanent loss of myofibrils and motor endplates, and the degree of atrophy increases with the duration of denervation. Chronic denervation also results in Schwann cell (SC) senescence. Growth hormone (GH) therapy has the potential to accelerate and augment axonal regeneration, while also acting directly on denervated muscle and SCs to minimize atrophy prior to reinnervation. The purpose of this study is to assess the effects of GH therapy on axonal regeneration, SC and muscle maintenance, and end-organ reinnervation in a nerve injury and repair model.

**Methods:** Male Sprague-Dawley rats underwent sciatic nerve transection-and-repair and femoral nerve transection without repair and received either daily subcutaneous GH (0.4 mg/day) or no treatment (N=8 per group). To assess GH activity, serum IGF-1 levels were measured at baseline, 2 weeks and 5 weeks, and body weights were measured weekly. All animals were sacrificed at 5 weeks and tissues were collected for analysis. Axonal regeneration was assessed by sciatic nerve histomorphometry distal to the repair site. SC proliferation within the denervated femoral nerve was determined by counting the number of cells that co-stain for Ki67 and S100. Muscle atrophy was determined by quantifying skeletal muscle fiber cross-sectional area within the gastrocnemius muscle. Percent reinnervation of motor endplates within soleus muscle was calculated.

**Result:** GH treated animals demonstrated greater percent increase in body weight (14.2±0.84 vs. 7.5±0.02, p<0.65), greater number of regenerating myelinated axons (13876±2036 vs. 8645±3279, p<0.05), greater percent reinnervation of motor endplates (75.8±8.7 vs. 38.2±22.6, p<0.05, figure 1), and greater muscle fiber cross-sectional area (738.5±214.4 vs. 536.8±162.6, p=0.053, figure 2) as compared to controls. Serum IGF-1 levels and quantification of proliferating SCs are still pending.

![Figure 1](image1.jpg) Neuromuscular junction staining of soleus muscle in GH-treated animals (left, middle) and control (right).

![Figure 2](image2.jpg) Laminin staining of cross-sections of non-denervated (left), GH-treated (middle), and untreated (right) gastrocnemius muscle.

**Conclusions:** GH therapy may improve outcomes following nerve injury and repair by accelerating axonal regeneration, minimizing muscle atrophy and SC senescence, and promoting muscle reinnervation.
100. Prospective Evaluation of Sensitivity and Specificity of CTS-6 for Diagnosis of Carpal Tunnel Syndrome
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Introduction: Carpal tunnel syndrome (CTS) is a compressive neuropathy and accounts for 90% of all cases of compressive neuropathy. Current AAOS Clinical Practice Guidelines give a Grade of Recommendation B to the use of electrodiagnostic studies in the setting of positive clinical or proactive. Graham et. al published a clinical diagnostic criteria for CTS where he found six clinical criteria that were statistically significant in the probability of diagnosing CTS. Subsequently, Graham demonstrated that electrodiagnostic studies do not change the likelihood of diagnosing CTS secondary to the high probability that can be estimated with CTS-6. Our prospective study aims define the diagnostic validity of CTS-6 when compared to the reference standard of electrodiagnostic testing.

Methods: Eighty-five consecutive patients over a three month period whom were referred for electrodiagnostic studies were prospectively enrolled into the study. A blinded, certified electrodiagnostic technician performed all electrodiagnostic testing. A distal motor latency > 4.2 ms or distal sensory latency > 3.2 ms was considered positive. A hand fellow, not involved in the electrodiagnostic examinations and trained to independently examine patients calculated the CTS-6 score. A score of 12 or greater was considered a positive diagnosis of CTS and less than 12 was negative. Sensitivity and specificity were calculated using electrodiagnostic testing as the reference standard.

Results: Fifty five of 85 patients tested positive for CTS with EMG/NCS and of those 49 tested positive for CTS using CTS-6. Thirty patients found not to have CTS based upon electrodiagnostic studies and 24 of those tested negative using CTS-6. The calculated sensitivity and specificity of CTS-6 was found to be 0.89 and 0.80 respectively.

Discussion: Current AAOS Guidelines give a strong recommendation to proceeding with electrodiagnostic studies in the setting of clinical finding as a confirmatory test. Graham devised a new clinical diagnostic test that challenges the routine of electrodiagnostic studies when diagnosing CTS. No prospective studies have evaluated the diagnostic validity of CTS-6 when compared to electrodiagnostic studies as the reference standard. In our study we found CTS-6 to have similar sensitivities and specificities to other confirmatory imaging tests. An accuracy of 86% for CTS-6 is respectable, but likely not high enough to suggest that CTS-6 can replace EMG/NCS. The relatively low specificity of 80% means that CTS-6 does not function as well as EMG/NCS as a good confirmatory test. The high sensitivity of 89% means that it is a relatively strong screening test.
101. Does Pre-Operative Electrodiagnostic Testing Predict Time to Resolution of Symptoms After Carpal Tunnel Release
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Background: Previous studies have found weak or no correlation between pre-operative electrodiagnostic (EDX) studies and functional and/or subjective outcomes after carpal tunnel release (CTR). However, these studies examined outcomes at 6 months or 1 year, potentially missing early differences in recovery. Our anecdotal experience found that patients with mild carpal tunnel syndrome recovered more quickly than those with severe carpal tunnel syndrome. The purpose of this study is to determine if EDX studies predict time to resolution of symptoms after CTR.

Methods: 61 consecutive patients undergoing open CTR were prospectively enrolled. Preoperative presence of nocturnal symptoms and daytime numbness/tingling were documented. Preoperative EDX studies were reviewed and classified as mild, moderate, or severe. After open CTR, patients were contacted by phone within 48 hours, at 1 week, and then at 2-week intervals for up to 9 months or until both nocturnal and daytime symptoms had resolved. Kaplan-Meir survival curves were constructed and compared using the Wilcoxon and log rank test.

Results: Daytime numbness and tingling in patients with mild carpal tunnel syndrome resolved in a median of 0.4 (95% IQR 0.4-1.4) weeks, moderate carpal tunnel syndrome in a median of 0.4 (95% IQR 0.4-1.4) weeks, and severe in a median of 0.4 (95% IQR 0.4-1.4) weeks, p = 0.002. Nocturnal symptoms resolved in a median of 0.4 (IQR 0.4-1.4) weeks for patients with mild CTS, median of 0.4 (IQR 0.4-1.4) weeks for patients with moderate CTS, and median of 0.4 (IQR 0.4-0.4) weeks for patients with severe CTS, p=0.3. All symptoms resolved in a median of 0.4 (IQR 0.4-1.4) weeks for patients with mild CTS, 0.4 (IQR 0.4-1.4) weeks in moderate CTS, and 4.0 (IQR 0.4-24.0) weeks in patients with severe CTS, p = 0.04.

Conclusion: Patients with mild or moderate CTS, based on preoperative EDX studies, experience a faster time to resolution of daytime numbness and tingling when compared to patients with severe CTS. Nocturnal symptoms resolved quickly in both groups. The results of this study are in contrast to previous studies that found little to no value of EDX in predicting postoperative functional and subjective outcomes, likely due to the early time points used in the current study. This is not mean to be an indication to obtain EDX, but could be used to counsel patients if obtained for other reasons.
102. Early Active Motion Versus Protective Splinting Following Open Carpal Tunnel Release Surgery
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**Introduction:** Splinting after open carpal tunnel release remains a controversial issue, with significant variability in practice among surgeons. We carried out a single blinded, randomized controlled trial to assess differences in outcome and complication rates between splinted and non-splinted patients following open carpal tunnel release.

**Materials & Methods:** One hundred and sixty-two patients undergoing open carpal tunnel release by a single surgeon using a standardized technique were prospectively randomized to Protective Splinting (77 patients, splinted two weeks post-operatively) or Early Active Motion (85 patients, no splinting). Visual Analog Pain Scale, Boston Carpal Tunnel Questionnaire (BCTQ), and the Disabilities of the Arm, Shoulder, and Hand (DASH) Questionnaire were completed pre-operatively, and at two, six, and twelve weeks post-operatively. Daily range of motion activities were prescribed for all patients throughout the study. Complications (pillar pain, scar sensitivity, surgical site infection, bowstringing of flexor tendons) were recorded at each follow-up appointment. Duration until return to modified and full duties at work was also recorded. Statistical analysis was performed using a repeated measures analysis of variance for continuous variables between groups and Pearson Chi-Square for binary outcome complication data. Return to modified and full duties at work was assessed using a Student’s t-test. Significance was set at a p < 0.05 for all comparisons.

**Results:** There were no significant differences in post-operative pain (p=0.973), Boston Carpal Tunnel symptom severity (p=0.828), functional severity scores (p=0.773), or DASH scores (p=0.642) between the Protective Splinting and Early Active Motion groups. In addition, there were no significant differences in the occurrence of pillar pain (p=0.930), infection (p=0.104), wound dehiscence (p=0.882), or scar hypersensitivity (p=0.465) between groups. Bowstringing of the flexor tendons was not observed in any patients in either group. There were no difference in return to modified (p=0.199) and full (p=0.471) duties at work between Protective Splinting (21.1 and 32.7 days, respectively) and Early Active Motion (16.7 and 36.3 days, respectively) groups.

**Conclusions:** No significant differences were identified in the subjective or objective outcome measures between patients in the Protective Splinting group and the Early Active Motion group. In addition, there was no significant difference in complication rate or return to work times between the two groups of patients. This data further suggests that there is no benefit to immobilization post-operatively following open carpal tunnel release surgery but also suggests that there was no detrimental effect of splinting as has been show in some previous studies.
103. Ultrasound Evaluation of the Median Nerve After Carpal Tunnel Release
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Introduction: Carpal tunnel syndrome is the most common entrapment neuropathy of the upper extremity, affecting 5.8% of women and 0.6% of men. Nerve conduction studies have long been used in the diagnosis of this condition, although recent reports have advocated the use of high-resolution ultrasound as a useful non-invasive alternative. The purpose of this study is to determine if there are measurable changes in the ultrasound cross-sectional area (CSA) of the median nerve following carpal tunnel release in a previously studied patient cohort that had undergone preoperative median nerve CSA measurement.

Materials and methods: 65 patients underwent ultrasound CSA measurement of the median nerve in our office as part of a previous study. A retrospective review of the patient records identified 30 patients who ultimately underwent carpal tunnel release. These patients were contacted and invited to undergo repeat ultrasound of the median nerve and CSA measurement. 10 patients agreed to participate in this study, and a median nerve CSA measurement of the operated side was performed in 11 wrists.

Results: Average age of returning patients was 58 years (range) and 30% were male. None of the respondents were affected with diabetes. Mean CSA of the median nerve prior to carpal tunnel release was 11.8 mm2. Average CSA post-CTR was 9.5 mm2. Seven wrists had a decrease in the median nerve CSA measurement following CTR, and one patient had no change in the CSA measurement.

Conclusions: Ultrasound examination of the median nerve at the wrist has been shown in previous studies to have comparable specificity and sensitivity to nerve conduction studies for diagnosis of carpal tunnel syndrome. The current study demonstrates that the CSA of the median nerve decreases after CTR in the majority of patients, but does not return to normal values in most patients. Larger prospective studies are necessary to characterize the role musculoskeletal ultrasound may have in monitoring the median nerve following carpal tunnel release.

Figure 1 – Median Nerve measurements

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<th>Wrist #</th>
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<th>US CSA (mm2) - POST CTR</th>
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Background: As healthcare in the United States continues to find more efficient and cost effective methods of treatment, physicians must evolve modalities and habit. Carpal tunnel syndrome is the most common compressive neuropathy with 56-87% of patients experiencing bilateral symptoms. Simultaneous bilateral carpal tunnel release has been proven to be both safe and effective. Our aim is to conduct a cost analysis of simultaneous versus staged bilateral carpal tunnel release through a retrospective chart review.

Hypothesis: We hypothesize simultaneous bilateral carpal tunnel release is more cost effective and time efficient for both patient and surgeon than staged bilateral carpal tunnel release.

Specific Aims: Analyze the cost of simultaneous bilateral carpal tunnel release and staged bilateral carpal tunnel release by evaluating: patient cost, work time lost, number of follow-up visits required, and physician fees.

Methods: Retrospective review of 198 patients who had bilateral carpal tunnel release performed between August 2009 and March 2014 by a single surgeon. Simultaneous versus staged procedures were compared with respect to billed charges, fees collected, days until return to work (with and without limitations), and the number of post-operative visits.

Results: 198 patients had bilateral carpal tunnel release performed between August 2009 and March 2014 by a single surgeon. Simultaneous versus staged procedures were compared with respect to patient charges, patient amount paid, days until return to work (with and without limitations), and the number of post-operative visits. Mean amount billed and total fees collected were both significantly reduced in the simultaneous versus the staged procedures ($4,312.09 vs. $4,364.46 and $733.87 vs. $1,003.43, p<0.05). Days returning to work (with and without limitations) were significantly reduced in the simultaneous procedures relative to the staged procedure (13 and 21 vs. 27 and 45 days respectively, p<0.001). The numbers of post-operative follow up visits were also reduced in the simultaneous procedure when compared to the staged procedure (1.45 vs 3.46 visits, p<0.001).

Conclusions: It is evident simultaneous bilateral carpal tunnel release is more efficient and cost-effective than bilateral staged release. Simultaneous release is beneficial for the patient in terms of work days missed and cost. The surgeon benefits from fewer postoperative office visits are necessary and the remuneration per hour is increased. Overall, simultaneous bilateral carpal tunnel release benefits patients and surgeons in terms of cost-effectiveness and time efficiency when compared to staged release of bilateral carpal tunnel syndrome.
105. PEG-fused Allografts Produce Rapid Behavioral Recovery After Segmental Nerve Loss
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Introduction: Every year in the United States approximately 360,000 people suffer from peripheral nerve injuries and roughly 12% of operations performed for traumatic neuropathy involve patients with segmental nerve loss. Of these operations less than 50% show meaningful recovery. Presently, the most dependable method of repair for such major deficiencies is autologous nerve grafts. Substitutes to nerve autografting are being pursued due to donor site morbidity and limited functional recovery. Polyethylene Glycol (PEG) has demonstrated an ability to improve behavioral outcome after nerve transection as well as nerve autografting. We hypothesized that the previously established PEG therapy could improve functional outcome after nerve allografting.

Materials and Methods: In this experiment we used a segmental rat sciatic nerve injury model in which we restored a 0.8-1.0 cm gap with a 1.0 cm nerve segment from a separate rat (allograft) using microsurgical techniques. The experimental animals were treated with a combination of solutions including Plasmalyte A (calcium free saline), Methylene Blue, Polyethylene Glycol (PEG), and Lactated Ringers (calcium containing saline); control animals received all solutions except for PEG. Animals underwent weekly (1w-6w) behavioral assessments using the Sciatic Functional Index. At 6 weeks post-op animals were perfused and fixed for thick cross sections.

Results: Following removal of 0.8-1cm segments of rat sciatic nerves, we report that micro-sutured allografts treated with polyethylene glycol (PEG) rapidly and permanently restore axonal continuity within minutes as assessed by action potential conduction (p<0.001) and intracellular diffusion of dye (Fig 1). Behavioral functions are largely restored (80%) within 2-4 weeks as measured by the sciatic functional index (SFI) in PEG treated animals and are associated with increased number of axons in PEG-fused allografts (p<0.001).

Conclusions: Our data suggests that use of microsutures, allografts, and PEG-fusion procedures might produce a paradigm shift in the clinical treatment of traumatic injuries to peripheral nerves for which the current gold-standard for simple cuts is micro-suture of the severed ends.

Figure 1 – Control nerve (left) and PEG-fused nerve (right) loaded proximally with fluorescent intra-axonal dye (Texas-Red) immediately following in-vivo repair. White arrows indicate proximal site of nerve severance.
Chaining Nerve Grafts With An Additional Suture Line Has Limited Impact On Axonal Regeneration
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Introduction: In cases of limited donor supply, the end-to-end coaptation of multiple nerve grafts (autograft or acellular nerve allografts-ANAs) to repair long nerve gaps has been performed. In this study, we sought to evaluate the effect of an added suture line on nerve regeneration and functional return when using either an autograft or ANA “chained” together.

Materials & Methods: Rat sciatic nerve was transected and repaired with 2cm nerve grafts. Nerve grafts consisted of either isografts (2cm: single, or a 1cm segment repaired to a 1cm segment: chained) or ANAs (single or chained). At an endpoint of 8 weeks post surgery, EDL muscle force and mass was measured, and nerve was harvested for quantitative histology (histomorphometry). In a separate parallel study, the same procedures and groups were employed, where nerves were harvested 2 weeks following graft implantation to assess gene expression changes using qRT-PCR. Collagen I, CD31, Ang-2, Jag1, VEGF and Dll4 expression levels at the middle of the grafts were determined.

Results: Contractile extensor digitorum longus (EDL) muscle force production was comparable between nerve isografts and ANAs as well as between single or chained groups. EDL muscle mass recovery was significantly increased by using a nerve isografts compared to ANAs, regardless of using a coapted nerve chains. Myelinated axon numbers assessed in nerve distal to the grafts were comparable between single and chained isografts and the single ANA, not the chained ANA.

Assessment of axonal regeneration within the grafts revealed stark differences. Myelinated axon numbers in the distal graft and proximal graft were similar between isografts groups (single or chained). However, ANAs, either single or chained, demonstrated decreased myelinated axon numbers in the distal graft compared to the proximal graft. The ratio of myelinated axon numbers in the distal graft compared to the proximal graft was ~85-100% in isografts but ~55-68% in ANAs. Gene expression analysis within grafts is ongoing to determine (1) why ANAs decrease axonal regeneration within nerve grafts and (2) differences between single and chained grafts.

Conclusions: Minimal axonal loss and no functional deficit were identified with an additional suture line in an isograft repair. Axonal regeneration across an additional suture line in ANAs has a moderate effect on axonal regeneration. The need to coapt multiple isografts to achieve a desired length does not limit axonal regeneration over short distances and is advantageous compared to the alternative ANA.
107. Magnesium Microfilaments inside Traditional Nerve Conduits Improve Nerve Regeneration Characteristics

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Introduction: The use of biomaterials for the reconstruction of long nerve gaps lacks clinical efficacy using current techniques. The placement of filaments inside traditional conduits has been proposed to provide physical support to guide cells across the gap. We have previously used magnesium (Mg) metal microfilaments as “cables” to act as physical support for nerve regeneration and as biodegradable implants that release Mg^{++} ions. We now test the hypothesis that Mg metal microfilaments can assist nerve regeneration across longer nerve gaps.

Materials & Methods: Short or long (6 or 15 mm) nerve gaps were created in the sciatic nerves of 44 adult male Lewis rats. Poly(caprolactone) nerve conduits were sutured into the gaps and filled with Mg microfilaments (99.9% pure, 250µm diameter), titanium microfilaments (250µm diameter) or saline filler alone (empty). Groups were: 1) empty conduits (6 and 15mm, n=7 each), 2) Mg (6mm, n=7; 15mm, n=8), 3) titanium (6mm only, n=7), or 4) isograft nerve controls (donor rats, 15mm, n=8). After sacrifice (6 weeks for short, 14 weeks for long gaps), the reconstructed nerve was excised, fixed and imaged by micro computed tomography (microCT) to determine extent of Mg degradation. Gastrocnemius muscles were removed and weighed. After imaging, nerves were halved and treated with either osmium to enhance contrast and imaged by microCT or paraffin embedded, sectioned and stained with H&E or immunostained for axons (anti-NF200).

Results: Mg degradation (seen via microCT) appeared accelerated (gaps at 6 weeks and almost no metal at 14 weeks). This was thought to be due to metal fatigue from processing. With short nerve gaps, there was no difference in muscle recovery or anti-NF200 staining between empty, titanium or Mg groups. Titanium filaments did not degrade, but this inert physical support also did not appear to improve regeneration characteristics (p>0.05). In long gap experiments, use of Mg microfilaments showed improvement over empty controls in terms of greater cross sectional area of total regenerating tissues (3.7 vs 2.4 mm^2, p<0.001) and greater area of axonal (anti-NF200) staining (62000 vs 25000 pixels, p<0.05). Muscle regeneration was not improved with Mg or empty groups at 14 weeks, but was with isografts (p<0.001).

Conclusions: Mg microfilaments improved the histologic characteristics of nerve bud regeneration through conduits across long nerve gaps, but did not improve muscle recovery at 14 weeks. Further research will focus on decreasing Mg degradation and assessing the effects of Mg^{++} ions on nerve regeneration.
Bilateral Regenerative Peripheral Nerve Interface Function Correlates with Hind Limb Kinematics during Treadmill Locomotion

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Introduction: Regenerative Peripheral Nerve Interfaces (RPNIs) are neurotized autologous free muscle grafts equipped with electrodes to record myoelectric signals for prosthetic control. In vivo characterization of voluntary RPNI signaling is critical when designing prosthetic device controllers. RPNIs are known to reliably produce high fidelity electromyography (EMG); however, RPNI signaling has not been matched with joint movements during walking when foot flexor and extensor signals are provided by RPNIs. We seek to define the relationships between Control and RPNI group signaling using kinematics and joint gait analysis during voluntary treadmill walking.

Methods: Three experimental groups of two rats were devised (Figure 1): Control, rat hind limbs remained intact; RPNI, rat left extensor digitorum longus and right soleus muscles were transferred to the ipsilateral thigh and reinnervated with the transected peroneal and tibial nerves, respectively; Denervated, rats underwent peroneal and tibial nerve transections. In all groups, bipolar wire electrodes were positioned on the muscles. Evaluations occurred 4-5 months post-surgery. Rats walked on a treadmill. A synchronized videography system was used to identify hip, knee, ankle, and toe joint angles bilaterally, with acquired EMG. Within each group, normalized joint angles and EMG were cross-correlated.

Results: Control and RPNI group EMG signals were periodic with gait. Control group hind limb movements were normal with normal EMG signal periodicity. RPNI and Denervated groups exhibited compensated gait with marked inability to dorsiflex or plantarflex the left and right hind feet. RPNI signal periodicity had different timings from Control due to gait compensations (Figure 2). EMG was highly repeatable within rat and within left and right legs (Control: r=0.88; r=0.91) and (RPNI, r=0.75; r=0.79); but, RPNI signaling was of lower amplitude than controls. Cross-correlation of the EMG from Control and RPNI groups indicated RPNI signaling predicts peroneal and tibial nerve firing that is proportionally similar to Control. The Denervated group demonstrated low amplitude random signaling, unrelated to gait.

Conclusion: This study determined that in vivo EMG signaling of Control and RPNI rats is periodic and highly correlated with hind limb joint angles during walking. EMG signaling by RPNIs correctly matched with compensations during walking.

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Histomorphometric Evaluation of Median Nerve Injury in Wistar Rats Treated with GM1

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Background: The aim of this study was to compare the morphologic alterations between traditional neurorrhaphy and neurorrhaphy combined with intraperitoneal administration of GM1 after median nerve injury of Wistar rats, using histomorphometric analysis.

Method: Twenty-two male Wistar rats suffered microsurgical median nerve damage. Rats were further subdivided into two experimental groups: Group I (10 animals) treated with external epineurial neurorrhaphy and Group II (12 animals) treated with epineurial neurorrhaphy combined with intraperitoneal GM1.

Results: Microscopic analysis containing distal stumps revealed that Group II animals had more regenerated axons with slightly thicker myelin sheath than Group I animals and had a more homogeneous and organized regeneration pattern, with a looser endoneurium in the central nerve fiber. A significant difference (p=0.0056) in mean axonal diameter of the distal segment was observed. Group II had larger and more axons (28%) than Group I.

Comparison with existing method(s): Traditional axonal regeneration index, obtained by axon counting in both segments was added to the diameter of axonal myelin layer.

Conclusion: Since nerve regeneration depends upon the association between the number of regenerated axons and myelin sheath diameter, data indicates that Group II is more highly myelinated than Group I. There is strong evidence (p=0.0536) that GM1 used as an adjuvant in peripheral nerve surgery improves axonal regeneration pattern.

Keywords: GM1, median nerve, axonal regeneration, morphometry, rats.
110. Motion Deficits of Thumb Opposition and Circumduction Due to Carpal Tunnel Syndrome
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Introduction: Carpal tunnel syndrome (CTS) is associated with sensory and motor impairments resulting from the compressed and malfunctioning median nerve. These impairments affect the thumb which is indispensable to hand function and is required to move in multiple directions through coordinated articulations at its three joints. CTS sufferers often experience clumsiness while performing daily tasks, but the pathokinematics of the thumb associated with CTS remain unclear. The purpose of this study was to evaluate thumb motion abnormalities associated with CTS. It was hypothesized that CTS would result in translational and angular motion deficits during thumb opposition and circumduction.

Methods: Eleven patients with CTS (49.5 ± 9.6 years) and 11 age- and gender-matched healthy controls (48.9 ± 7.6 years) participated in this study. Translational and angular motion of the thumb was obtained using marker-based video motion analysis during thumb opposition and circumduction tasks. Translational metrics included thumb tip path length and thumb tip position, normalized according to subject specific palm width (PW); angular kinematics were quantified by examining 6 angular degrees of freedom.

Results: Analyses revealed translational and angular motion deficits for patients with CTS. In comparison to control subjects, the path length traveled by the thumb tip for CTS patients was approximately 30% less during opposition and 25% less during circumduction (p < 0.001). Specifically, CTS patients were unable to reach a similar maximum ulnar position of their thumb tip during opposition, with an ulnar deficit of 0.3 PW (p < 0.05). The angular range of motion for the CTS group was 36-41% less for the metacarpophalangeal and interphalangeal joints in extension/flexion compared to the control group across both opposition and circumduction (p < 0.05). These kinematics abnormalities were present even though there was no difference in pinch strength between the two groups (53.1±18.1 N for patients with CTS and 57.2±18.1 N for controls, p = 0.56).

Conclusions: Motion deficits of the thumb are present for CTS patients while completing tasks of opposition and circumduction. Improving the understanding of thumb pathokinematics associated with CTS may help clarify the clumsiness in hand function related to CTS given the critical role of the thumb in dexterous manipulation. Furthermore, such advanced kinematic analyses may be used to assess functional improvement following median nerve decompression.
111. Reverse End-to-Side Anterior Interosseous Nerve to Ulnar Nerve Transfer for Severe Ulnar Neuropathy at the Elbow
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Introduction: Ulnar nerve injury or severe nerve compression at the elbow is a difficult clinical problem as decompression or repair at this level may result in variable reinnervation of intrinsic hand muscles. Distal reverse end-to-side nerve transfers (anterior interosseous nerve to ulnar motor fascicles) have been suggested to “supercharge” or augment intrinsic hand muscle recovery while axons regenerate from the level of the elbow and have a significant theoretical advantage. There is little published about the efficacy of this technique.

Materials & Methods: Consecutive patients presenting between June 2013 and December 2013 who had repair of an ulnar nerve injury or severe compressive neuropathy (McGowan Grade III) at the elbow were considered for reverse end-to-side AIN to ulnar nerve transfer at the wrist. Consenting patients underwent nerve transfer by a single surgeon and followed post-operatively with electrodiagnostic studies and clinical evaluation. Changes in MRC grade and evidence of early intrinsic muscle reinnervation (6 months) on EMG were evaluated. Pinch strength and intrinsic hand function were also evaluated. In addition, strength and neurophysiology changes were measured with the forearm in neutral position and pronation (simulating AIN function).

Results: Six patients were eligible for review. Four patients suffered an ulnar nerve laceration with microscopic repair at the elbow and two had a severe compressive neuropathy. Average time from initial injury to transfer was 37.5 months (2 – 120 months). All patients had no recruitable motor units on pre-operative EMG of hand intrinsic muscles. All patients had intrinsic muscle wasting pre-operatively, which persisted post-operatively. Fifty percent (3/6) had an improvement in MRC grade by 6 months. Eighty three percent (5/6) of patients showed evidence of intrinsic muscle reinnervation at a time earlier than expected for regeneration from the elbow level (< 6 months). Eighty three percent (5/6) showed improvement in needle EMG studies with nascent units recruited during active forearm pronation, suggesting contribution of the AIN to the ulnar nerve motor fascicles.

Conclusion: The timing of clinical and electrophysiologic recovery suggests the AIN to ulnar reverse end-to-side nerve transfer enhances the results of surgery for severe ulnar neuropathy at the elbow.
112. In-situ Decompression of Ulnar Nerve Entrapment: A Controlled Randomized Study Comparing Decompression using Two Small Transverse Incisions with Standard Open Decompression
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Introduction: Ulnar Nerve entrapment is the second most common nerve entrapment in the upper limb in adults. Many methods of treatment have been advised including transposition, in-situ decompression as well as endoscopic decompression.

Method: We performed in-situ ulnar nerve decompression for 60 patients with ulnar nerve entrapment who were randomized into 2 groups. In group I decompression was performed through basically 2 transverse incisions each less than 2 cm long which were placed 4 cm above and 3 cm below the medial epicondyle and centered on the course of the ulnar nerve which was decompressed and all potentially compressing structures were released from the medial intermuscular septum down to the 2 heads of the flexor carpi ulnaris muscle. In group II decompression was performed through a standard curvilinear approach averaging 8 cm based anterior to the medial epicondyle.

Patients who had symptoms of ulnar nerve instability, cubitus valgus, elbow osteoarthritis or significant local scaring were considered to be indicated for ulnar nerve transposition and were excluded from the study.

Results: Outcome measures used included Visual Analogue Scale (VAS) for pain, time of return to work, Disabilities of the Arm, Shoulder, and Hand (DASH) score, Gabel and Amadio score, and grip and pinch strengths as well as local scar tenderness. At final follow up, which averaged 6.3 months (5-8.5 months), there was no statistically significant difference in either the DASH score, the Gabel and Amadio outcome scores or grip and pinch strength between both groups. However group I showed significantly less pain on VAS scores, faster return to work, less scar tenderness and less disfiguring scar.

Conclusion: These results are similar to those of endoscopic release of the ulnar nerve but avoiding the need for special equipment and thus less added time and expense as well as a long learning curve.

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Purpose: Restoration of hand function in people with cervical spinal cord injury (SCI) is critical to their independence and quality of life. Traditional surgeries (tendon transfers/tenodesis) are, however, remarkably under-utilized despite their well-established benefits. This study examines a series of SCI patients who underwent the relatively novel application of nerve transfers to improve hand function. The purpose was to investigate patient knowledge about treatment options and preliminary perceptions regarding nerve transfers.

Methods: A qualitative study design was used to assess five post-surgical cervical SCI patients 6-10 months after nerve transfer surgery. A semi-structured interview was performed. Questions addressed access to health information, barriers to treatment, and perceptions of treatment options including nerve transfers.

Results: Patients reported that they received most of their health information from physicians and the internet. The major barriers to information about and access to care for upper extremity surgery were jargon and access to medical professionals. Only 3/5 reported even having previously heard about the traditional surgical treatments (tendon transfers/tenodesis). Limited post-operative downtime was the highest ranked advantage of the nerve transfer surgery. Reliability and perceived lower risk also made the nerve transfer surgery appealing. While 2/5 had at least one negative experience related to the surgery (paresthesias, transient donor site weakness), all patients reported they would have the surgery again, are considering surgery on the contralateral arm, and would recommend the surgery to others.

Conclusion: Tendon transfers and tenodesis are under-utilized options to improve hand function in SCI patients despite their benefits in improving independence and self-care. In our patient population, barriers to information regarding upper extremity surgery, including jargon and access to medical professionals, appear to contribute to this under-utilization. In comparing traditional options to nerve transfers, the lack of downtime and perceived increased reliability made nerve transfers attractive. Moreover, despite being early in their recovery (it typically takes 12-18 months to see gain in function after nerve transfer surgery), all of the patients have a positive impression of the surgery. These data provide preliminary guidance for further prospective investigation of patient perceptions of surgery to improve hand function in the setting of cervical SCI. The long term goals of this work are better dissemination of information regarding treatment and patient/provider education in this setting.
Double Distal Nerve Transfer for Hand Reconstruction after Lower Brachial Plexus Injuries
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Hypothesis: Lower brachial plexus injury (LBPI) remains a clinical challenge. Early distal nerve transfer may provide useful thumb and finger functional recovery.

Material & Methods: Four patients were treated with distal double nerve transfer for LBPI. There were 3 males and one female, 3 of them involved in the right side. The mean age at surgery was 22 (17-26) years old. The procedure includes transferring a pronator branch of the median nerve (PBMN) to the anterior interosseous nerve (AIN) and a supinator branch of the radial nerve (SBRN) to the posterior interosseous nerve (PIN). The mean time of delay from injury to surgery was 7.5 (5-13) months. The mean follow-up was 19 (6-36) months.

Results: The first 2 patients achieved M4 of EPL/EDC and M4 of FPL/FDP. The other two more recent patients showed signs of motor recovery 6-9 months after surgery. There was no functional loss of forearm pronation or supination after surgery.

Conclusion:

- Simultaneous double distal transfer of a PBMN to AIN and a SBRN to PIN is a reliable technique for patients with LBPI.
- The advantages of this procedure include: 1) transfers can be performed through one incision with minimal intraneural dissection; 2) the transferred nerves are very close to the targeting muscles; 3) there is no need for nerve graft; 4) both transfers are in-phase with ease in post-op therapy.

References:

115. Outcomes from an Ongoing Multicenter Registry Study on the Use of Processed Nerve Allograft as Compared to Contemporary Controls for Sensory, Mixed, and Motor Nerve Reconstructions
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Introduction: The RANGER registry is an active database designed to collect injury, repair, safety and outcomes data for processed nerve allograft (PNA), Avance® Nerve Graft, AxoGen, Inc. In 2013, a control arm (MATCH) was added to the registry to allow for comparisons of outcomes between conduit and nerve autografts. Here we report our cumulative findings from the ongoing registry on the safety and efficacy of processed nerve allograft with comparisons of outcomes to conduit and nerve autografts.

Methods: The RANGER registry is designed to continuously monitor and incorporate data using standardized data collection into a centralized database. For the control arm, a medical record review was conducted at participating centers to identify subjects repaired with conduit or nerve autograft according to the IRB approved protocol. Outcome measures were reported for the cumulative PNA dataset and then stratified for comparisons to controls. PNA repairs with gaps <30mm were compared to conduit and gaps >20mm were compared to the nerve autograft group. Meaningful recovery was defined by the MRCC scale at S3/M3 or greater.

Results: Quantitative outcomes data was available in 109 subjects with 152 PNA repairs. The mean age was 41±16 (18-70). The mean gap was 21±12 (5-65) mm. Recovery of meaningful sensory function was reported in 84% of the repairs (118 sensory/18 mixed). The mean static and moving 2PD was 8±3mm and 7±3mm respectively. Return to light-touch or greater was demonstrated in 47 of 65 repairs reporting SWMF scores. Recovery of meaningful motor function was reported in 68% of repairs (22 mixed/9 motor). There were 8-M3, 6-M4, and 7-M5. No related adverse experiences were reported. PNA was further stratified for comparisons to controls. Meaningful recovery was reported in 49% and 64% in the conduit and nerve allograft groups. See Table 1.

Conclusions: Outcomes from the registry continue to demonstrate the successful use of Avance® Nerve Graft in sensory, motor, and mixed nerve defects between 5 and 65mm. Meaningful recovery at MATCH sites for PNA exceed that of tube conduits and are comparable to nerve autograft. This study is currently in open; additional data incorporated into the registry and MATCH control arms will allow for continued analysis on the role of processed nerve allografts, tube conduit, and nerve autograft in the treatment algorithms for peripheral nerve injuries.
Poster Session

P1. Epineurial Windows are Necessary for Donor Axons to Regenerate Across an Autograft into Recipient Denervated Rat Nerve Stumps: the Axons Grow Equally in Proximal and Distal Directions
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Introduction: 1) Whilst donor axons may cross an end-to-side repair without creating an epineurial window, epineurial windows may permit more axons to grow into a denervated nerve stump. Would window size affect axon growth across two end-to-side neurorrhaphies (cross-bridges), through an autograft and into a recipient denervated nerve stump? 2) Sprouting of donor axons was reported after end-to-side neurorrhaphy. What is the relative contribution of sprouting and regeneration of donor nerves into the denervated nerve stump? 3) As donor axons prevent atrophy of denervated Schwann cells and facilitate axon regeneration after delayed nerve repair, we ask whether these axons grow either side of the bridges in the denervated stumps.

Methods: 1) Common peroneal (CP) autografts, 1-9 in number and either 6 or 3.2mm in length, were secured with Tisseel glue between 10mm lengths of a donor tibial nerve and a recipient CP distal nerve stump. Epineurial windows of ~1.5mm, 2 and 4mm were opened for comparison of tibial nerve growth through 1 and 3 bridges and, in a subset of rats, the CP nerve stump was ligated 20mm distal to the bridges. Motor and sensory neurons were backlabelled with fluorescent dyes 3 months later to count the tibial neurons that had sprouted (contained dye applied both to the tibial donor nerve and the CP nerve stump) and/or regenerated axons proximal and/or distal to the bridges.

Results: 1) The numbers of donor tibial neurons that regenerated axons into recipient denervated CP nerve stump increased with the diameter of the epineurial windows: <1%, ~5% and ~34% of the motoneurons regenerated axons through 3 bridges placed between ~1, 2, and 4 mm windows, respectively. 2) Few motor and sensory tibial neurons sprouted axons into the CP stump, the majority regenerating their axons. 3) The same numbers of motor and sensory tibial neurons regenerated axons in the recipient CP nerve stump proximal and distal to the bridges and, the axon numbers were equal on either side irrespective of whether the CP stump was ligated distally. The latter finding eliminated possible retrograde neurotrophism.

Conclusions: Epineurial windows are necessary for donor axons to regenerate across an autograft into a recipient denervated nerve stumps. Axons grow equally in proximal and distal directions. They sustain chronically denervated Schwann cells to improve nerve regeneration.

3. Ladak et al Neurosurg 68:1654
**P2. Leprous Neuropathy Screening: Field Testing of Sensory Testing Devices in Ecuador**  
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**Objectives:** Leprous neuritis represents an important cause of disability worldwide. Screening is typically performed with Semmes-Weinstein monofilament (SWMF) or ballpoint pen testing (BPT). However, important rates of under-diagnosis have been reported with these modalities. The Pressure-Specified Sensory Device™ (PSSD) has emerged as an effective way to assess peripheral nerve function. We aimed to determine the diagnostic accuracy of these three screening modalities.

**Design:** A cross-sectional study analyzed a consecutive sample of patients screened for Leprous neuritis during a mission trip to Los Ríos, Ecuador. Patients meeting the WHO criteria for Hansen’s disease and complaining of neuropathy symptoms were classified as Leprous neuritis patients. Patients without any sign of Hansen’s disease were used as controls. The same investigator performed bilateral ulnar nerve screening with the PSSD, SWMF (0.07g, 0.4g, 2g, 4g, 10g, 300g) and BPT in all patients. Sensitivity and specificity were calculated and compared across tests.

**Results:** A total of 71 patients (142 nerves) were evaluated. The mean age of the population was 39.4 ± 20.1 years. Three (4.2%) patients were excluded due to a potentially confounding cause of neuropathy (diabetes). Compared to the 10g SWMF and the BPT, the PSSD was found to have significantly higher sensitivity (78.3% vs. 0% with p<0.001, for both) with comparable specificity (97.8% vs. 100% with p>0.999, for both). Compared to the 0.07g SWMF (lightest in our series), the PSSD showed similar sensitivity (78.3% vs. 65.2%, p=0.514) but significantly higher specificity (97.8% vs. 51.1%, p<0.001).

**Conclusions:** The PSSD provides optimized diagnostic accuracy for detecting leprous neuritis compared to SWMF and BPT. Future prospective studies should assess the impact of early leprous neuritis management and should aim to determine the inter-rater variability associated with PSSD screening.
P3. Congenital Anterior Transposition of the Ulnar Nerve at the Elbow: A Rare Anatomic Variant
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Introduction: Cubital tunnel syndrome is the second most common compressive neuropathy of the upper extremity. Anatomic variants of the ulnar nerve at the elbow are rare.

Methods: Two cases of congenital transposition of the ulnar nerve, identified intra-operatively during cubital tunnel release, are presented here. One, a 66-year-old male who presented with typical cubital tunnel symptoms with no history or upper extremity trauma or surgery. The other, a 22-year-old female with cubital tunnel symptoms and a history of a both bone forearm fracture as a child.

Results: In these cases, the ulnar nerve was identified behind and penetrated through the intermuscular septum about 3-5 cm proximal to the medial epicondyle at the elbow. It then ran anterior to the pronator-flexor mass before entering the forearm between the ulnar and humeral heads of the flexor carpi ulnaris.

Discussion: Although a rare anatomic anomaly, congenital anterior transposition of the ulnar nerve is potentially under reported and has great clinical importance. In individuals with cubital tunnel syndrome, diagnosis and surgical treatment may be negatively impacted if the electrodiagnostic and ultrasound technician or surgeon fails to recognize the aberrant anatomy. Upper extremity surgeons should also be mindful of this condition when performing elbow arthroscopy or medial epicondyle release to prevent inadvertent injury to the nerve.
P4. Differences in Inflammatory Cytokine Production in Nerve Regeneration and Allograft Rejection and Effects on Nerve Regeneration
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Purpose: Nerve regeneration is a complicated process made even more complex by the involvement of the immune system. The interaction between nerve regeneration and the immune system is a critical part of nerve regeneration after any injury, and is most dramatic during recovery following limb transplantation. This new field of reconstructive surgery relies on further research to improve patient outcomes and can serve as a powerful model to study the mechanisms of interaction between nerve regeneration and immune function. We have investigated the differences in cytokine signaling during nerve regeneration between the

Methods: Rats underwent sciatic nerve transection and repair in isolation (intact limb) or in the context of syngeneic or allogeneic transplantation. Orthotopic hindlimb transplants were performed between (Lewis) syngeneic or (BN to Lewis) allogeneic rat strain combinations. Allogeneic transplants in different groups were either kept on immunosuppression or allowed to reject. Cytokine levels were analyzed using multiplex assay and quantitative PCR and nerve regeneration quantified by nerve histomorphometry.

Results: Several patterns of pro-inflammatory cytokines were noted to be up-regulated in the distal nerve following cut and repair. Anti-inflammatory cytokines including IL-10 were down regulated in distal nerve segments as well. Differences were seen between inflammation caused by cut and repair and syngeneic transplant vs allogeneic transplants. Several cytokines were found to be specific for inflammation involved in rejection. One of these cytokines IL-6 was also found to up-regulated even in the presence of immunosupresion and no clinical signs of rejection. These findings correlated with impaired nerve regeneration by histomorphometry in allogeneic transplants even while on immunosuppression.

Conclusions: Inflammatory cytokines are activated and anti-inflammatory cytokines down regulated following cut and repair of any nerve. Additionally, specific cytokine patterns are initiated in nerves generating in the context of a limb transplant. These inflammatory changes seem to have a deleterious effect on nerve regeneration even in the absence of clinical rejection. Further understanding of the complex interplay between inflammation and nerve regeneration will allow us to make improvements in the clinical outcomes of patients with both nerve injuries and limb transplants.
P5. Schwann Cell Transfer: Does Source and Passage Matter?
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Introduction: Schwann cells (SCs) have been shown to facilitate regeneration when applied locally at the nerve repair site or inside artificial conduits. Due to its antigenicity autologous cells should be used. In current literature only SCs derived from sciatic nerves have been used in both in vitro and in vivo studies. This poses an obstacle for clinical translation. We developed a protocol to isolate and expand SCs from sural nerve, the most common donor nerve, and compared the yield, purity and bioactivity of these cells to sciatic nerve derived SCs.

Methods: Sciatic nerve and sural nerve were harvested from adult female Lewis rats. A course of predegeneration of the nerves was employed before tissue digestion. Dissociated cells were plated and expanded. Serum tapering was used to eliminate fibroblast contamination. Sural nerve derived SCs (SuSCs) and sciatic nerve derived SCs (SciSCs) were harvested when culture reached subconfluency. Cells were counted and SC purity quantified. Expressions of P75, S100 and GFAP were measured via immunofluorescence staining. Cell bioactivity was determined by the extent of PC12 cell differentiation when co-cultured with SCs. Percentage of neurite-bearing PC12 cells, number of neurites per cell, and neurite length were used as indicators. These parameters were compared between SuSCs and SciSCs. Furthermore, SuSCs culture was passaged till 3rd passage. SC purity, biomarker expression and bioactivity of different passages were compared.

Results: The amount of cells harvested from per mg of sciatic nerve was much more than that from sural nerve (1.52E+05 vs. 3.34E+04). Purity of SciSCs and SuSCs were both above 94%. Expressions of P75, S100 and GFAP were 94.22%, 95.07% and 94.34% for SciSCs and 97.19%, 97.37%, and 98.06% for SuSCs. PC12 cell differentiation in SuSCs co-culture and in SciSCs co-culture was similar. Percentage of neurite-bearing PC12 cells, number of neurites per cell, and neurite length were 40%, 4.74/cell and 61µm for SuSCs and 42%, 3.78/cell and 68µm for SciSCs. For multiple passage cultures, cell purity ranged from 97.2% to 88.6% from P0 to P3. Cell marker expression showed similar trend. Quantification and comparison of bioactivity of cells from different passages is ongoing.

Conclusions: Culturing SCs from sural nerve can achieve comparable purity, cell marker expression and cell bioactivity. Cell yield from sural nerve is lower than that from sciatic nerve. Passaging of cells can increase cell number from sural nerve culture without significantly compromising SC purity, phenotype and bioactivity.
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Introduction: Sciatic nerve injuries are devastating and result in loss of lower leg function and impaired ambulation. While nerve transfers are commonly used to restore upper extremity function, their use in lower extremity nerve injuries is rare. To demonstrate the restoration of tibial nerve function with nerve transfer from terminal quadriceps motor branches, an anatomical study and two clinical cases were performed.

Methods: In six cadaver legs, we noted the length and the branching patterns of the terminal motor branches of the femoral nerve to the vastus medialis and lateralis muscles, and the neurolysis length of the gastrocnemius and the sural nerves. Histomorphometry was performed on both the donor and recipient nerves. Clinically, the nerve transfers included transfer of the terminal branches of vastus medialis and lateralis to the medial and lateral gastrocnemius branches respectively in the distal thigh, and sensory transfer of the saphenous nerve to the sural nerve.

Results: A consistent branching pattern and location of the saphenous nerve and terminal femoral motor nerve branches in the thigh were noted. The terminal motor branches to the vastus medialis and lateralis were identified at 13.2±1.7 and 16.7±3.5 cm superior to the medial and lateral patella, respectively. The neurolysis lengths of the medial and lateral gastrocnemius branches and the sural nerve were 8.4±1.2, 8.2±2.7, and 11.4±3.2 cm respectively, and allowed for anterior transposition for direct transfer from the femoral donors. Transfer of the vastus lateralis terminal branch required a short nerve graft in most specimens. In two patients at one year follow-up, MRC grade 3/5 gastrocnemius function was restored with active plantar flexion and improved ambulation. Because muscle reinnervation was first noted 6-8 months after surgery, these results are early and greater strength and coordination are anticipated. One patient has even returned to competitive sports with the use of a footdrop splint. An advancing Tinel’s is found at the distal lower leg in both patients, indicating sural nerve reinnervation. A planned transfer of the sural nerve to the distal tibial nerve at the tarsal tunnel for plantar sensation has been performed in one patient.

Conclusions: This study confirms the anatomical feasibility and clinical success of femoral to tibial nerve transfers to restore gastrocnemius and sural nerve function after a devastating sciatic nerve injury. Use of the femoral nerve for nerve transfer has untapped potential and could also be considered to restore ankle dorsiflexion in cases of common peroneal palsy.
P7. Early Nerve Surgery in Adult Brachial Plexus Traction Injuries - Why Wait?
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Introduction: Timing for surgical intervention in case of adult brachial plexus traction injuries (ABPTI) is traditionally delayed for several months to await spontaneous recovery to occur. Spontaneous recovery, however, will not occur in case of root-avulsion or rupture. Results of nerve surgery decrease with increasing interval between trauma and operation. Since 2009, we endeavour to operate ABPTI as soon as possible in patients with evidence or high suspicion of root avulsion. Here we present our first experience and preliminary results.

Materials & Methods: Between 2009 and 2011 42 ABPTI patients were operated, for whom 35 follow-up data were available. The biceps muscle was the main target of the nerve reconstruction using nerve grafting or nerve transfer. Early nerve reconstruction was defined as surgery performed within two weeks of the trauma.

Results: Five of 35 ABPTI patients were operated early. All patients had at least one root avulsion as diagnosed on preoperative MRI and confirmed intra-operatively. Reinnervation of the biceps muscle was pursued by grafting 2 times and by transfer 3 times. All five ‘early’ patients recovered biceps force ≥MRC 4, as compared with 12 of 30 patients who were operated late.

We experienced the following advantages of early surgery. The absence of scar formation facilitated identification of proximal stumps. Additionally, distally retracted plexus elements could be repositioned thereby shortening graft length. Direct electrical stimulation of distal stumps of ruptured or avulsed nerves showed muscle responses, because Wallerian degeneration was not yet complete, which facilitated anatomical identification. Dorsal root ganglions and anterior root filaments could be easily identified.

Conclusions: Early surgery in ABPTI is feasible and has surgical advantages. In our experience, early surgical exposure is more convenient, leading to superior reconstruction strategy, and maybe the ability to use shorter nerve grafts. Our preliminary results suggest that result may be superior.

A treatment algorithm will be presented to decide which patients are eligible for early surgical intervention for ABPTI.
P8. Dynamic Changes of Acetylcholine Receptors (AChR) and Creatine Kinase (CK) Activity in Complete Denervated Muscle and Protective Effect of Laser Phototherapy
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Background: Post-traumatic prevention of muscle atrophy is a major challenge in restorative medicine. When muscles are denervated, as in cases of complete peripheral nerve injury, they deteriorate progressively. Denervated muscles can account for significant differences in the extent of AChR and CK activity during the denervation period.

This study was designed to assess the status of skeletal muscles during long-term denervation processes, by investigating changes in the level of AChR and CK activity in the denervated gastrocnemius muscle of the rat. The influence of low power laser irradiation (laser phototherapy) on muscle degenerative process was also analyzed.

Methods: The study was conducted on 96 rats: 48 that received laser treatment and 48 untreated controls. The gastrocnemius muscle was denervated by removing a 10mm segment of the sciatic nerve. Low power laser irradiation was delivered transcutaneously to the right gastrocnemius muscle 30 min for 14 consecutive days. Under general anesthesia, the rats were euthanized at seven time points: 7, 14, 21, 30, 60, 120, and 210 days, with and without laser treatment respectively. AChR was quantified by the 125I-a-bungarotoxin. CK activity was measured by a specific spectrophotometric method.

Results: Muscle denervation results is progressive degradation of AChR and CK content. After 4 months for AChR and 2 months for CK content we found partial preservation in both components until 7 months after complete muscle denervation. Laser treatment had a significant therapeutic effect on the denervated muscle during the first 21 days for AChR and the first 30 days for CK activity.

Conclusions: We suggest that survival of denervated muscle is longer than previously considered. In the early stages of muscle atrophy, laser phototherapy may preserve the denervated muscle by maintaining CK activity and the amount of AChR close to its initial level before injury.
Peripheral Nerve Reconstruction of the Sciatic Nerve in Rats Using Chitosan Hollow Tubes Versus Standard of Care with Nerve Graft

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Objective: Traumatic injuries of peripheral nerves represent a major cause for morbidity and disability affecting about 3% of all trauma patients. In cases of peripheral nerve injuries with a nerve defect, autologous nerve grafting is the treatment of choice, however, with extensive nerve loss the clinical outcome of neural repair is often unsatisfactory. The aim of this study was to evaluate, in a preclinical perspective, an innovative biohybrid artificial nerve scaffold for regeneration of injured peripheral nerves.

Methods: In the current study the authors have analyzed 21 rats in two groups comparing nerve reconstruction of the sciatic nerve using chitosan hollow tube versus standard of care with autologous nerve graft. The sciatic nerve was transected in the left hind limb in all rats. In 11 rats chitosan hollow tube was used with a 10mm gap between the proximal and distal ends. In the control group autologous nerve graft was used with end-to-end anastomosis representing the standard of care for nerve reconstruction. Outcome was evaluated during the follow up period using functional sciatic index, electrophysiology, and ultrasonography. After 90 days muscle weight was analyzed along with histology of the proximal and distal parts of the nerves.

Results: Somato-sensory evoked potential and compound muscle action potentials tests along with functional sciatic index demonstrate similar results with no statistically significant difference between the groups. Ultrasonography imaging performed during the follow up period demonstrates nerve tissue proliferation inside the tube. Histology slides of the proximal and distal sciatic nerve 90 days after reconstructive procedure exhibit similar axon numbers and myelin thickness in both groups.

Conclusions: Peripheral nerve reconstruction of the sciatic nerve in rats using chitosan hollow tubes is comparable with autologous nerve grafting while harboring the potential for treatment of larger nerve gaps and functioning as a scaffold with filling material supporting nerve regeneration.
P10. WITHDRAWN
P11. Optimization of Decellularized Nerve Allografts: Comparison of Rat and Human Nerves
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1Department of Orthopedic Surgery, Microvascular Research Laboratory, Mayo Clinic, Rochester, MN; 2Department of Plastic, Reconstructive and Hand Surgery, Erasmus MC, University Medical Center, Rotterdam, Netherlands

Introduction: In previous animal studies, commercially available processed nerve allografts have been inferior to autograft nerve for motor recovery. We hypothesize that different processing and storage techniques may better maintain nerve ultrastructure, lower immunogenicity, and minimize cellular debris, resulting in an ‘optimized’ processed nerve. We tested several modifications to previously-described processing and storage protocols in both fresh rat sciatic and human sensory and motor nerves. The protocols evaluated used chemical detergents and irradiation as previously described, with the addition of variable exposure to the highly potent enzyme Elastase.

Materials & Methods: For this experiment 50 rat nerves and 70 human sensory and motor nerves were processed, followed by storage at either 4 or -80 °C for the duration of two weeks. Both processed and fresh control nerves were analyzed using immunohistochemical stainings and confocal microscopy. We evaluated basal lamina (laminin γ-1), Schwann cells (S100 protein) and immunogenicity (major histocompatibility complex class I, MHC-I). Ultrastructural integrity and amount of cellular debris were analyzed on cross-sections of the nerves stained with toluidine blue and by electron microscopy.

Results: Nerve gross morphology and internal structure and basal lamina were preserved with all decellularization protocols. Storage at -80°C severely altered nerve ultrastructure after any decellularization method. Elastase was found to significantly reduce the immunogenicity (MHC-I) and the amount of Schwann cells (S100), while maintaining good structural properties. Increased concentrations of the enzyme enhanced the decellularization process. It also significantly diminished cellular debris. Significant differences were found between rat and human nerve outcomes using the different decellularization protocols. However, both showed the same negative effect of the freeze storage of the nerve after the decellularization process.

Conclusions: Elastase, when added to nerve processing reduced its immunogenicity, diminished cellular debris and better removed Schwann cells while maintaining ultrastructure. Storage at -80°C heavily damaged nerve ultrastructure. A following in vivo study will be needed to demonstrate superior functional outcome with the optimized processed nerve allograft.
P12. Calcitonin improves nerve regeneration after transection injury and repair
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Plastic Surgery, Medical College of Wisconsin, Milwaukee, WI

**Purpose:** Investigate the effect of calcitonin on transected and repaired peripheral nerves

**Methods:** Sixteen rats underwent sciatic nerve transection followed by direct repair then were divided into two groups (n=8). The calcitonin group had a calcitonin-filled mini-osmotic pump implanted subcutaneously parallel to the repaired nerve. The control group was repaired without implantation of the pump.

Comparison between the groups were then made: 1) Compound muscle action potential recordings of the extensor digitorum longus (EDL) muscle; 2) Tetanic muscle force test of EDL; 3) Nerve calcium concentration; 4) nerve fiber count and calcified spots count.

**Results:** Injured sciatic nerve treated with calcitonin showed greater recovery compared to the injured untreated group.

**Conclusions:** The calcitonin-filled mini-osmotic pump improved functional recovery of injured sciatic nerves by accelerating calcium absorption from the repaired nerve which has potential clinical applications.
P13. Comparing Epimysial Patch with Intramuscular Hook Electrodes for Electromyography Recording of Regenerative Peripheral Nerve Interfaces
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Plastic Surgery, University of Michigan, Ann Arbor, MI

**Purpose:** Advanced motorized prostheses with multiple degrees of freedom have the potential to improve upper extremity amputee function, but reliable interface technology for volitional control is lacking. We developed a Regenerative Peripheral Nerve Interface (RPNI) consisting of a unit of free muscle neurotized by a transected peripheral nerve and an implanted epimysial electrode. RPNI biologic signals are capable of controlling myoelectric limbs by reliably transmitting electromyographic (EMG) signals, but the most appropriate electrode type and configuration has yet to be determined. We therefore compare epimysial patch electrodes with intramuscular hook electrodes using monopolar and bipolar configurations to determine which recording strategy transduces EMG signals with highest fidelity.

**Methods:** Using a rat model, two bipolar epimysial patch electrodes (n=4) (Figure 1) or two bipolar intramuscular hook electrodes (n=4) were applied to RPNI s of transferred extensor digitorum longus (EDL) muscle, neurotized by the divided peroneal nerve, and wrapped in small intestinal mucosa (SIS). Cables were tunneled subcutaneously to a head cap for recording. Twitch stimulation pulses were applied to either the peroneal (signal of interest) or tibial nerves (signal noise) and in vivo evoked EMG signals were recorded in monopolar and bipolar configurations. Data were collected at the time of electrode placement and at subsequent monthly intervals for four months.

**Results:** Recorded mean EMG peak amplitudes ± SEMs are listed in Table 1. Compared to monopolar recording strategy, a bipolar configuration decreased signal noise as determined by tibial nerve stimulation for both epimysial patch and intramuscular hook electrodes (p<0.01) (Figure 2). With peroneal nerve stimulation, the decrease in peak-to-peak EMG voltage was less (better) for patch electrodes when comparing monopolar to bipolar recording strategies. Intramuscular hook electrodes were more prone to failure as a result of cable breakage, migration, and extrusion.

**Conclusion:** Epimysial patch electrodes were superior to intramuscular hook electrodes with respect to maximizing signal amplitude, minimizing signal noise, and being less prone to failure in a rat model.

**Acknowledgment:** This work was supported by DARPA (N66001-11-C-4190).
Table 1: Mean EMG peak amplitudes (μV ± SEMs. Recordings were made using intramuscular hook electrode and epimysial patch electrodes during peroneal or tibial nerve stimulation using either monopolar or bipolar configuration. Abbreviations: DOS, date of surgery; 1mo, 1 month post-implantation; 2mo, 2 month post-implantation; 3mo, 3 months post-implantation; 4mo, 4 months post-implantation.

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Figure 1: Epimysial patch implantation

Figure 2: Maximal signal noise during tibial stimulation. Monopolar is compared with bipolar electrode configuration using (A) intra-muscular hook and (B) epimysial patch electrodes. Values indicate mean ± SEM. *P<0.05, **P<0.001. Abbreviations: DOS, date of surgery; 1mo, 1 month post-implantation; 2mo, 2 months post-implantation; 3mo, 3 months post-implantation; 4mo, 4 months post-implantation.

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Introduction: Photochemical tissue bonding (PTB) creates sutureless, watertight bonds between two apposed tissue surfaces that have been stained with photoactive dye and illuminated with a 532nm laser. Building on previous success in end-to-end repair, we have recently shown that, when applied to large gap injury requiring nerve grafting, PTB can result in superior outcomes in comparison to conventional suture fixation. Following major multi-limb injury and amputation, demand for autogenous nerve graft may exceed that which can be supplied by the patient. Acellular nerve allograft (ANA) is an alternative option in these circumstances although outcomes are typically inferior to autograft. It is the aim of this study to assess the efficacy of PTB when used with ANA.

Methods: 20 sciatic nerves were harvested from Sprague Dawley rats and sent to AxoGen Inc for processing. An additional 20 male inbred Lewis rats were randomized into 2 groups (n=10). All rats had 15mm left sciatic nerve defects created and repaired with processed ANA. 1 group had nerves secured using conventional epineurial suture. The remaining group had ANA secured using PTB. Following surgery, walking track analysis was performed at monthly intervals and sciatic function index (SFI) calculated. Following sacrifice after 150-days, repaired nerves were excised for histomorphometric analysis. Left (experimental) and right (control) gastrocnemius muscles were excised for calculation of muscle mass retention. Statistical analysis between groups was performed using the unpaired t-test.

Results: Sciatic function index did not differ significantly between standard repair and PTB groups after 5-months follow up (-80.3+/-.4.2 vs. -78.3+/-.5.0 respectively; p=0.3). Following sacrifice, all nerves were in continuity and, on gross observation, showed evidence of regeneration. Those nerves repaired photochemically had less extraneural scar tissue formation in comparison to standard epineurial suture. Gastrocnemius muscle mass retention did not differ significantly between standard repair and PTB groups (53.3%/+/-6.9 vs. 55.2%/+/-5.5 respectively; p=0.5). Histomorphometric analysis is in progress.

Conclusion: Although PTB has proved superior to conventional suture when used for isograft fixation, this advantage appears to be lost when applied to ANA. The beneficial effects of PTB likely relate to its ability to create a water-tight seal, preventing the leakage of neurotrophic factors. The absence of schwann cells and other cellular components in ANA may reduce the impact of this effect and explain the observed loss of efficacy in this study. PTB remains an alternative, rapid method of fixation for end-to-end nerve repair and nerve graft reconstruction.
P15. Peripheral nerve regeneration using novel bioengineered peptide amphiphile nanofibers: In Vivo studies
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Department of Plastic and Reconstructive Surgery, REBAR Lab, University of California, Los Angeles, Los Angeles, CA

Background: Peripheral nerve injuries can result in lifelong disability. Primary nerve repair is used for short nerve defects. Though autologous nerve can bridge longer defects, the harvesting procedure creates donor site morbidity. Nerve conduits lack an aligned internal scaffold to support and guide axonal regeneration. A peptide amphiphiles (PA) can self-assemble into aligned nanofibers, and can potentially mimic the native internal architecture of peripheral nerve. A bioactive epitope, RGDS (Arg-Gly-Asp-Ser) can be incorporated into PA nanofibers and have been shown to promote neuronal cell adhesion, growth, and migration. We have reported the favorable ability of PA nanofibers to support the proliferation of Schwann cells, key components in peripheral nerve healing. In this study, we devised a PA construct for use in a peripheral nerve critical sized defect model.

Methods: Rat sciatic nerve defects were created and reconstructed with autologous nerve, Poly (lactide-co-glycolide acid) (PLGA) conduits filled with various forms of aligned PAs, or left unrepaired. Motor and sensory recovery were determined and compared among groups. Our results demonstrate that Schwann cells are able to adhere to and proliferate in aligned PA gels, with greater efficacy in bioactive PAs compared to the backbone-PA alone.

Results: In vivo testing revealed recovery of motor and sensory function in animals treated with conduit/PA constructs comparable to animals treated with autologous nerve grafts. Functional recovery in conduit/PA and autologous graft groups was significantly faster than in animals treated with empty PLGA conduits. Histological examinations also demonstrated increased axonal and Schwann cell regeneration within the reconstructed nerve gap in animals treated with conduit/PA constructs.

Conclusion: These results indicate that PA nanofibers may represent a promising biomaterial for use in bioengineered peripheral nerve repair.
**P16. Morphologic Characterization and Lifespan of Terminal Schwann Cells**
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*Plastic Surgery, Washington University in St. Louis, St. Louis, MO*

**Purpose:** Functional reconstruction of peripheral nerve injuries is limited by a critical therapeutic window during which native musculature can be reinnervated. Non-myelinating Schwann cells at the neuromuscular junction (NMJ) called terminal Schwann cells are relatively understudied and may contribute to the temporal restrictions on muscle reinnervation. In addition the lifespan of terminal Schwann cells (and all Schwann cells) is unknown. We describe our preliminary morphologic observations of the development and lifespan of this unique cell type.

**Methodology:** The sternomastoid muscles of wild type and transgenic S100-GFP mice were evaluated with confocal microscopy at different developmental timepoints from embryonic day 14.5 to a maximum age of 29 months. Neuromuscular junctions were evaluated with immunostaining using S100 antibody (for glial cell identification), alpha-bungarotoxin (for motor endplate staining), DAPI (for nuclear staining), and neurofilament (for axonal staining).

**Results:** Terminal Schwann cells are identified at the NMJ beginning around postnatal day 6 or 7. Approximately 3 to 5 terminal Schwann cells are noted per NMJ. In mature NMJs, terminal Schwann cells have characteristic round nuclei with a centrally located, round nucleolus. No obvious morphologic changes are noted in the NMJs of 9 month old mice compared to younger adults, but by 14 months of age, terminal Schwann cells and motor endplates become disjointed; NMJs often have endplates in isolation or terminal Schwann cells in isolation, with fewer NMJs having colocalization of both elements. By 29 months of age, fewer terminal Schwann cells are present at each NMJ with some NMJs having an absence of terminal Schwann cells. Motor endplates become fragmented with increasing age beyond 12 months.

**Conclusions:** Terminal Schwann cells can be identified at the NMJ with characteristic morphologic features. Interruption of the colocalization of motor endplates with terminal Schwann cells is notable with age beyond 14 months. Quantitative studies are underway to better define this phenomenon.
P17. Long-term Observation of Respiratory Function After Unilateral Phrenic Nerve and Multiple Intercostal Nerve Transfer for Avulsed Brachial Plexus Injury
Mou-Xiong Zheng, MD; Wen-Dong Xu, MD; Yan-Qun Qiu, MD; and Jian-Guang Xu, MD
Hand Surgery Department, Huashan Hospital, Fudan University, Shanghai, China

Objective: Either phrenic nerve transfer (PNT) or multiple intercostal nerves transfer (MIT) alone was reported having no significant impact upon pulmonary function in short or medium term. But it has rarely been reported whether the combination of PNT-MIT could influence respiratory function in the long term. In this study, pulmonary and diaphragmatic function were compared between PNT and PNT-MIT after 7 to 19 years (mean 10 years) postoperatively.

Methods: 23 adult patients with brachial plexus avulsion injuries (BPAI) underwent PNT-MIT were compared with 19 corresponding adult patients who underwent PNT only. Pulmonary function testing, phrenic nerve conduction study, chest fluoroscopy were performed to assess ventilation, diaphragmatic response and excursion. In the PNT-MIT group, further comparison was performed to investigate whether transferred intercostal nerves number and the timing of MIT would influence the results.

Results: In PNT-MIT group, forced vital capacity (FVC), forced expiratory volume in one second (FEV1) and total lung capacity (TLC) were 73.69%, 72.04% and 74.81% of predicted without significant differences from PNT group. Diaphragmatic paralysis permanently existed with hemidiaphragm elevation of 1 to 1.5 intercostal spaces (ICSs) and near one ICS reduced excursion. But no statistically significant difference was found between PNT and PNT-MIT groups. Furthermore, in the PNT-MIT group, three and four intercostal nerves transfer resulted in no further decrease in pulmonary function than two intercostal nerves. No significant difference was found when PNT-MIT was performed at the same stage or at an interval of 1 or 2 months.

Conclusion: In the long term observation, PNT-MIT didn’t result in additional impairment in respiratory function in adult patients compared with PNT alone. Two to four intercostal nerves transfer performed for 1 to 2 months delay after PNT is a safe method for treating BPAI.
Side-to-side nerve bridges support donor axon regeneration into chronically denervated nerves and are associated with characteristic changes in Schwann cell phenotype and re-myelination

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**Introduction:** Chronic denervation resulting from long nerve regeneration times and distances is a major contributor to suboptimal regenerative outcomes following nerve injuries. Recent studies showed that nerve grafts between an intact donor nerve and a denervated distal recipient nerve stump (termed side-to-side nerve bridges) enhanced the regenerative success after delayed nerve repair. In this study, we addressed the cellular aspects of axon growth across these bridges to evaluate the basis for this ‘protection’ of the chronically denervated Schwann cells by the donor nerves.

**Materials & Methods:** In Sprague Dawley rats whose neurons express green fluorescent (GFP), three side-to-side nerve bridges were placed bilaterally over a 10 mm distance between opposing epineurial windows of an intact tibial (TIB) nerve and a distal denervated common peroneal (CP) nerve stump. Numbers of axons that grew across the bridges were counted in cross-section after 4 weeks. Immunofluorescent imaging of axons and Schwann cells were imaged and semi-quantitated over a 4 month period.

**Results:** Side-to-side nerve bridges supported the growth of donor axons across and into the denervated CP nerve stumps. Denervated Schwann cells dedifferentiated to a proliferative, non-myelinating phenotype within the bridges as well as the recipient denervated CP nerve stump after injury. Schwann cells demonstrated strong p75 immunoreactivity and redifferentiated to a myelinating phenotype with expression of myelin basic protein after ingrowth of donor axons. Side-to-side nerve bridges promoted a relative increase in wet muscle mass of tibialis anterior and extensor digitorum longus muscles in the anterior hindlimb compartment as compared the mass of the muscles whose common peroneal nerve was not ‘protected’ by cross-bridges.

**Conclusions:** This study documents the pattern of donor axon regeneration and myelination into the denervated recipient nerve stump that supports a mechanism where ingrowing axons sustain a pro-regenerative state in a denervated nerve pathway that would otherwise have deteriorated in the face of chronic denervation.
P19. Selective Nerve Root Transection Produces a Permanent, Partial Nerve Injury Model
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Department of Surgery, Division of Plastic and Reconstructive Surgery, Washington University School of Medicine, St. Louis, MO

Introduction: This study's goal was to develop a partial, non-regenerative nerve injury model that results in permanently reduced muscle force to allow the study of therapeutics to improve motor function. Reduction of the motoneuron pool to 20% normal values is required to produce a measureable loss of muscle force in rats. We hypothesized that transection of one or more of the L4-6 nerve roots would cause a permanent measurable reduction in muscle force.

Methods: Eighty rats were randomized into four groups (n=20) that underwent variations of nerve root transections. Group I and II had the L4 or L5 nerve root transected respectively, and a silicone cap placed proximally to prevent regeneration. Group III had both L4&5 roots and group IV had the L4&6 roots transected and capped. Retrograde labeling of the tibial and peroneal nerves (n=12 per group) was performed at 3 weeks. Normal values were established in a sham surgery group. Tibial and peroneal nerves were harvested for histomorphometry at 3 and 12 weeks to evaluate the presence of myelinated axons. Muscle force testing (n=8 per group) was conducted to provide functional data corroborating the reduced counts. Neuman-Keuls post-hoc analysis was performed.

Results: In group III (L4&5-cut), the tibial mean motoneuron count was 6% of control, and peroneal nerve had no motoneurons. In group IV (L4&6-cut) mean motoneuron counts in the tibial and peroneal were 20% and 8% respectively (Figure 1). Large myelinated axon counts confirmed these results. In group III, the muscle mass of gastrocnemius and extensor digitorum longus (EDL) were significantly reduced v. sham (p<0.05). In group III, mean gastrocnemius force was to 1.1N (9.6% of sham), a statistically significant reduction. EDL force was 0.0 N in group III, confirming motoneuron and myelinated axon counts (Figure 2). No significant reduction in muscle force was observed in other nerve injury groups.

Figure 1: Tibial Nerve

![Tibial Nerve Graph](image)

Peroneal Nerve

![Peroneal Nerve Graph](image)

Each letter represents significant difference p<0.05 from other letters.
Summary Points:

• This partial nerve injury model produces reproducible and stable decrease in muscle force.

• Unlike previously described partial nerve injury models, this novel model avoids the rat's innate neuroregenerative capability and produces a stable platform from which to evaluate therapeutics to increase motor function.
P20. Functional Restoration Following High level Injuries of Upper Extremity Including Both Median and Ulnar Nerve
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Aim: High level injuries of upper extremity including both median and ulnar nerve are challenging. Repair of high level nerve injuries have suboptimal results despite meticulous surgical technique. As a result, new surgical options are being searched for better outcomes. In this study, we present 4 cases of median and ulnar nerve injuries due to gunshot wounds which were treated successfully with nerve transfers.

Patients and Method: The study is performed in Gulhane Military Medical Academy. Four patients suffering from both median and ulnar nerve injuries due to gunshot wounds were included in the study. The ulnar nerve motor function is restored by a nerve transfer from extensor carpi ulnaris branch of radial nerve. Anterior interosseous nerve function and pronator teres function were restored nerve transfers from brachialis branch of musculocutaneous nerve and extensor carpi radialis branch of radial nerve, respectively. Opponens pollicis function is restored by nerve transfer from extensor digiti quinti branch of radial nerve. The functional outcome is evaluated with EMG, lateral pinch and pulp pinch strength, grip strength and Jebsen hand function test.

Results: EGM evaluation revealed a functional nerve coaptation in all patients. When compared with the preoperative measurements, lateral pinch, pulp pinch, grip strength and Jebsen hand function test results showed a marked increase in the postoperative period.

Table : Postoperative functions of both median and ulnar nerve injuries

<table>
<thead>
<tr>
<th>Case</th>
<th>Lateral pinch</th>
<th>Pulp pinch</th>
<th>Grip Strength</th>
<th>Jebsen hand function</th>
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</table>

*Data indicates the percentage of hand function of injured hand according to the uninjured hand during the preoperative and postoperative period.

Especially, functional results of patients with both median and ulnar nerve injury was found to be encouraging.

Conclusion: Nerve transfers provide a new option for reconstruction of high nerve injuries of forearm. Especially, it remains the only option for patients with median and ulnar nerve injury.
AAHS/ASPN/ASRM Joint Outstanding Paper Session

AAHS #1 Patient Factors Associated with Complications within 30 days of Hand Surgery; an Analysis of 9,969 Patients Using the 2006-2011 ACS-NSQIP Datasets
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Introduction: The ACS-NSQIP database collects detailed and validated data on patient demographics, co-morbidities, and 30-day postoperative outcomes on patients undergoing operations in most subspecialties. This dataset has been previously used to delineate specific complication risks and risk factors in a number of surgical subspecialties, but has not yet been used for hand surgery. While the risk of early complications following hand surgery is generally believed to be low, it is important to define these risks quantitatively, and to identify patient groups who are at higher risk for complications so that preventive measures can be employed.

Materials and Methods: ACS-NSQIP data from 2006-2011 was queried using 293 hand-specific CPT codes. Descriptive statistics were calculated for the population, and potential risk factors and patient characteristics contained within the NSQIP database were analyzed for their association with complications in the 30-day postoperative period. The most common complications were identified, and significantly associated variables were determined.

Results: 204 hand-specific CPTs were represented in the data. Of these, 81 resulted in at least one complication. The overall 30-day complication rate for hand surgery was 2.7%. Women had fewer complications than men, and there were significant differences between races. Age and BMI did not correlate significantly with complication rates. Significant increase in complication rates were associated with insulin-dependent diabetes (10%), pre-operative dyspnea (5.4%), COPD (7.4%), hypertension (4.2%), peripheral vascular disease (14.9%), renal failure (44.1%), preoperative steroid use (10.5%), bleeding disorder (16.7%) and emergent surgery (10%). Increased surgical wound class was associated with increased rate of complications. Lower complication rates were associated with operations done under local or regional anesthesia. Decreased operating time and anesthesia time were significantly associated with decreased rate of complications. The most common complications were superficial and deep surgical site infections, urinary tract infection, unplanned intubation, sepsis, pneumonia, and wound disruption.

Conclusions: This study utilized a large, prospective national database to characterize the 30-day complication profile and risk factors for surgery of the hand. Overall, the incidence of complications is low, approximately 2.7%. However, rates are significantly elevated in certain sub-groups and with some perioperative conditions. The most common complications are listed and quantified. This information is valuable in counseling patients preoperatively, and in identifying groups of patients on whom risk reduction efforts should be focused.
AAHS #2 Can Platelet-rich Plasma Impact the Formation of Flexor Tendon Adhesions?
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Division of Plastic Surgery, Penn State Hershey Medical Center, Hershey, PA

Background: Because of its regenerative potential, platelet-rich plasma has been studied extensively. Chickens contain nucleated thrombocytes that contain many of the growth factors contained within mammalian platelets. We aimed to separate the thrombocyte-rich plasma (TRP) from avian whole blood, deliver this growth factor-rich concentrate to a traumatic flexor tendon laceration and evaluate its effect on flexor tendon healing - specifically the formation of peritendinous adhesions.

Methods: 9 chicken surgeries were performed on 18 digits (3rd, 4th digit). Prior to surgery, the digits were randomized to undergo laceration and repair - in an area homologous to zone two in the human hand -followed by the addition of thrombocyte-rich plasma (treatment) or closure without TRP (control). 5 cc of whole blood, separated via the Biomet GPS III system generated 1 cc of thrombocyte-rich concentrate. Post-operatively, all chicken feet were immobilized using a plaster cast. Three weeks later, subjects were euthanized and the tendons were examined histologically by five independent, study-blinded pathologists for the presence of connective tissue, peritendinous adhesion formation and the presence of a peritendinous space. These specimens were graded on a scale of 1 to 5 and a mean value for each specimen was calculated. The difference in severity of tendon adhesions between the treatment and control groups was calculated using a paired t-test.

Results: Mean adhesion score for the treatment group was 3.40 and mean adhesion score for the control group was 3.36 (p= 0.90). Mean Olympic adhesion scores (highest and lowest score not included) for the treatment and control tendons were 3.40 and 3.44, (p=0.91) respectively. A score of 3 suggests moderate adhesions, peritendinous space preserved in more than 50 % of the circumference and a score of 4 denotes severe adhesions, peritendinous space obliterated in more than 50 % of the tendon circumference. Adhesion formation varied between hosts, but there were no differences between treatment and control groups or between digits 3 and 4.

Discussion: The efficacy and reproducibility of platelet-rich plasma delivery is controversial. Laboratory evidence suggests that the addition of platelet-rich plasma may aid in tendon healing, but thus far there is no literature detailing its effect on adhesion formation. Though we have shown no difference in adhesion formation between control and study tendons, there are two significant limitations to this study: our ability to reliably quantify the growth factors within the thrombocyte-rich fraction and to consistently deliver the same volume to the repair site.
Introduction: Total root avulsion of the brachial plexus remains to be a major reconstructive challenge. This study aims to evaluate the functional outcomes of brachial plexus patients with unilateral total root avulsion, who were reconstructed with CC7 spinal nerve transfer.

Method and Materials: 168 patients who suffered from total brachial plexus palsy, and underwent reconstruction with CC7 spinal nerve transfer from 1985 to 2013 were analyzed. We then selected and analyzed the characteristics and the achievements of the 10 patients who attained the best functional outcomes.

Results: The average age was 24.8 years old. 89 patients had their dominant upper limb affected. All but 4 had more than one body part injured, 21% of them suffered from concomitant fractures in their affected upper limbs. 40 patients had vascular injuries on their affected limbs. The average time from injury to initial nerve reconstruction was 133.5 days and the average time from initial injury to CC7 transfer was 263 days.

Neither significant nor permanent donor site morbidity was noted. The average follow-up period was 5 years

For the 10 patients with best functional outcomes, no significant difference in their basic characteristic, their injury or their time from injury to initial nerve reconstruction was noted when compared with the other patients. A majority attained an education level of upper high school or above. They attended more follow-sessions with a longer average follow-up period of 8.2 years

6 patients had their CC7 transfer to both median and musculocutaneous nerves while 4 patients had their CC7 transfer to their median nerve only. This ratio is comparable to the remaining patients. 8 out of 10 patients underwent more than 1 surgery, which was significantly more than the rest of the patients. All patients with CC7 transfer to musculocutaneous nerve could achieve an elbow flexion motor grading of 4. 3 out of 4 patients with CC7 transfer to median nerve only had a finger flexion of grade 3. 9 patients had a finger flexion of at least grade 2. They all had protective finger sensation. Their self-perception improved post nerve reconstruction. They also had a significant improvement in DASH and Michigan Hand score.

Conclusion: CC7 is a good treatment option for patients with total brachial plexus injuries. Patients who are young and have high education status and motivation appear to achieve better functional results in the long term.
AMERICAN SOCIETY FOR PERIPHERAL NERVE | 2015 ANNUAL MEETING

ASPN #2 A Quantitative Analysis of the Sensory and Motor Fibres of the Brachial Plexus in Man
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1Division of Plastic and Reconstructive Surgery, CD Laboratory for Restoration of Extremity Function, Vienna, Austria; 2Systemic Anatomy, Medical University of Vienna, Vienna, Austria; 3Division of Plastic and Reconstructive Surgery, Medical University of Vienna, Vienna

Introduction: Any surgical nerve reconstruction must take into account amount of individual nerve fibres at any given level of injury. To date, however, literature on qualitative and quantitative assessment of motor axons of the peripheral nerves of the upper extremity is scarce. Furthermore, none of these studies have depicted the topography of motor fibres along the entire course of these peripheral nerves. The aim of the present study is to present the total number of motor fibres of the brachial plexus from each root down to the level of its corresponding branches.

Material and Methods: Nerve samples have been harvested from 12 organ donors immediately after death. From 8 incisions ranging from the neck to the wrist a total of 36 nerve samples were gained per organ donor. A special immunohistochemical protocol was applied to visualize the specific structure of interest within the nerve cross section. Antibody against neurofilament served to determine the total amount of myelinated and unmyelinated axons. Antibody against choline acetyltranferase (ChAT) was used to detect cholinergic/motor fibres. Histology sections were then scanned and evaluated with a digital software program to allow quantification of each cross section. These numbers were cross checked in an animal model with standard retrograde tracing methods. Finally, the quality of this method was also cross checked with staining ventral and dorsal roots of organ donors at spinal cord level.

Results: As expected the majority of any given peripheral nerve contains afferent fibers. To our surprise, however, only around 10% of all axons in a mixed peripheral nerve are efferent fibers. In a “pure” peripheral motor nerve (thoracodorsal nerve) one third of the axons are cholinergic. In a pure cranial motor nerve the motor portion rises to about 60%(accessory nerve) but still has a significant afferent fibre population. The control experiments in a rodent animal model show good correlation between retrogradely labelled motor neurons with ChAT positive labels in the peripheral nerve section.

Conclusion: Here we present for the first time a quantitative analysis of all afferent and efferent fibres of the brachial plexus and its consecutive nerves. The surprising finding is that even “pure” motor nerves with a suspected high number of motor fibres (thoracodorsal nerve) only have a relatively small number of efferents. Since this ratio is relatively constant for motor nerves at different levels of the extremity these results challenge the traditional view of fiber distribution and innervation density in man.
ASRM #1 Evaluation of Viability and Structural Integrity after Whole Eye Transplantation
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\textsuperscript{1}University of Pittsburgh; \textsuperscript{2}University of California San Diego; \textsuperscript{3}Harvard University; \textsuperscript{4}Fourth Military Medical University

BACKGROUND: Approximately 37 million people throughout the world suffer from blindness. Whole eye transplantation (WET) gives the opportunity to provide viable retinal ganglion cells and the entire optical system to recipients with vision loss and irreversible injury to the eye. A key obstacle to WET is the poor regenerative ability of the optic nerve. Recently, several groups have demonstrated optic nerve regeneration, showing promise for eye transplantation. There has been difficulty in establishing a consistent small animal model for basic science research in WET. We previously established and published a functional face transplant model in the rat, and have recently expanded our model to include the whole eye, optic nerve and its blood supply.

Methods: All syngeneic transplants were performed in Lewis (RT1\textsuperscript{1}) rats. The donor flap, pedicled by the common carotid artery and external jugular vein, is composed of all ocular tissue distal to the optic chiasm, a portion of the temporal bone, and the skin tissues of the eyelids and external ear. The recipient site was prepared by removing a similar region of skin tissue and the eye socket content, with the optic nerve cut at the base of the globe. A nerve apposition between the donor and recipient optic nerve was performed. Slit lamp examination and Optical coherence tomography (OCT) imaging of the cornea, lens and retina were performed weekly after transplantation. Histological sections of the eye were analyzed post-mortem.

Results: 6 out of 8 rats survived the surgical procedure while maintaining visual transparency of the anterior eye. All eyes were viable via slit lamp examination. OCT imaging confirmed transparency of the cornea and lens, preservation of the structural layers of the retina, and blood flow throughout the eye. Histology confirmed neovascularization of the cornea as well as preservation of the structural integrity of the retina, with the exception of degeneration of the retinal ganglion cell layer.

Conclusion: We have established a viable orthotopic model for vascularized whole eye transplantation in the rat. Maintenance of structural integrity and viability were confirmed by slit lamp examination, OCT, and histology. The model is ideal for examining viability, functional return and immunology in whole eye transplantation.

Figures
ASRM #2 Implications of Intracranial Facial Nerve Grafting in the Setting of Facial Reanimation
Bridget Harrison, MD; Khalil Chamseddin, MS; Gangadasu Sagar Reddy, MD; Shai Rozen, MD
UT Southwestern Medical Center

**Background:** Most intracranial tumors involving the facial nerve are extirpated with nerve preservation, but when resected, and if feasible, intracranial facial nerve grafting is performed. Results likely depend on multiple factors such as age, anatomic location, pre-operative facial palsy, radiation, and gap-length. Results can vary from complete palsy to varying degrees of tonicity, synkinesis, effective motion, and ocular protection.

**Purpose:** Evaluate the varying degrees of facial reanimation by facial region after intracranial nerve grafting and identify implications for future facial reanimation and pre-operative consultation.

**Methods:** Between the years 1997-2012, twenty-seven patients underwent intracranial nerve grafting after tumor extirpation. Of the 26 candidates, 14 completed evaluations. All patients were prospectively evaluated by three physical therapists specializing in facial nerve rehabilitation and scored with Facial Disability Index (FDI), and two regional grading systems - Facial Nerve Grading System 2.0 (FNGS 2.0), and SunnyBrook Facial Grading Score (SFGS). Additionally, all patients underwent still photos and videography to assess quality of motion and tonicity in repose. Demographic and surgical variables were analyzed as to their possible effect on end results.

**Results:** The average age was 43 (22-66). The average time interval between nerve grafting to evaluations was 44 months (12-146). Average total FDI was 67.5% comprised of the Physical Function and Social/Well-Being portions averaging 62.8% and 72.6% respectively. Subdivisions of the physical function score with worst outcomes were eye dryness/tearing and difficulty speaking. Best outcomes were recorded in teeth brushing, eating, and drinking. FNGS 2.0 demonstrates best outcomes in Eye and Oral Commissure portions and worse in Brow and Nasolabial fold. Final FNGS 2.0 grade average was 4.3 (1-5) i.e. moderately severe dysfunction. The SFGS reveals 64.3% have oral resting symmetry, but only 28.6% resting symmetry in eye and nasolabial fold. Symmetry in voluntary movement revealed gentle eye closure and lip pucker as best – 3.6 and 3.0 respectively, while brow lift as worst - 1.0 and open mouth smile at 2.0 (5-25). Total synkinesis score averaged low at 3.6 (0-15).

**Conclusion:** Intracranial nerve grafting does not provide consistently good facial animation but may provide periocular protection, although not symmetry. It does afford good symmetry of the midface in repose, thus potentially improving results of midface reanimation surgery by providing improved baseline tonicity with minimal synkinesis. This information is important during patient discussions if intracranial facial nerve resection and grafting is anticipated or in the interim between nerve grafting and planned future facial reanimation.
The SPA Arrangement of the Branches of the Upper Trunk of the Brachial Plexus
Amgad S. Hanna, MD
Neurosurgery, University of Wisconsin, Madison, WI

Introduction: All brachial plexus diagrams in every textbook or paper draw the branches/divisions of the upper trunk in the following sequence from cranial and posterior to caudal and anterior: Suprascapular nerve (S), anterior division (A), then posterior division (P), respectively. This is such a wrong concept.

Method: Twelve cadavers (24 brachial plexuses) were dissected. We performed both supra and infraclavicular exposures. The clavicle was resected to identify the divisions of the brachial plexus.

Result: In all dissections the origin of the posterior division was in a more cranial and dorsal plane in relation to the anterior division, which was more caudal and ventral. In most dissections, the suprascapular nerve branched off distally from the upper trunk (UT) giving it the appearance of a trifurcation, and taking off just cranial and dorsal to the posterior division.

Conclusion: The branching pattern of the UT most commonly takes the form of a trifurcation with the following arrangement from cranial and posterior to caudal and anterior: Suprascapular nerve (S), posterior division (P), and anterior division (A), respectively. Hence the mnemonic SPA can be used to avoid confusion. The application of this is huge. Supraclavicular exposure of the brachial plexus exposes only the trunks and divisions. Recognizing the 'SPA' arrangement of the branches helps identifying the correct targets for neurotization, especially that these 3 branches are the most common targets for brachial plexus repair. Understanding the anatomy means better surgical planning, and better patient outcomes. There is nothing worse than grafting to the wrong targets if we don't understand the anatomy.

Key Words: brachial plexus, suprascapular nerve, upper trunk.

Figure Legend:

22. Obstetrical Brachial Plexus Palsy: Can Excision of Upper Trunk Neuroma and Nerve Grafting Improve Function in Babies with Adequate Elbow Flexion at Nine Months of Age?

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\(^1\)University of Pittsburgh, Pittsburgh, PA; \(^2\)School of Clinical Medicine, University of Cambridge, Cambridge, United Kingdom

**Introduction:** Accepted indications for exploration in obstetrical brachial plexus palsy (OBPP) vary by center. Most agree that full elbow flexion against gravity at 9 months of age excludes a baby from surgical intervention. In infancy there are certain movements that are difficult to assess and less common for a baby to use such as shoulder external rotation and forearm supination. However the latter are extremely important for normal function as they grow.

**Methods:** A retrospective review of all obstetrical brachial plexus patients treated at a major multidisciplinary children's center was performed from 2009 to 2014. All patients were evaluated by a single PT and a single surgeon and the Toronto Active Movement Score (AMS) system was used. Those patients who had operative intervention were selected out for further review. Further analysis identified a cohort with isolated upper trunk lesions who had an active movement scale score of 5 or better for elbow flexion. Data analysis was performed on this group to look for improvement in overall function.

**Results:** 23 underwent operative intervention. 5 out of the 23 (22%) had isolated upper trunk lesions and elbow flexion AMS scores of 5/7 or better at the time of exploration. All 5 had weakness in shoulder abduction, flexion, external rotation as well as forearm supination. The average age at operative intervention was 10.8 months. Average follow-up was 22 months. All 5 had excision of neuroma between c5 and c6 and the upper trunk. Reconstructions were with sural nerve graft to either the anterior and posterior divisions of the upper trunk or, alternatively, the upper trunk proximal to the takeoff of the suprascapular nerve. In the case of the former a spinal accessory to suprascapular transfer was also performed. All patients except one, who was 4 months postop at time of evaluation, either returned to or exceeded their preoperative scores in the above mentioned shoulder and forearm movements. Their elbow function was preserved or improved (Table 1).

**Conclusion:** In select cases of isolated upper trunk OBPP, operative intervention despite adequate elbow function can improve overall patient shoulder and forearm functional movement. There is no evidence that excision of neuroma with nerve grafting causes loss of elbow function or worsening of overall outcome.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Shoulder Abduction</th>
<th>Shoulder Flexion</th>
<th>Shoulder External Rotation</th>
<th>Elbow Flexion</th>
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*Scores based on Toronto Active Movement Scale (AMS)
23. Pain in Children with Obstetrical Brachial Plexus Palsy and Primary Microsurgical Reconstruction
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Introduction: Our understanding of the pain experienced by children with obstetrical brachial plexus palsy (OBPP) is limited. The purpose of this study was to determine the prevalence and characteristics of pain experienced by children with OBPP who have undergone microsurgical reconstruction.

Materials and Methods: A prospective case series of children between 6 to 18 years with a diagnosis of OBPP who had microsurgery at less than 12 months of age with nerve grafting or neurotisation was conducted. Pain was evaluated using the Faces Pain Scale - Revised (FPS-R) and the Adolescent Pediatric Pain Tool (APPT). Sensory symptoms in the affected limb were also collected.

Results: Sixty-five children were evaluated: 28 (43%) upper plexus and 37 (57%) total plexus palsy. The mean age was 11.0 ± 3.3 years. The point prevalence of pain was 24.6%. The reported lifetime prevalence of pain was 66.2%. Seventy-one percent reported that their affected extremity felt different at least once in their lifetime. The average intensity of those with pain (n=43) was 40 ± 19 mm on a 100 mm visual analog scale. Seventy percent of children reported that symptoms occurred every day or at least once a week. The anatomical distribution of the pain was throughout the affected upper extremity irrespective of the severity of injury, with the exception of children with upper plexus injuries who did not report pain in their hand. A mix of words typically used to describe neuropathic or musculoskeletal symptoms were chosen by the children to represent their pain. However, the children were more likely to report words associated with neuropathic symptoms when asked about the sensation in their limb as opposed to pain.

Conclusions: Children with OBPP who had microsurgical reconstruction commonly report pain. This pain is typically frequent, but episodic and low in intensity. The descriptions of the type of pain experienced include terms typical of both neuropathic and musculoskeletal origins.
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**Background:** Contralesional hemisphere compensation can be an important recovery mechanism for upper extremity paralysis after hemispheric injuries, especially for large lesions. However, possibly due to insufficient connections from the contralesional hemisphere to the affected upper extremity, the quality of spontaneous compensation is weak and the degree is quite limited. Here, we postulate to increase this connection for greater compensation ability of the contralesional hemisphere by surgically creating a peripheral nerve cross between the unaffected and affected upper extremities.

**Methods:** Ten young hemiplegic patients received the cross neck C7-C7 nerve transfer surgery. Another ten patients with matched pathological conditions were assigned as the control group receiving only rehabilitation therapy. Sensorimotor functions of bilateral upper extremities were assessed. The Modified Ashworth Scale and Quality of Upper Extremity Skills Test were used for evaluating spasticity and functional use of the affected upper extremity respectively. Transcranial magnetic stimulation, functional MRI, and PET scans were used for evaluating brain activity associated with functional recovery of the affected upper extremity.

**Results:** Both flexor spasticity release and motor functional improvements in the affected upper extremity were observed in all ten surgery patients. There was no permanent loss of sensorimotor function of the unaffected upper extremity. Neurophysiological and neuroimaging studies showed that the functional recovery of affected upper extremity was associated with the activation of the contralesional sensorimotor cortex in surgery patients.

**Conclusions:** This cross neck C7-C7 nerve transfer approach may open a door to promote functional recovery of upper extremity paralysis in hemispheric neurological injuries.
25. Functional Outcome following Single-Root Reconstruction of the Upper Trunk in Erb’s Palsy
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**Background:** Obstetric brachial plexus palsy (OBPP) commonly presents with isolated upper trunk paralysis, but avulsion of either the C5 or C6 root is rare. In these situations, only one root is available for intraplexal reconstruction of the upper trunk. The purpose of this study was to determine the functional outcome of patients with Erb’s palsy, who required single-root reconstruction of the upper trunk.

**Methods:** We performed a retrospective cohort study of patients with OBPP undergoing a primary nerve operation between 1993 and 2009. All data was collected prospectively. Patients were included if they had isolated upper trunk injuries (C7, C8 and T1 intact) and avulsion of either the C5 or C6 nerve root. Functional outcome was assessed using the Active Movement Scale (AMS) at a minimum follow-up of 4 years. Post-operative movement in patients with single-root reconstruction of the upper trunk was then compared to a control group of patients where two roots were available for upper trunk reconstruction (n=18), using the Wilcoxon rank-sum test.

**Results:** Ten patients with OBPP (7 female, 9 unilateral) were included in the final cohort. Average birth weight was 8.9 ± 1.5 pounds, and mean age at surgery was 10.4 ± 0.9 months. The C6 root was avulsed in 9 patients (90%), and the C5 root in 1 patient. Surgical reconstruction of the upper trunk entailed neuroma resection and interpositional nerve grafting from the single available root (mean number of sural nerve cables, 6 ± 1). At a mean follow-up of 10.1 ± 5.0 years, all patients achieved AMS scores of 7/7 for elbow flexion. Average scores for shoulder abduction, shoulder flexion, and forearm supination were 6.0 ± 1.7, 6.0 ± 1.7, and 5.8 ± 1.9 respectively. The poorest recovery was seen in external rotation of the shoulder, with a mean AMS score of 2.9 ± 2.2. Post-operative AMS scores at 4 years follow-up did not significantly differ between patients undergoing single- versus two-root reconstruction of the upper trunk.

**Conclusions:** Reconstruction of the upper trunk via interpositional nerve grafting from a single nerve root for C5 or C6 avulsions can restore excellent elbow flexion and good shoulder function. Functional recovery in this patient group is similar to that which can be achieved when two donor nerve roots are available for grafting. Active external rotation remains difficult to achieve in all cases of C5-C6 injuries requiring operative intervention.
26. The Musculofascial Lengthening Technique for Submuscular Transposition of the Ulnar Nerve in Patients with Persistent or Recurrent Cubital Tunnel Syndrome: a Retrospective Case Series

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Introduction: Persistence or recurrence of cubital tunnel syndrome (CuTS) occurs in up to 10-25% following primary decompression interventions. There are several strategies for revision surgery, including subcutaneous or submuscular transposition. Only a small number of studies have investigated the effectiveness of these procedures in patients with persistent or recurrent ulnar neuropathy.

In this study, we present the results of 34 cases of submuscular transposition of the ulnar nerve for persistent or recurrent CuTS using the musculofascial lengthening technique described by Dellon and Coert (also known as the Z-lengthening technique).

Materials & Methods: Between January 2003 and January 2014, submuscular transposition of the ulnar nerve for recurrent or persistent CuTS was performed in a total of 40 adult patients. Six patients were excluded because of concomitant radiculopathy, polyneuropathy of the upper extremities and lost to follow up after 2 weeks. The objective outcome was assessed with the Likert scale. A satisfactory result was defined as Likert 1 (complete recovery) and 2 (almost complete recovery). In addition patient self-reported outcome was assessed with the questionnaire reported by Novak et al.

Results: All patients were followed with a mean duration of 7.4 months (+/- 6.2, range 1.5 – 30.6). Successful clinical outcome (Likert 1 and 2) was observed in 65% and complete recovery in 27%. All symptoms improved significantly after revision surgery (paraesthesias= 0.000, pain= 0.004, sensibility= 0.002 and strength 0.016).

Results of the questionnaire, which was obtained at a mean follow-up period of 2.62 years (+/- 2.55, range 0.21 – 9.59), showed that 47% of the patients had subjectively improved of which 22% completely. Seventy-two percent were satisfied or partly satisfied. Subjectively the reduction in pain, paresthesias and pain at the elbow were statistically significant (p< 0.012, 0.000, 0.39 respectively).

Conclusions: This retrospective case series shows that submuscular transposition of the ulnar with the musculofascial lengthening technique can be an effective treatment for patients with recurrent or persistent cubital tunnel syndrome. To obtain more evidence a prospective study and/or randomized trial comparing the submuscular and subcutaneous transposition is needed.

References:


27. The Psychological Impact of Brachial Plexus Injury
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**Introduction:** We hypothesized that brachial plexus injury (BPI) profoundly impacts patients psychologically. To inform, improve upon and develop standard best practices for multidisciplinary treatment of BPI, this pilot study collected clinical data about the psychological effects of BPI, specific patient psychosocial concerns and associated sources of psychological distress, and subsequent impact on daily functioning.

**Materials & Methods:** Between February 2013 and January 2014 during scheduled pre-operative surgical assessments and/or post-operative appointments, the staff social worker at a major brachial plexus center conducted mixed-methods psychosocial interviews with 39 patients to assess impact of BPI on psychological functioning. Open-ended interview questions explored how BPI affected perception of self and ability to function in interpersonal, familial, occupational, discretionary, and necessary daily living situations. Further questions investigated how adapting to and accepting BPI influenced mood and variance thereof over time. Patients (n=14) were evaluated for posttraumatic stress disorder (PTSD), depression, and substance use through the respective validated scales: Post Traumatic Stress Disorder Checklist-Specific (PCL-S) Patient Health Questionnaire-8 (PHQ-8), National Institute on Drug Abuse Quick Screen (NIDA Quick Screen).

**Results:** Mean age was 38 and 71.8% were men. BPI strongly impacted psychological well-being as evidenced by patient report and higher prevalence of PTSD and depression than US population norms (6.8% and 16.6% respectively). Open-ended interview questions revealed sources of psychological distress ranging from substantial negative impact on self-worth, to increased dependency upon others, to loss of employment. Results of the PCL-S and PHQ-8 supported clinical data from interviews (Table 1). 28.6% met diagnostic criterion for PTSD. Although 50% showed symptoms of depression, only 28.6% exhibited clinical depression. Of the 39 interviewed, 20.5% divulged suicidal thoughts. Consistent with other studies investigating the impact of comorbid PTSD and depression on suicidality, severity of suicidal ideation was most prevalent in depressed patients currently experiencing PTSD. Patients disclosed social alcohol and tobacco use but no significant substance abuse patterns were noted.

**Conclusions:** BPI significantly influences psychological well-being and ability to function in daily life. In addition there is a high prevalence of PTSD, depression, and suicidal ideation as a result of BPI. Subsequently, BPI patients have unique psychosocial concerns and psychological challenges requiring continued psychological attention throughout treatment and further study to develop multidisciplinary standard best practices for comprehensive BPI treatment.
Introduction: There is general agreement among brachial plexus surgeons that patients’ muscular recovery plateaus within 2-3 years after reconstructive surgery. We were unable to find literature to document objective improvement of strength and range of motion following adult brachial plexus reconstruction after this arbitrary 2-3 year window. We hypothesized that patients who underwent brachial plexus re-innervation would continue to gain strength and ROM beyond 2-3 years of follow-up.

Materials and Methods: Six male patients who had undergone plexus reconstruction prior to 2005 at our institution were available for clinical examination at 10 or more years postoperatively. The average age at the time of operation was 41 years (range 17-73). The mean preoperative time was 175.3 days. Two cases presented with C5-C6 paralysis, three cases presented with complete five level injuries, and one case presented with isolated axillary nerve injury. The average time for long-term follow-up was 9.4 years (range 5-15.5). Functional evaluation was based on the assessment of active range of motion (ROM) in degrees and the British Medical Research Council (BMRC) scale for muscle strength.

Results: We compared shoulder and elbow BMRC grade and ROM in patients who underwent plexus surgery at 2 years and average 9.4 years follow-up. EMG confirmed re-innervation of all target muscles at an average of 9 months postoperatively. Average shoulder abduction was 45±11 degrees (range 20-80) at 2 years postoperatively as compared to 90±31 degrees (range 30-160) at 9.4 years postoperatively, and average shoulder BMRC grade increased from 3.3 (range 3-4) at 2 years to 3.9 (range 3-4.5) at final follow-up. Average elbow ROM increased from 102±40 (range 0-140) degrees at 2 years to 124±19 (range 90-140) degrees at 9.4 years, and average elbow BMRC grade increased from 3.3±1 (range 0-4.5) at 2 years to 3.9 (range 2-4.5) at final follow-up. Overall, shoulder abduction showed statistically significant improvement in the 9.4 year follow-up group when compared to the 2 year follow-up group (p<0.05, Fisher’s exact tests).

Conclusion: We conclude that patients continue to gain range of motion and strength well after 2-3 years of follow-up, contrary to conventional opinion. While the precise mechanism is not known, it is likely due to terminal collateral sprouting, or to an unknown or as yet undescribed mechanism. We recommend that patients be encouraged to continue strengthening exercises well after the initial recovery period, and that more long-term data be collected to expand upon these observations.
29. Long-term Ongoing Cortical Remodeling after Contralateral C7 Nerve Transfer

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Background: Contralateral C7 nerve transfer is developed for the treatment of brachial plexus injury patients. In the surgical procedure, the affected recipient nerve would connect to the ipsilaterial motor cortex and the dramatic peripheral alteration may trigger extensive cortical reorganization. But little is known about the long term results after such specific nerve transfer. The purpose of this study is to investigate the long term cortical adaptive plasticity after brachial plexus injury and contralateral C7 nerve transfer.

Methods: In this study, nine healthy male volunteers and five male patients who suffered from right BPAI and underwent contralateral C7 transfer for more than 5 years were involved. fMRI was used for the investigation of the long-term cerebral plasticity.

Results: The neuroimaging results suggested that the ongoing cortical remodeling procedure after contralateral C7 nerve transfer could last for a long period, at least for 5 years. The motor control of the reconstructed limb may finally transfer from the ipsilateral hemisphere to the contralateral hemisphere solely instead of the bilateral neural network activation.

Conclusions: It was believed that the cortical remodeling may last for a long period after peripheral rearrangement and the successful cortical transfer is the foundation of the independent motor recovery.
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Introduction: Isolated axillary nerve injury with resultant deltoid paralysis can be treated with a triceps motor branch transfer to the axillary nerve or interpositional nerve grafting across the damaged lesion. Currently, there is no consensus on the optimal treatment. The purpose of this study was to evaluate our experience treating isolated axillary nerve injuries and compare outcomes between different treatment methods with respect to motor function, range of motion and patient reported outcomes.

Methods: Thirty-five isolated axillary nerve injury patients that underwent operative intervention (neurolysis, interpositional grafting or triceps motor branch transfer) with at least 1 year of follow-up between 2001-2014 were retrospectively reviewed. Functional outcomes, including post-operative medical research council (MRC) grade, shoulder abduction and disability of the arm, shoulder and hand (DASH) scores, were compared using one-way ANOVA or non-parametric comparisons, as appropriate. Predictors for successful outcome (MRC ≥ 3), including age, body mass index (BMI), injury-to-surgery interval were also compared between groups.

Results: The most common surgical intervention was the triceps motor branch transfer (63%, 22/35), followed by interpositional sural nerve grafting (20%, 7/35; mean graft length = 8.7cm, range 4-12) and neurolysis (17%, 6/35). Patients were younger in the grafting group: 24 years (s.d. 11) versus 31.5 (s.d. 17) and 49 (s.d. 22) in the transfer and neurolysis groups, respectively (p < 0.05). At a mean follow-up time of 22 months, average post-operative MRC scores were not significantly different between the transfer (3.4, s.d. 1.3) and the nerve graft (3.9, s.d. 0.5) and neurolysis groups (4.5, s.d. 1). DASH scores were significantly lower following interposition grafting compared with nerve transfer (3.9 versus 23) with no difference in shoulder abduction between groups. Successful outcome (MRC ≥ 3) was seen after one or more years in 100% of neurolysis and nerve graft patients and only 67% of nerve transfer patients; however this finding did not reach statistical significance (p = 0.058).

Conclusions: This is one of the larger series of isolated axillary nerve injuries. Despite being the most commonly used surgical intervention, triceps motor branch transfer does not demonstrate improved functional outcomes having the lowest average post-operative MRC score, proportion of successful outcomes (MRC ≥ 3) and significantly greater DASH scores. The main limitation of this study is the small number of interpositional graft patients; however, these data suggest that this technique results in similar and potentially improved functional outcomes over nerve transfer for isolated axillary nerve injury.
31. Motor and Sensory Donor Axons Growing Across Two End-to-side Neurorrhaphies Through an Autograft ‘Protect' Chronically Denervated Schwann Cells to Improve Nerve Regeneration After Delayed Nerve Repair in Rats

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Introduction: End-to-side neurorrhaphy where a transected nerve is repaired to the side of an intact nerve may circumvent long delays for nerve regeneration through long autografts or artificial conduits\(^1\). Side-to-side bridging with two end-to-side neurorrhaphies is another option when only the distal stump is available\(^2\). The relative contributions of motor and sensory axons growing into the recipient denervated distal nerve stump have not yet been determined. Mackinnon’s group reported that very few motoneurons regenerated axons through an end-to-side neurorrhaphy\(^3\). We asked the questions: 1) How many motor and sensory neurons send their axons through autologous side-to-side bridges between an intact donor nerve and an adjacent chronically denervated nerve stump? 2) Do these donor nerves alleviate the negative effects of chronic Schwann cell denervation by promoting effective nerve regeneration and, in turn, target reinnervation?

Methods: 1) Common peroneal (CP) autografts were secured with Tisseel glue between 10mm lengths of a donor tibial nerve and a recipient CP distal nerve stump, three between epineurial windows and 5-9 bridges after removing the epineurium. Retrograde dyes were applied ~6mm proximal and distal to the bridges for enumeration of sensory and motor neurons that grew axons into the 3 month chronically denervated CP nerve stump. 2) Transected CP nerve stumps were opposed for surgical repair. Motor and sensory neurons that had regenerated their axons after 5 months were enumerated and isometric force recordings were made to count the motor nerves that reinnervated muscle.

Results: 1) Motor and sensory neurons regenerated axons in equal numbers from a donor tibial nerve across 1-9 bridges both proximal and distal to the bridges in the recipient chronically denervated CP nerve stump. 2) Three but not more bridges significantly increased regeneration of motor and sensory CP nerves after delayed CP nerve coaptation. The numbers of CP motoneurons that regenerated their axons increased 3-fold and fully reinnervated flexor muscles with complete recovery of their mass.

Conclusions: Both motor and sensory nerves grow through bridges toward recipient denervated nerve stumps. They effectively ‘protect’ chronically denervated Schwann cells to significantly improve sensory regeneration and promote regeneration and muscle reinnervation of all motoneurons. Partial occupancy of chronically denervated nerve stumps with donor axons promotes a growth permissive state, possibly via neuregulin signaling.

32. Non-Invasive Ultrasound as a Longitudinal Tool for Analysis of Nerve Regeneration
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Introduction: Functional recovery after nerve trauma can be measured using various techniques. Today’s golden standard in experimental studies is the muscle mass ratio, which is invasive and requires a sacrificial procedure. We propose a new, innovative, non-invasive method to obtain the muscle atrophy with the use of ultrasound. The reliability and validity of obtaining the muscle atrophy by ultrasound was already established for the gastrocnemius muscle. This study takes the next step in creating this new method: developing the ultrasound evaluation techniques for smaller muscles in the leg. Secondly, the ultrasonographic findings will be correlated and compared with the actual force. Lastly, the innervation of the muscle was studied with histology.

Methods: For this experiment fifty rats underwent a 10 mm autograft sciatic nerve reconstruction. With a two-week interval, 5 animals where tested with a total follow up period of 20 weeks. The functional recovery of the muscles of the hind limb measured with the new innovative and non-invasive ultrasound was compared with the muscle mass ratio, today’s most widely accepted assessment technique in this field, and the isometric tetanic force. In addition, neuromuscular junctions were histologically analyzed to study the innervation on the muscle level. The different evaluation techniques were compared using correlation analysis and the inter-rater reliability of the ultrasound assessment was determined.

Results: Four weeks after denervation the muscle showed extensive atrophy resulting in a decrease of muscle mass up to 30%. In the following weeks the muscle mass recovered, but it reached not higher than 75% of the original values. The ultrasound showed good correlations with the muscle mass ratio for both the tibial (r=0.85) and gastrocnemius muscles (r=0.89). The interrater reliability of the ultrasound of the tibial muscle showed a good correlation of r=0.88. The correlation of ultrasound with the isometric tetanic force was found to be 0.62.

Discussion & Conclusion: This study shows a new non-invasive method to analyze the functional recovery of the rat after nerve reconstruction. Ultrasound proves to be a valid and alternative method to obtain the muscle atrophy for both the gastrocnemius and tibial muscle. Compared to the golden standard, muscle mass ratio, ultrasound shows high correlations. Analyzing isometric tetanic force, ultrasound shows a lower, but still significant correlation, as compared to weight. We propose that ultrasound can be used as an alternative of muscle mass ratio to obtain study muscle atrophy after nerve injury in a less invasive, animal friendly manner.
33. Migraine Headache as a Novel Risk Factor for Carpal Tunnel Syndrome

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**Introduction:** Compression neuropathies have been associated with one another, and migraine headaches are being successfully treated by nerve decompression. The goal was to evaluate whether an association exists between migraine headache and carpal tunnel syndrome (CTS), the most common compression neuropathy.

**Materials and Methods:** Data from the cross-sectional 2010 National Health Interview Survey Sample Adult module were used to calculate nationally-representative prevalence estimates and 95% confidence intervals of CTS and migraine headache. Multivariate logistic regression was used to calculate adjusted odds ratios (aOR) and 95% confidence intervals (CI) for the degree of association between migraines and CTS controlling for known demographic and health-related factors. Analysis was restricted to adults at least 18 years old, to those with complete data for all variables, and to those of race/ethnicity groups large enough to be included in regression models.

**Results:** Of 27,157 respondents, 25,880 (95.3%) were included in the analysis. CTS was associated with older age, female gender, obesity, diabetes, and smoking. CTS was less common in Hispanics and Asians. Migraine headache was associated with younger age, female gender, obesity, diabetes, and current smoking. Migraine headache was less common in Asians. Migraine prevalence was 34% in those with CTS, compared to 16% in those without CTS (aOR 2.60, 95%CI 2.16-3.13). CTS prevalence in patients with migraine headache was 8%, compared to 3% in those without migraine headache (aOR 2.67, 95%CI 2.22-3.22).

**Conclusions:** Migraine headache is associated with carpal tunnel syndrome in a nationally-representative database. Because migraine headache is more prevalent at younger ages, and CTS more prevalent at older ages, migraine headache may serve as a predictor of developing future CTS. Identification of those with migraine headache may allow for earlier diagnosis, treatment, or even prevention, of CTS. Longitudinal and genetic studies with physician verification of migraine and CTS are needed to further define this association.
34. Latissimus Dorsi Tendon Transfer to Restore shoulder Abduction; A New Technique
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Shoulder abduction is an essential movement for placement of the human hand in space and thus for upper limb function. The deltoid muscle functions as the main shoulder abductor. In our study we describe a new technique to compensate for loss of deltoid function using a latissimus dorsi tendon transfer augmented with an ipsilateral semitendinosis tendon graft. The tendon graft is passed over the acromion and attached to the deltoid insertion. Postoperatively a shoulder spica in 90° abduction was used for six weeks followed by a removable abduction brace for another 6 to 8 weeks. This technique was used in 5 case of loss of deltoid function. Two of which were isolated axillary nerve injuries while the other 3 were in patients who had a C5 root injury and were treated with a nerve transfer which was unsuccessful in restoring deltoid muscle function. All patients regained active shoulder abduction which averaged 98° (85° to 118°) and were satisfied with the outcome of the procedure. The strength of abduction was measured and compared to the contralateral normal side using a hand-held dynamometer. The average strength regained was 62% of the normal side. We believe that this procedure can be a useful technique for restoration of a significant range and strength of active shoulder abduction.
35. Morphometric and Functional Analysis of Axonal Regeneration after End-to-End and End-to Side Neurorrhaphy in Rats
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Background: End-to-side neurorrhaphy is controversial in the literature, with discussion about the degree of recovery. In this study, nerve regeneration was assessed in rats after end-to-side neurorrhaphy by morphometric analysis, electromyography, electron microscopy and retrograde horseradish peroxidase (HRP) and Fluoro-gold® (FG) transport, and compared to end-to-end neurorrhaphy and sham operation.

Methods: 37 animals were operated and divided randomly into four groups: group 1 - sham, group 2 - end-to-end neurorrhaphy, group 3 - end-to-side neurorrhaphy with an epineural window (w/w), and group 4 - end-to-side neurorrhaphy without an epineural window (wt/w). Three months after surgery, HRP was injected in the peroneal muscles. After 48 hours, nerve segments and lumbar spine segments were collected. Electromyography data were compared between groups and FG uptake was compared in 20 other animals. Analysis of variance (ANOVA) with Tukey-Kramer correction was used for group comparison.

Results: The fiber count after end-to-end neurorrhaphy was higher than after end-to-side neurorrhaphy w/w (q = 5.243 and p <0.01) or wt/w (q = 4.951 and p <0.01). The sham group had a higher perimeter than group 3 (q = 7.211 and p <0.001) and group 4 (q = 6.971 and p <0.001). HRP labeling showed a difference between group 2 and end-to-side neurorrhaphy w/w (q = 5.291 and p <0.01) and wt/w (q = 5.617 and p <0.01). There was also a difference in mean area labeled with FG. Furthermore, the amplitudes of the action potentials were significantly higher in groups 1 and 2.

Conclusions: There was nerve regeneration in all groups studied. However, the end-to-end neurorrhaphy group had better reinnervation than the end-to-side neurorrhaphy groups
36. Signals from Adjacent Antagonistic RPNIs are Independent during Voluntary Walking
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Introduction: Regenerative Peripheral Nerve Interfaces (RPNIs) are neurotized muscle grafts that transduce peripheral nerve action potentials to electrical signals suitable for prosthesis control. Each RPNI controls a single movement. At least two independent RPNIs are required for agonist/antagonist control; however, it is unknown whether signals from adjacent RPNIs are independent. Our purpose was to determine whether two, adjacent RPNIs, with one reinnervated by a foot dorsiflexor nerve and the second reinnervated by a foot plantar-flexor nerve provide independent activation.

Methods: For RPNI group rats (n=2), two muscles were grafted to the left thigh and implanted with electrodes; one was neurotized with the transected tibial nerve, the other by the transected peroneal nerve. Control rats (n=2) had bipolar electrodes implanted onto soleus and extensor digitorum longus left leg muscles. Rats walked on a treadmill, were videographed and raw electromyography (EMG) signals were recorded. Rectified EMG was integrated, normalized to time, and expressed as percent of total stepping cycle activity for each stance and swing. Data are 16 stepping cycles for each rat.

Results: EMG activity for RPNI and Control rats displayed alternating patterns of activation during stance and swing (Fig 1). We found significantly greater tibial nerve activity during stance compared to swing and greater peroneal nerve activity during swing compared to stance for each nerve in RPNI and Control rats (p<0.00001) (Fig. 2). Out of 32 stepping cycles both RPNIs were active when expected 31 times—96.9% sensitivity (Fig. 2). Independence was found for RPNI activation by comparing tibial nerve reinnervated muscle activity during stance to peroneal nerve reinnervated muscle activity during stance. The strength of the significant difference indicates signaling is independent (p<0.00001). The same significant difference was found for tibial nerve reinnervated RPNI swing compared to peroneal nerve reinnervated RPNI swing (p<0.00001). Independent activity was also found for the Control group muscles.

Conclusion: Two, adjacent RPNIs, peroneal nerve reinnervated RPNI muscle and tibial nerve reinnervated RPNI muscle, were found to independently activate during quadripedal stepping cycles. These data support RPNI independence and the feasibility of using RPNIs to provide agonist/antipodal control signaling for myoelectric prostheses.

Acknowledgment: DARPA (N66001-11-C-4190).
Figure 2. Summarized and individual gait phase EMG activity represented as a percent of total stepping cycle activity. (A) Bars represent average gait phase EMG activity. Each bar is a mean ± 1 SD value of 32 gait cycles from two rats within each group. Stance and swing values were different (p<0.00001) for each nerve within both groups. Tibial n. stance EMG activity was significantly higher (p<0.00001) than swing activity for both Control and RPNi. Peroneal n. activity was significantly higher during the swing gait phase (p<0.00001) compared to the stance gait phase for both groups. During the stance phase, tibial n. activity was higher (p<0.00001) compared to peroneal n. activity for both groups. During the swing phase, peroneal n. activity was higher (p<0.00001) compared to tibial n. activity for both groups. (B-E) Individual gait phase data from 16 gait cycles per rat. Stacked bars present total gait cycle EMG activity subdivided to stance and swing gait phases. Red line marks 50%. Both RPNi were active (majority of signaling occurring in expected gait phase) when expected, according to Control, for 31/32 gait cycles. (B) Control—tibial n. (C) Control—peroneal n. (D) RPNi—tibial n. (E) RPNi—peroneal n.

Fig. 2 (A) Brackets above bars indicate significant differences (Paired t-test within group, p<0.00001; or independent t-test between groups, p<0.00001).
**ASPN/ASRM Combined Scientific Session**

**ASPN #1 Clinical and Cytogenetic Study in Patients with Möbius Syndrome at the General Hospital**
Manuel Gea González, MD¹; Pablo Arrieta-Joffe, MD¹; Marcia Perez-Dosal, MD¹; Gabriela Ortíz de Zarate, MD²; Alexander Cardenas-Mejia, MD¹

¹Plastic and Reconstructive Surgery, Hospital General "Dr. Manuel Gea González", Mexico City; ²Genetics, Hospital General, Mexico City

**Introduction:** Möbius syndrome is a rare congenital disease characterized by facial paralysis associated with an absence of abduction of the eyes for abnormalities in VI and VII cranial nerves. The pathogenesis has different hypotheses that include genetic, vascular, and teratogenic causes. There are few reports in the literature and especially in Latin America to describe the clinical and genetic characteristics of these patients.

**Methodology:** We analyzed 92 patients with the diagnosis of Möbius syndrome in its 3 presentations. All patients underwent a complete clinical examination by a multidisciplinary team formed by orthopedist, ophthalmologist, otolaryngologist, orthodontist, neurologist, plastic surgeon, pediatrician, and geneticist. They underwent CTG banded karyotype to identify structural chromosome abnormalities.

**Results:** Thirty-nine patients (42%) were male and 52 (58%) female. Clinical manifestations were found with unilateral or bilateral facial paralysis with VI nerve involvement in 100% of patients, associated with strabismus in 58.8%, 35.29% clubfoot, simple syndactyly 19.61%, 13.73% cleft palate, micrognathia 13.73%, Poland syndrome 11.7%, among others. Cytogenetic analysis showed normal karyotype in 91 patients and a reciprocal translocation between chromosome 4 and 10 in one patient. Eleven cases of reported intake of misoprostol during the first trimester.

**Conclusions:** So far this study is the largest global cohort reported in a single hospital of patients with Möbius syndrome. Variability of the clinical presentation justifies the management of these patients as a multidisciplinary team. This study opens the door for new studies that allow us to understand the pathophysiology of this disease and its response to different treatments. Keywords: Moebius syndrome, Möbius syndrome, facial nerve, congenital facial palsy, abducens nerve.
Corneal Reinnervation: A Minimally Invasive Approach

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Division of Plastic and Reconstructive Surgery, University of Toronto, Toronto, ON, Canada

Introduction: Corneal sensation is a necessary component of corneal maintenance. Corneal anesthesia renders the cornea susceptible to non-healing epithelial ulceration, scarring and perforation. Previously, unilateral corneal reinnervation has been achieved with direct neurotization using the contralateral supraorbital and supratrochlear nerves. However, this technique requires an extensive dissection and bicoronal incision. We present the early results of a less invasive corneal neurotization technique in young patients.

Materials & Methods: An end-to-side coaptation was performed from the donor supratrochlear nerve to a sural nerve graft. The graft was tunneled subconjunctivally to the perilimbal area and draped around the cornea. Corneal anesthesia was evaluated preoperatively and postoperatively in the center of the cornea and in four corneal quadrants using a Cochet-Bonnet esthesiometer.

Results: Preoperatively, all corneas sustained complications related to corneal anesthesia and lacked any detectable sensation preoperatively. Five eyes underwent the procedure (aged 9-34 years), with one patient undergoing bilateral reconstruction. Figure 1 shows the time progression of the esthesiometry measurements in the four pediatric eyes completed to date, with A and B representing the patient with bilateral reconstruction. A score of 60 is considered indistinguishable from normal, and a score of 15 is required for protective corneal sensation. No complications occurred postoperatively. The adult patient, aged 34, is demonstrating improved corneal sensation 6 months post-operatively.

Conclusions: Corneal sensory reconstruction restores corneal sensation and ocular defense mechanisms, protecting vision in patients with previously anesthetic corneas. This can be achieved with minimal morbidity using sural nerve grafts and this novel method avoids the cosmetically objectionable bicoronal scar. This is the first report of restoration of corneal sensation using a sural nerve graft for unilateral and bilateral anesthetic corneas. Furthermore, this technique may enable future corneal transplantation in patients with irreversible corneal scarring.

Figure 1.
Impact of Decellularized Small Intestinal Submucosa on Regeneration, Reinnervation, and Revascularization of Freely Transferred Muscle

Frances M. Walocko, MSE; Elizabeth A. Mays, BSE; Cheryl A. Hassett, BS; Jana D. Moon, BS; Nicholas B. Langhals, PhD; Paul S. Cederna, MD; Melanie G. Urbanchek, PhD

1Department of Surgery, Section of Plastic Surgery, University of Michigan, Ann Arbor, MI; 2Plastic Surgery, University of Michigan, Ann Arbor, MI

Introduction: To improve reliability of myoelectric prosthetic devices, we developed the Regenerative Peripheral Nerve Interface (RPNI) for neuroprosthetic control. In our standard model, decellularized small intestinal submucosa (SIS) secures an epimysial electrode to the RPNI. However, RPNI viability and signal transduction require muscle regeneration, reinnervation and revascularization, and SIS may affect these essential processes. We determined the effects of SIS on RPNI viability by comparing muscle fiber regeneration, reinnervation, and revascularization of freely transferred muscle wrapped in SIS to those without SIS.

Materials & Methods: Rats were assigned to Standard RPNI (n=6) or RPNI without SIS (n=6) groups. RPNIs were surgically constructed with autologous unilateral, free muscle transfer to the ipsilateral upper thigh using the extensor digitorum longus (EDL) muscle. The proximal end of the divided peroneal nerve was implanted into the muscle. The EDL muscle was then either wrapped in SIS (Standard RPNI) or not (RPNI without SIS) (Figure 1). Three months postoperatively, RPNI compound muscle action potential (CMAP), force, fatigability, and histology were assessed.

Results: All rats survived the surgery and did not differ in age or body mass. RPNIs without SIS had 42% increased CMAP amplitude and 52% decreased rheobase (minimal current required to reach depolarization threshold), illustrating increased muscle reinnervation (Table 1). Although RPNIs without SIS produced similar maximal forces, surprisingly, they recovered 80% of maximal force after 20-minutes of fatiguing work, while Standard RPNIs only recovered 50% (Table 2). Thus, fatigue testing exposed a previously hidden limitation of the SIS RPNI wrap. The 8.9% greater muscle mass seen in RPNIs without SIS may also indicate enhanced regeneration, reinnervation, and revascularization. Additional RPNI testing, histology, and statistical analyses are in progress.

Conclusion: RPNIs without SIS have improved RPNI muscle viability and signal production during both brief and continuous CMAP activation when compared with Standard RPNIs. With study completion, we shall present complete SIS impact results for regeneration, reinnervation, and revascularization of freely transferred muscle.

<table>
<thead>
<tr>
<th>Table 1: Summary of Evoked CMAP Data</th>
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<tr>
<td><strong>Standard RPNI</strong></td>
</tr>
<tr>
<td>Number of rats evaluated to date</td>
</tr>
<tr>
<td>Body mass, mg</td>
</tr>
<tr>
<td>Rheobase, mA</td>
</tr>
<tr>
<td>Latency, msec</td>
</tr>
<tr>
<td>Amplitude, mV</td>
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<tr>
<td>Velocity, m/s</td>
</tr>
<tr>
<td>CMAP area, mV/ms</td>
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<tr>
<td>CMAP duration, msec</td>
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</tbody>
</table>

Values presented are means ± 1 standard deviation. Abbreviations: mg, milligrams; mA, milliamperes; msec, milliseconds; mV, millivolts; m, meters; s, seconds.

<table>
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<tr>
<th>Table 2: Muscle Force and Fatigue Testing</th>
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<tr>
<td><strong>Standard RPNI</strong></td>
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<tr>
<td>Number of rats evaluated to date</td>
</tr>
<tr>
<td>Maximum isometric tetanic force, mN</td>
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<tr>
<td>Force recovered after fatigue, mN</td>
</tr>
<tr>
<td>Percent force recovered after fatigue</td>
</tr>
<tr>
<td>Wet muscle mass, mg</td>
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<tr>
<td>Muscular cross-sectional area, mm²</td>
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Values presented are means ± 1 standard deviation. Abbreviations: mN, millinewtons; mg, milligrams; mm, millimeters.
Purpose: Contralateral C7 (CC7) transfer for brachial plexus injuries (BPI) can benefit finger sensation but remains a controversial technique due to a wide variation in motor results. We reported our 20-year experience of using CC7 transfer for BPI. All of which had at least 4 years follow-up.

Patients and Methods: 137 adult BPI patients underwent CC7 transfer from 1989 to 2006. Out of these patients, 101 fulfilled the inclusion criteria for this study. All surgeries were performed by a single surgeon. A vascularized ulnar nerve graft (VUNG), either pedicle or free, was used for CC7 elongation. The VUNG was transferred to the median nerve (Group I, one target) in 55 patients, and to the median and musculocutaneous nerves (Group II, two targets) in 23 patients. In another 23 patients (Group III, two targets, two stage), the CC7 was transferred to the median nerve (17 patients) or to the median and musculocutaneous nerve (6 patients) during the first stage, followed by functioning free muscle transplantation (FFMT) for flexor digitorum profundus restoration for finger flexion.

Results: Finger flexion strength $\geq$ M 3 was considered a successful functional result.

The success rates of CC7 transfer were 54.50%, 39.10% and 74% for Group I, II, and III respectively. Additionally, the success rate of elbow flexion (strength $\geq$ M3) in Group II was 83%.

Conclusion: In reconstruction of total brachial plexus root avulsion, adopting the Group III technique of using CC7 transfer to the musculocutaneous and median nerve, followed by FFMT in the early stage (<18 months) for finger flexion may be the best option. Such technique can potentially improve motor outcome of elbow and finger flexion in a shorter rehabilitation period (3-4 years), and most importantly provide finger sensation to the completely paralytic limb.
ASRM #2 Rectus Abdominis Motor Nerves as Donor Option for Free Functional Muscle Transfer
Aaron Mull, MD; Michael Nicoson, MD; Thomas Tung, MD
1Washington University School of Medicine; 2Kleinert and Kutz Hand Associates

Background: To evaluate rectus abdominis motor nerves as potential donor nerve options for free functional muscle transfer in the reconstruction of brachial plexus injuries.

Methods: Four human cadavers were obtained and dissected to find intercostal nerves 3-8, the thoracodorsal nerve, and medial pectoral nerves. Biopsies were taken for histomorphometric analysis. The intercostal nerves were biopsied at the midaxillary line, anterior axillary line, and midclavicular line.

A retrospective chart review was performed from 2001-2013 to include all free functional muscle transfers by a single surgeon in the same institution. Of the 34 total patients identified, 5 were eliminated for restoration of finger flexion only, 2 were eliminated because they were not performed solely by the senior author, 1 was eliminated as the patient had two separate donors, 2 were eliminated because the patients had non-standard nerve transfers, and 1 was eliminated for short follow up time.

Results: Preliminary results from two cadavers demonstrate average nerve counts for lower intercostal+rectus abdominis (7-8) at the midaxillary, anterior axillary, and midclavicular line were 5554, 5124, and 2465, respectively. For higher intercostal nerves (3-6), the average nerve counts at the midaxillary, anterior axillary, and midclavicular line were 2250, 1432, 1186, respectively. The average nerve count for medial pectoral nerves and anterior branch of the thoracodorsal nerve was 2718 and 4520, respectively.

Nine patients were lost to follow up or not enough time had elapsed since reconstruction. Of the remaining 14 remaining, the average recovery of elbow flexion was MRC grade 3/5 for high intercostal nerves (1 patient), 3.3/5 for lower intercostal + rectus abdominis nerves (5 patients), and 2.6/5 for distal accessory nerve (5 patients). Each of these required two stages with a nerve graft. Postoperative elbow flexion for medial pectoral nerve donors was 4/5 (2 patients), and for anterior branch of thoracodorsal nerve was 4/5 (1 patient). These were single stage operations.

Conclusions: Rectus motor nerves have more nerve counts than thoracodorsal, medial pectoral nerves, and more than double the nerve counts of intercostal nerves. The use of rectus abdominis motor nerve branches restores elbow flexion that is comparable to intercostal nerves and distal accessory nerve as donors for a staged reconstruction requiring a long nerve graft. In cases where multiple intercostal nerves are not available as donors (rib fractures, phrenic nerve injury), rectus abdominis nerves provide an option for motor reconstruction without adversely affecting respiration.
ASRM #3 The TRAIL Flap: Bridging the Gap Between Breast Reconstruction and Cancer Treatment via a Gene Therapy Flap
Mauricio De la Garza; SD Mendenhall; CE Harrison; La Cox; NM Cosenza; Jd Reichensperger; MW Neumeister
Southern Illinois University School of Medicine

Introduction: Breast cancer is a global problem with an incidence increasing from 1 in 11 women in 1975 to 1 in 8 today. The rat superficial epigastric flap has been proposed to deliver gene therapy. Tumor necrosis factor-related apoptosis inducing ligand (TRAIL) has been shown to have selective apoptosis-inducing effects in cancer cells with long-term tumor-free survival and without causing damage to normal cells. Our research proposes a treatment to safely eradicate human breast cancer using a combination of TRAIL gene therapy with a traditional autologous flap technique similar to what is currently used in human breast reconstructive surgery.

Methods: Therapeutic efficacy of adenoviral vector expressing green fluorescent protein (Ad/g-TRAIL) over luciferase-tagged (l-t) MDA-MB-231 human breast cancer cells was tested in vitro and in vivo and compared to controls. In vitro paracrine effect of TRAIL was tested via transfection of fibroblasts on a transwell. Annexin V-affinity assay using flow cytometry evaluated apoptosis. A luminometer multi-detection system quantified l-t MDA-MD-231 expression of luciferase. A TRAIL Human ELISA Kit measured tissue TRAIL expression. In vivo tumor mass formation was evaluated following subcutaneous injection of l-t MDA-MD-231 human breast cancer cells within a female nude rat superficial epigastric flap model. Eradication of breast cancer was analyzed following TRAIL transduction. A Xenogen in vivo bioluminescence imaging system assessed the tumor mass evolution by transcutaneous quantification of luciferase expression. Microscopic cellularity was assessed with hematoxylin and eosin (H&E) stains as well as with DAPI on immunofluorescence.

Results: In vitro, l-t-MDA-MB-231 cells underwent apoptosis when treated with Ad/g-TRAIL virus in contrast to the control cells that continued to replicate. A paracrine apoptotic effect by TRAIL was confirmed. Apoptosis rates correlated directly to amount of TRAIL expression and indirectly to remaining viable l-t-MDA-MB-231 tumor cells. In vivo, macroscopic formation of a tumor mass was grossly noticeable and quantifiable via bioluminescence days after injection of l-t-MDA-MB-231 into the flap. The in vivo tumoricidal effects of TRAIL showed decreasing transcutaneous quantification of luciferase expression. This correlated with a significantly smaller tumor mass on gross measurements. H&E and DAPI immunofluorescence confirmed decreased cellularity on flaps treated with TRAIL when compared to control.

Conclusion: Human breast cancer can be safely eradicated following transduction of TRAIL gene therapy on a rat superficial epigastric flap that resembles human breast reconstructive surgery.
37. Use of Nerve Allograft in Targeted Motor Reinnervation
Peter S. Wu, MD, MSc ; Brian T. Carlsen, MD
Division of Plastic Surgery, Mayo Clinic, Rochester, MN

Introduction: Targeted motor reinnervation (TMR) is a relatively new approach to improving function after upper limb loss. TMR involves surgically re-routing divided nerves to defunctionalized muscles in the residual limb for the purpose of generating electromyographic signals for prosthetic control. Commercially available nerve allograft has been successfully used to bridge nerve gaps, but its use in TMR has not been described. We present a case of a transhumeral amputee who successfully underwent a targeted motor reinnervation procedure using nerve allograft.

Materials/Methods: A 50-year old, right hand dominant woman presented several months after a left transhumeral amputation from a rollover automobile crash. The patient is a nonsmoker, an avid outdoorsman, and uses her hands extensively as an engineer. She had previously undergone amputation revision at an outside facility. Pre-operatively Tinel’s signs were used to localize signals corresponding to median, ulnar and radial innervation. Her biceps and triceps muscles were noted to be present and intuitively functional. A large posterior skin flap was used to cover an anterior defect presumably due to the obliquity of the amputation and loss of anterior soft tissue. The patient underwent TMR of her radial nerve to the long head of the triceps for intuitive hand open signal. Anteriorly, it was recognized intra-operatively that the nerve transection was very proximal (at the level of the medial cord) and there was insufficient length to reach the short head of the biceps. An interpositional human nerve allograft (2-3mm x 3cm Avance. Axogen Inc., Alachua, FL) was used to bridge the nerve gap to the motor branch of the short head of the biceps. Due to a size mismatch, the excess fascicles of the graft were directly implanted into the muscle.

Results: Six months following this procedure, the patient demonstrated voluntary muscle contractions in the distributions of the radial and median nerves consistent with their motor reinnervation. The patient was subsequently able to use a left upper extremity myoelectric prosthesis.

Conclusions: Although the use of patient autograft is traditionally the preferred conduit for motor nerve reconstruction for segmental nerve injury, our patient successfully underwent targeted motor reinnervation with allograft. Further study is needed to determine whether clinical differences exist between autograft and allograft for the purposes of TMR, however we demonstrate that nerve allograft is a feasible option if autograft is unavailable.
38. Tissue Engineered Nerve Grafts Serve as a Living Scaffold to Facilitate Regeneration and Functional Recovery Following a 5 cm Nerve Lesion in Swine

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1Neurosurgery, University of Pennsylvania, Philadelphia, PA; 2Axonia Medical, Inc, Kalamazoo, MI

Surgical repair strategies following peripheral nerve injury (PNI) are currently inadequate, especially following major lesions involving the loss of segments several centimeters in length. We have developed tissue engineered nerve grafts (TENGs) that are lab-grown nervous tissue comprised of neurons spanned by long axonal tracts. Axon “stretch growth” - a natural axon growth mechanism that we replicate in custom mechanobioreactors - is used to rapidly generate long, aligned axonal tracts (5-10 cm in 14-21 days). Three-dimensional TENGs are then created by embedding the living axonal tracts in an extracellular matrix. In rodent and porcine models of PNI, we previously found that TENGs serve as a “living scaffold” to accelerate and direct host axon regeneration and Schwann cell infiltration across short nerve gaps. In the current study, TENGs were used to bridge major 5 cm lesions in the deep peroneal nerve (~20 cm from distal muscle target) in comparison to the sural nerve autograft in minipigs. We found that TENG neurons/axons survived long-term (absent immunosuppression) and directly interacted with host axons and Schwann cells. In particular, we found that TENGs accelerated acute axonal regeneration across the 5 cm lesions based on direct axon-facilitated axon regeneration, demonstrating that this new mechanism of axon regeneration that was observed in 1 cm lesions was scaled to more challenging 5 cm lesions. Remarkably, this allowed a sub-population of host axons to cross the graft within 5 weeks (averaging 1.6 mm/day) - before host Schwann cells had infiltrated the graft. By 3 months following TENG repair, the bulk of host axons had crossed the graft and there was a reconstituted compound nerve action potential. Initial muscle reinnervation and evoked hoof twitch were achieved by 7 months following TENG repair, and at this time the repaired nerve contained a high density of myelinated host axons. Currently, the extent of functional recovery and regenerated nerve morphometry are being compared between TENGs and sural nerve autografts at 9-12 months post-repair. These results demonstrate that our unique tissue engineering strategy accelerated regeneration based on axon-facilitated axon regeneration, which recapitulates an axonal pathfinding mechanism used during embryonic development and allowed for an initial bolus of axon regeneration to occur independent of host Schwann cells. Overall, TENGs enabled rapid axon regeneration, target reinnervation and functional recovery when used to bridge a challenging 5.0 cm nerve lesion, demonstrating the efficacy of TENGs to facilitate nerve regeneration following major PNI.
39. Spectrochemical Analysis of Peripheral Nerve Myelin for use in Living Biological Systems
Joey Grochmal, MD; Wulin Teo; Hardeep Ghambir, PhD; Ranjan Kumar; Peter K. Stys, MD; Rajiv Midha, MD, MSc, FRCS(C)
Neuroscience, University of Calgary, Calgary, AB, Canada

Introduction: Current experimental techniques for the chemical analysis of peripheral nerve myelin are not well applied in living biological systems. We introduce the technique of myelin analysis using spectral confocal fluorescence microscopy of the solvatochromic myelin-incorporated dye Nile Red, and then apply our method to the study of myelination by Schwann cells and Schwann cell-like stem cells (Skin-derived precursor Schwann cells) both in vitro and in-vivo.

Hypothesis: Our hypothesis is that myelin conferred by SKP-SCs and SCs will reliably move through states of progressive chemical maturity as the tissue gradually approaches an adult phenotype, and that this progression can be characterized by Nile Red emission spectra in living systems.

Methods: In vitro analysis- We co-cultured BFP transduced SCs and SKP-SCs with isolated Thy-1 GFP DRG explants. Cell derived myelin was stained with Nile Red and imaged at sequential time points with spectral confocal microscopy, then analyzed using ImageTrak spectral analysis software (Peter Stys).

In-vivo analysis- GFP positive SCs and SKP-SCs were injected into the sciatic nerves of Lewis rats 9 days post doxorubicin injury (focal demyelination injury). Rats were sacrificed at 12,15,18,21, and 24 days post injection and sciatic nerves were fixed, frozen, and sectioned (4 nerves/cell group/time point). SKP-SC and SC Nile Red stained myelin was identified on cross-sections with circumferential GFP co-localization, and imaged and analyzed as above.

Live rodent analysis- The above experimental paradigm was repeated using BFP transduced SCs and SKP-SCs, and live sciatic nerve imaging (SD Thy-1 GFP rat) was performed sequentially following topical exposure to 50uM Nile Red at 15,21, and 27 days post cell-injection. Cell-derived myelin figures were identified and analyzed as above.

Results: All three experimental paradigms demonstrate that myelin polarity changes in a reliable fashion with progressive maturity, irrespective of paradigm or cell type used. Preliminary in-vitro data suggests that these changes may in part reflect interaction of immature myelin with cytoplasmic lipid-rich vacuoles. Preliminary results also suggest that SKP-SC conferred myelin may progress to chemical maturity faster than SC myelin.

Conclusions: PNS myelin analysis by Nile Red emission spectra can yield spectro-chemical information regarding myelination in living systems, while Nile Red can function as a non-toxic probe for live cell and animal imaging. Sequential and reliable changes in NR emission patterns with progressive myelin maturity can be used to measure myelination "velocity" by SC grafts, while single cell imaging yields novel clues as to the cellular machinery of myelin formation.
40. Daily Electrical Muscle Stimulation Enhances Functional Recovery and Upregulates Muscular Brain Derived Neurotrophic Factor (BDNF) Following Nerve Transection and Repair in Rats
Michael P. Willand, PhD; Jennifer J. Zhang; Cameron D. Chiang; Elyse Rosa; Stephen WP Kemp; Margaret Fahnestock; Gregory H. Borschel; Tessa Gordon
1Division of Plastic and Reconstructive Surgery, The Hospital for Sick Children, Toronto, ON, Canada; 2Department of Psychiatry & Behavioural Neurosciences, McMaster University, Hamilton, ON, Canada

Introduction: The use of chronic electrical muscle stimulation for treating partially or completely denervated muscle is controversial. Recently, we used a daily electrical muscle stimulation paradigm over a two week period after nerve injury and immediate repair. We showed that muscle stimulation significantly increases the number of motor nerves reinnervating muscles and axon outgrowth within the distal nerve stump. Activity-dependent intramuscular trophic factor release acts on regenerating axons, which may explain the increased early regeneration in stimulated muscle. However, chronic electrical muscle stimulation applied throughout the entire reinnervation period has not been previously assessed. In the present study we hypothesized that stimulation would enhance functional recovery over three months and that stimulation enhances early intramuscular trophic factor release.

Methods: Six groups of Thy1-GFP transgenic male rats underwent tibial nerve transection and immediate repair using two epineurial sutures. One group of rats underwent daily electrical muscle stimulation of the gastrocnemius with a paradigm producing 600 equally separated contractions throughout one hour, delivered 5 days per week. Rat gastrocnemius muscles were electrically stimulated for 1, 2, or 3 months and then we assessed muscle force, contractile properties, motor unit numbers, and wet muscle weight. Rats in the 3 month group were serially evaluated using a tapered beam test to evaluate skilled locomotion. Muscles underwent immunohistological examination of motor end plate reinnervation. Two additional groups of rats were subjected to the same nerve injury and were used to investigate early intramuscular trophic factor release following two weeks of electrical muscle stimulation or no treatment.

Results: The number of motor units was significantly increased after daily muscle stimulation at all three time points (1, 2, and 3 months). Mean motor unit sizes were significantly smaller in stimulated muscles, suggesting that muscle stimulation may inhibit terminal sprouting as reported by others. This may allow for a more natural course of reinnervation resulting in improved functional recovery. Indeed, skilled locomotion tests showed that stimulated muscles enhanced and maintained recovery at levels no different than normal functioning rats, whereas non-stimulated controls became progressively worse and did not recover to baseline. After two weeks of stimulation, BDNF was significantly upregulated in stimulated muscle compared to non-stimulated muscle.

Conclusions: Treatment of denervated muscle using electrical stimulation significantly enhanced muscle reinnervation, and upregulation of BDNF may explain this enhancement. As the muscle continues to reinnervate, tailoring the stimulation paradigm to improve muscle force and fatigability may further enhance muscle recovery.
Paired Regenerative Peripheral Nerve Interfaces Predict Rat Limb Locomotion
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1Plastic Surgery, The University of Michigan, Ann Arbor, MI; 2Mechanical Engineering, The University of Michigan, Ann Arbor, MI

Introduction: Regenerative Peripheral Nerve Interfaces (RPNI) are neuritized autologous free muscle grafts equipped with electrodes to record myoelectric signals for prosthetic control. Signals collected from single RPNI implanted in the rat hind limb display strong relationships to joint angles during walking. However, myoelectric prosthesis controllers need at least two signals to produce agonist – antagonist paired actions that control and limit movement. Our purpose is to determine the predictability of hind limb joint angles from dual RPNI myoelectric activity during walking.

Methods: Two groups of three rats each were experimentally formed. In the Control group, patch electrodes were positioned on the left extensor digitorum longus and soleus muscles. Rats in the RPNI group received two free muscle transfers to the ipsilateral left thigh and re-innervation with a proximal tibial nerve fascicle and fully transected peroneal nerve, respectively. Electrode wires were run subcutaneously to a headstage. Evaluations were performed 4-5 months post-surgery. Rats walked on a treadmill at constant pace. Synchronized videography and data acquisition were used to identify joint angles and acquire myoelectric signals. The hind limb ankle joint angle predictive capacity of the paired RPNI was assessed as a weighted linear combination of the dual RPNI myoelectric activity using multidimensional linear regression.

Results: Myoelectric signal patterns were highly repeatable within rats and within groups (Table 1). Control group myoelectric signals were periodic with observed joint angles and reflected typical agonist and antagonist activation patterns. The RPNI group signal pattern was also periodic with gait, though, as expected, signaling was lower than controls. The activation of the agonist antagonist myoelectric signals within RPNI rats exhibited a constant phase delay, proportional to walking speed. A comparison of the predicted to the observed hind limb joint angles from the myoelectric activity of the paired RPNIIs indicated consistent and accurate walking pattern generation (Figure 1).

Conclusion: This study determines that in vivo myoelectric activity of RPNI antagonistic pairs is periodic and can predict hind limb joint angles observed during walking. RPNIIs paired signal patterning, repeatability, timing, and amplitude during walking are appropriate for prostheses control algorithm development.

Acknowledgments: DARPA (N66001-11-C-4190).

| Table 1: Summary of results obtained from Control and RPNI Rat Cohorts |
|---|---|---|
| | Control | RPNI |
| Descriptives | | |
| No. of Rats Evaluated to Date | 2/3 | 2/3 |
| Weight (g) | 377 ± 3 | 381 ± 19 |
| No. of Steps Evaluated | 32 | 32 |
| Stance (% Gait) | 72 ± 7 | 75 ± 9 |
| Swing (% Gait) | 28 ± 6 | 24 ± 4 |
| Outcomes | EMG-Predicted vs Observed Ankle Joint Cross correlation (Pearson’s R) | 0.82 | 0.76 |
| Tibial vs Peroneal Muscle Graft: EMG Cross correlation (Pearson’s R) | 0.37 | 0.41 |
| EMG Activation in Stance (%) | Tibial | 65 ± 14 | 66 ± 9 |
| | Peroneal | 25 ± 8 | 24 ± 10 |
| EMG Activation in Swing (%) | Tibial | 34 ± 14 | 33 ± 9 |
| | Peroneal | 74 ± 8 | 66 ± 10 |
| EMG vs Ankle Joint Phase Delay (% Gait Time) | Tibial | 12 | 15 |
| | Peroneal | 51 | 58 |

Values are means ± SD. Abbreviations: No., number; g, grams; EMG, electromyography.
42. Effects of Electrode Presence on Regenerative Peripheral Nerve Interface (RPNI) Performance
Zachary P. French; Elizabeth A. Mays, BSE; Frances M. Walocko, BSE, MSE; Cheryl A. Hassett, BS; Jana D. Moon, BS; Nicholas B. Langhals, PhD; Paul S. Cederna, MD; Melanie G. Urbanchek, PhD
Section of Plastic Surgery, University of Michigan, Ann Arbor, MI

Background: A Regenerative Peripheral Nerve Interface (RPNI) is a piece of grafted autologous muscle that regenerates and becomes reinnervated by an implanted residual peripheral nerve. By convention, RPNIs have epimysial electrodes fixed to the muscle by a wrap of decellularized small intestinal submucosa (SIS). RPNIs rival “Targeted Muscle reinnervation” for controlling myoelectric prostheses because RPNI electrodes are in direct contact with muscle instead of skin. Our purpose is to determine whether epimysial electrodes remain stable, record signals, and allow the RPNIs to function optimally by comparison to RPNIs with no electrodes.

Methods: In rats (n=13), one extensor digitorum longus (EDL) muscle was transferred to the upper thigh and implanted with the transected peroneal nerve. Two groups were formed by either not placing (NO ELECTRODE) or placing fine wire, pad or patch electrodes (ELECTRODE) on the muscle. A wrap of SIS was placed around each RPNI. Rats recuperated for at least three months. Residual peroneal nerve stimulation was used to evaluate RPNI muscles during needle by compound muscle action potential (CMAP), muscle contractile force, and a 40 minute fatiguing protocol.

Results: At evaluation, all RPNI muscles of both NO ELECTRODE and ELECTRODE groups had a thin connective tissue layer covering the SIS while all electrodes were covered by capsules of biofilm > 1mm and 40% were dislodged or broken. Recording from implanted electrodes were inconsistent and of low quality. Despite poor conditions for the implanted electrodes, the NO ELECTRODE and ELECTRODE groups did not significantly differ in muscle mass, needle CMAP, maximal tetanic force, post fatigue tetanic force, or percent of maximum force recovered (Table 1). However, the NO ELECTRODE group produced significantly more force than the ELECTRODE group during the fatigue protocol starting at contraction number 460 out of 720 (Figure 1).

Conclusions: RPNI muscles were not adversely affected by the presence of an electrode until heavy work was performed by the muscle during fatigue producing excitation. Movement of the muscle against a stiff electrode may have damaged the RPNI muscle. Many electrodes became inoperable due to disengagement and biofouling.

Support: DARPA (N66001-11-C-4190).

Table 1. Summary of Descriptive and Force Data with p-values

<table>
<thead>
<tr>
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<th>NO ELECTRODE</th>
<th>ELECTRODE</th>
<th>Significance</th>
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<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Muscle Mass (mg)</td>
<td>220 ± 104</td>
<td>178 ± 33</td>
<td>0.34</td>
</tr>
<tr>
<td>Post-Operative Days</td>
<td>155 ± 167</td>
<td>292 ± 87</td>
<td>0.09</td>
</tr>
<tr>
<td>Maximum Tetanic Force (mN)</td>
<td>850 ± 530</td>
<td>353 ± 413</td>
<td>0.09</td>
</tr>
<tr>
<td>Max Force Post Fatigue Set 2</td>
<td>386 ± 468</td>
<td>194 ± 254</td>
<td>0.37</td>
</tr>
<tr>
<td>% of Max Force Recovered 2</td>
<td>45 ± 33</td>
<td>44 ± 32</td>
<td>0.97</td>
</tr>
<tr>
<td>Maximum CMAP Amplitude (mV)</td>
<td>2.1 ± 0.7</td>
<td>2.8 ± 3.5</td>
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Data are presented as means ± standard deviation. Comparisons were by independent t-tests with two-tailed p<0.05 considered as significant.

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Figure 1. Significantly more force (p<0.05) was produced in the NO ELECTRODE group after pulse 460 indicated by the boxes. These charts show the muscle force production throughout the fatigue protocols as a function of contraction number. The light line plot forces individually for each rat, while the dark line is a running average to which an exponential regression was fit.
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Background: A key method in evaluation of nerve regeneration is the evaluation of axon density in nerve cross sections. Formal quantification of axons is classically performed by toluidine blue staining even though this technique is considerably time-consuming and more expensive then immunohistochemical or immunofluorescence techniques that are used extensively in neuropathology. Immunofluorescence staining may have the additional advantages of differential axon count as well as fluorescent techniques of automated axon count.

Our hypothesis was that using immunofluorescent stains, evaluation of axon density is as accurate as Toluidine Blue staining.

Methods: Seven wild type rats underwent a unilateral crush injury of the sciatic nerve. The nerves were harvested at either 1 or 3 weeks post insult, including segments proximal and distal to the injury site and from the contralateral (uninjured side, designated control). Each segment was divided into two parts and fixated either for the toluidine blue (TB) staining or for 2 types of immunofluorescent staining with axon-specific antibodies (anti-NeuroFilament (NF) and anti-Protein-Gene-Product 9.5 (PGP)). Manual axon count was performed and compared between the groups using Pearson as well as Spearman’s rho correlation measures.

Results: We found a high correlation between the immunofluorescent stains and the toluidine blue for all segments including those distal to the injury. Comparing NF with TB, yielded a Pearson’s coefficient of 0.992 (p<0.001) for proximal, 0.857 (p=0.029) for distal and 0.977 (p=0.001) for control segments. Comparing PGP with TB yielded a Pearson coefficients of 0.767 (p=0.044), 0.915 (p=0.011) and 0.968 (p<0.001) for proximal, distal and control segments, respectively.

Discussion: We found that immunofluorescent staining resulted in comparable estimation of axon counts in peripheral nerve, as the toluidine blue stain, regardless of the injury. Immunofluorescence techniques may be a more affordable tool for the analysis of peripheral nerve injury and regeneration.
44. Histomorphometric Analysis of Facial Nerve Regeneration through Cross-face Nerve Grafts in Facial Reanimation Surgery
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Division of Plastic and Reconstructive Surgery, Medical University of Vienna, Vienna, Austria

**Introduction:** Facial palsy leads to functional and aesthetic impairments. An option to emotionally reanimate the paralyzed face is the use of cross-face nerve grafts. These grafts guide healthy facial nerve axons to innervate the paralyzed face, by innervating the denervated mimic facial muscle directly (one-stage procedure) or power a free muscle transplant (two-stage procedure). Biopsies of the donor nerve (healthy facial nerve), the autografts and the recipient branches were harvested for histomorphometric analysis.

**Materials and Methods:** From 2007 to 2013, 376 nerve biopsies of 84 patients, who underwent cross-face nerve grafting alone, or combined with free gracilis muscle transplantation, were harvested. 75% of patients presented with total and 25% with partial facial palsy. 57% of facial nerve deficits were complete and 43% incomplete. The most common etiologies of the facial palsy were iatrogenic/postoperative (45%), developmental (31%) and idiopathic (9.5%). Biopsies from the cross-face nerve grafting procedure (stage 1) were available in 72 patients and from the free muscle transplantation (stage 2) in 31 patients.

Nerve biopsies were fixed in a 2.5% glutaraldehyde solution, postfixed with 2% osmium tetroxide and embedded in epoxy resin. For histomorphometric analysis, the samples were assessed with a semiautomatic image-analysis system.

Statistic analysis was performed using descriptive analysis, non-parametric tests and Spearman rank coefficient. The two-sided alpha was set at 5%.

**Results:** The mean axon counts of the buccal facial nerve branch (smile) were 1826.23 (SD: 1497.7) and 576.5 (SD: 341.0) for the zygomatic branch (eye closure). The mean axon count of the sural nerve used as cross-face nerve graft on the donor side was 4575.5 (SD: 1993.8). The mean number of myelinated axons at the distal end of the cross-face nerve graft (stage 2) which innervated the free gracilis muscle from the buccal facial nerve branch was 806.6 (SD: 1348.733) and 175 (SD: 173.0) from the zygomatic branch. Nerve biopsies harvested at both stages, showed that 46% of axons of the buccal facial nerve branch (smile) and 33% of the zygomatic branch (eye closure) reached the free gracilis muscle. Patient characteristic did not significantly correlate with the axon counts.

**Conclusion:** Objective histomorphometric analysis of the buccal and zygomatic facial branches revealed that ~50% and ~30% of axons reach the distal end of cross-face nerve grafts for the reanimation the paralyzed face with free gracilis muscle transplants.
Introduction: Neuroma formation, which may occur due to nerve transection, causes severe pain and significantly impairs the quality of life. The purpose of this study was to assess the outcome of epineural sheath jacket (ESJ) application as a novel technique in neuroma prevention in the rat sciatic nerve model. Epineural sheath is a naturally occurring material, which supports nerve regeneration by expressing proneurogenic and proangiogenic markers.

Material and Methods: Thirty-six rats were divided into six experimental groups (n=6 each): Group 1: Nerve stump without any protection; Group 2: Nerve stump buried into the muscle; Group 3: Nerve stump covered with ESJ; Group 4: ESJ buried into the muscle; Group 5: ESJ filled with fat graft; Group 6: ESJ filled with fat graft and buried into the muscle.

A 20mm long sciatic nerve defect was created and fascicles were removed using pull out technique. The distal part of the conduit was ligated creating the ESJ, which was applied over the proximal nerve stump. Additionally, in groups 5 and 6 ESJ was filled with autologous fat. Animals were evaluated 6 months after surgery. Outcome assessment included: sensory pinprick, Tinel sign, autotomy score, histomorphometry, neural/connective tissue ratio (N/C) and retrograde neuronal labeling analysis (RNL).

Results: Significant adhesions were seen in Group 1 during nerve evaluation 6 months after surgery. The integrity of the ESJ was preserved in groups 3-6. In Groups 1 and 2 percentages with positive Tinel signs were significantly higher than in groups protected with ESJ. The average pinprick score was significantly increased in Groups 1 and 2, six months after surgery, which may indicate fibers sprouting, or hyperesthesia of the operated site. Nerve architecture was maintained in groups with ESJ protection, whereas in Groups 1 and 2 disorganization of the nerve fascicles was noticed using S-100 staining. N/C ratios evaluated by Masson trichrome staining, in groups 3 and 4 were comparable with the current gold standard of neuroma management – muscle burying (Group 2). RNL analysis revealed significantly higher number of neurons in dorsal root ganglion in groups with ESJ protection compared to the controls. Quantitative nerve histological analysis is currently in progress.

Conclusions: This study confirmed feasibility of ESJ application as a novel technique in neuroma protection. The protective effect of ESJ was proved by functional and histological analysis in groups 3-6 yielding comparable outcome to muscle burying - current gold standard of neuroma management.
46. Neuronal Transplants into the Distal Nerve Segment Maintain Pro-Regenerative Schwann Cells in a Model of Chronic Axotomy

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\textsuperscript{1}Neurosurgery, University of Pennsylvania, Philadelphia, PA; \textsuperscript{2}Axonia Medical, Inc, Kalamazoo, MI

Peripheral nerve injury (PNI) often results in a choreographed sequence of Wallerian degeneration and alterations in Schwann cell (SC) structure and function. Initially, SCs assume a pro-regenerative phenotype to form the bands of Bungner, which are aligned columns to facilitate axon regrowth. However, this pro-regenerative environment degrades over several months, ultimately blunting the extent of axonal regeneration and hence functional recovery. This is particularly relevant in cases of long segmental defects and/or proximal injuries where this pro-regenerative capacity is present over a shorter timeframe than necessary to support axonal regeneration to distal targets. In a novel approach, we utilize tissue engineering strategies to implant living neurons to prolong the pro-regenerative capacity of SCs in otherwise axotomized nerves. In the current study, we assessed the ability of allogeneic neuronal constructs to “babysit” distal nerve SCs following sciatic nerve axotomy in the rat. In this paradigm, tissue engineered constructs were attached to the transected distal nerve and the proximal nerve was capped to prevent host axonal ingrowth. Histological analyses were performed up to 16 weeks post-transection to determine distal nerve cytoarchitecture, graft survival, and graft axon integration with distal nerve. Over 2-4 weeks post-transection, distal nerve SCs formed stereotypical aligned regenerative columns. In control animals (not receiving neuronal implants), the density of these columns decreased over 6-8 weeks, with a marked reduction in SC presence and alignment after 9 weeks and widespread SC degeneration and a complete absence of alignment by 16 weeks. In animals receiving neuronal implants, we found surviving graft neurons – despite an absence of immunosuppression therapy – and substantial graft axon penetration deep into the distal nerve to interact directly with host SCs. Remarkably, this led to an abundance of SCs which maintained their pro-regenerative alignment and phenotype out to at least 16 weeks. These findings demonstrate the promise of living axonal constructs to maintain the regenerative capacity of resident SCs. For clinical deployment, we envision secondary “satellite” neuronal transplants at metered locations distal to a primary repair, with the sole purpose to maintain pro-regenerative SCs ahead of regenerating axons. We are currently assessing the ability of such satellite transplants to “babysit” distal SCs in support of host axonal regeneration following repair of major (≥4cm) nerve lesions in rodent and porcine models. This tissue engineering strategy may enable host axons to reinnervate long-distance targets and potentially facilitate functional recovery following currently untreatable PNI.
Obstetrical Brachial Plexus Palsy: Geographic Information Systems Data on a Single Cohort at a Major Children's Hospital

Christopher Lunsford, MD; Jack E. Brooker; Kristen Kurland, BA; Lorelei Grunwaldt, MD

1UPMC Children's, Pittsburgh, PA; 2School of Clinical Medicine, University of Cambridge, Cambridge, United Kingdom; 3H. John Heinz III College, Carnegie Mellon University, Pittsburgh, PA; 4University of Pittsburgh, Pittsburgh, PA

Introduction: Obstetrical brachial plexus palsy (OBPP) is very uncommon, occurring in approximately 1.5 of 1,000 total births. Factors related to OBPP previously studied include gestational age, birth weight, maternal parity, and duration of labor. It was noted that presenting cases were disproportionately from underprivileged backgrounds in this major treatment center, however, there are no socio-economic geographic studies.

Methods: An IRB approved retrospective review of all patients presenting to a major children's hospital multidisciplinary brachial plexus clinic was performed from 1992 to 2014. A total of 188 patients were initially reviewed. Data was geocoded by patient street address (n=177) and ZIP Code (n=180). Exclusion criteria included lack of address information. Data were analyzed for age, sex, race, location of birth hospital, address of residence, and proximity to birth centers.

Results: Overall, none of the referral hospitals had a higher than normal incidence of OBPP. 57 out of 177 (88.7%) of patients were from the same state and 52/177 (29%) were from the same county as the treating center. When geocoding OBPP patients within the city of the treating center at the neighborhood level (n=24), demographics show a lower than average socio-economic status of these neighborhoods (n=18) compared to the general population of the city. There was a higher undereducated population (16.09% vs 11.40%), lower percentage of residents with bachelor's degrees (21.21% vs 33.80%), and higher percentage of single mothers (22.32% vs 14.30%) in the OBPP cases versus city averages respectively (p=0.0039). When geocoding patients in the state, household income was lower ($47,650 vs $52,267), college degree attainment was lower (23.35% vs 27%) and the number of female households higher (26.47% vs 11.9%) than state averages respectively (p=6.97x10^-5). It was noted that more of the presenting cases were African American (13.7% vs 10.9%). Geocoded figures for African American patients showed considerably lower socio economic averages particularly female headed households (42.26% vs 26.08%) compared to the overall patient sample which may explain this racial preponderance.

Conclusion: Our findings suggest a higher incidence in low socio-economic neighborhoods in our cohort compared to regional references. This suggests that accessibility to healthcare, parental education, and marital status may be modifiable factors contributing to OBPP. The use of geospatial analysis for specific diagnoses may provide clues to etiology and suggest public policy responses to improve health care delivery.
48. MR-Neurography-Based Evaluation of Morphological Features of Damaged Dorsal Root Ganglia and Nerves in the Cervical and Brachial Plexus

Tessa Buckle, PhD¹; Thijs Engelen²; Berit M. Verbist, MD, PhD³; Mark A. van Buchem, MD, PhD³; Martijn J.A. Malessy, MD, PhD²; Fijis W.B. van Leeuwen, PhD¹

¹Radiology/Interventional Molecular Imaging Laboratory, Leiden University Medical Center, Leiden, Netherlands; ²Neurosurgery, Leiden University Medical Center, Leiden, Netherlands

Introduction: Nerve damage to the cervical or brachial plexus results in reduced sensory and/or motor function. Damage results in neuronal cell death, which in turn leads to a decrease in the volume of the dorsal root ganglion (DRG). Morphologic evaluation can potentially provide a non-invasive measure for the degree of functional recuperation after damage. The purpose of this study was to evaluate the use of D-prep MR neurography (MRN) in the evaluation of morphological changes in the DRGs and nerves of the cervical and brachial plexus.

Materials and Methods: Five healthy volunteers and eight patients (Schwannoma or MPNST between C7 and T1 (n=4) or trauma (n=4) underwent an MRI of the cervical and/or brachial plexus (3T; Philips Ingenia) using standard T2 STIR and a D-prep MRN sequence. The detectability and dimensions of DRGs from C1 through T1 and nerves of the cervical and brachial plexus were assessed with both approaches. Volumetric measurements of the DRGs were performed according to the Cavalieri principle.

Results: (Volumetric) measurements were feasible in all patients and volunteers. DRGs (especially of the cervical plexus) were more clearly visualized with D-prep MRN compared to T2 STIR. In the volunteers DRG volume increased from 30-60 mm³ in C2-C4 to 180-200 mm³ in C7-C8. Spinal nerves of the cervical plexus (C2-C4; diameter 19.1 +/- 3.6 mm) and/or brachial plexus (C5-C8; diameter 39.7 +/- 4.8 mm) could be accurately visualized, and traced downward from their ganglion. In patients dimensions of unaffected nerves and DRGs were comparable to the measurements in healthy volunteers. Neuropathy after trauma (sustained > 2 months prior to scan) resulted in a decrease in DRG volume at the affected location (24.5 +/- 6%) compared to the contralateral side. Tumor invasion prohibited measurements of directly affected nerves and DRGs. Compression of nerves in close proximity to the tumor co-occurred with local edema and an increase in DRG volume (compared to the contralateral side).

Conclusions: (Volumetric) measurements, based on D-prep MRN images, could be performed on all DRGs and nerves. As differences in DRG volume were seen after tumor-related nerve-compression and neuropathy after trauma, nerve specific MRI might serve as an non-invasive in vivo measure for the degree of nerve damage.