Introduction

Despite extensive research efforts, no therapeutic agents are clinically available for the treatment of peripheral nerve injuries. Insulin-like growth factor 1 (IGF-1) is an ideal therapeutic candidate as it can accelerate axonal regeneration and also minimize the deleterious effects of prolonged denervation on muscle and Schwann cells. However, given its short half-life, a practical delivery system is needed to stabilize the protein and provide sustained release to target tissues. Using a novel encapsulation method, we demonstrated sustained release of bioactive IGF-1 from nanoparticles, in vitro, and improved nerve regeneration and functional recovery, in vivo. Here we describe the fabrication, optimization, and characterization of a nanofiber hydrogel composite (NHC) carrier system to maintain IGF-1 nanoparticles at target tissue sites for the duration of drug release and avoid frequent re-dosing.

Methods

An injectable NHC with PCL nanofibers covalently bonded to hyaluronic acid was fabricated by electrospinning. We combined IGF-1 nanoparticles with various NHC formulation and measured IGF-1 release kinetics and bioactivity in vitro and in vivo. NHC biocompatibility, biointegration, and immune response were characterized. Finally, we used a rat model in which chronic denervation is induced prior to nerve repair in order to test the efficacy of the optimized IGF-1 nanoparticle-NHC delivery.

Results

The NHC architecture was found to mimic ECM fat (Figures 1A and 1B). It exhibited favorable biocompatibility with minimal inflammatory response 25 days post injection (Figures 1C, 1D, 1E, 1F). The composite system polarized the invading macrophages into an anti-inflammatory and pro-regenerative M2 phenotype (Figure 1G, 1H, 1I). As a carrier for IGF-1 nanoparticles, NHC conferred superior IGF-1 release kinetics, in vitro and in vivo, in comparison to fibrin gel and saline (Figure 1J). The optimized IGF-1 nanoparticle-NHC system was effective in reducing muscle atrophy and sustaining Schwann cell proliferation in the setting of prolonged denervation.
Conclusion

We introduce a novel drug delivery system in which IGF-1 nanoparticles are combined with a nanofiber hydrogel carrier to provide sustained local concentrations of bioactive IGF-1 within target nerve and muscle. This therapeutic approach has the potential to improve functional outcomes via enhanced axonal regeneration and maintenance of denervated muscle and Schwann cells. IGF-1 and the polymer components of the engineered delivery system are currently used in FDA-approved formulations and devices, which will facilitate clearance of regulatory hurdles.
**Purpose:** The molecular target of the FK506 neuroregenerative property following its application at a nerve injury and repair site, is still unknown. We investigated the role of FK506 binding proteins (FKBP)s, including FKBP12 and FKBP52, in the neuroregenerative action of locally applied FK506 using an *in vitro* three-dimensional compartmented cell culture system.

**Method:** The compartmented system consisted of a neonatal rat dorsal root ganglion (DRG) attached to the end of an acellular nerve allograft (ANA) that provided the native peripheral nerve scaffold (Fig.1). In the experimental groups, FK506 (100 ng/mL) was delivered exclusively to the growing neurites, the DRGs, or both *in vitro*. Such exclusive delivery was enabled by a silicone sheet that isolated the culture media surrounding the growing neurites and the DRG (Fig.1). The DRG-ANA constructs in the negative control group were not cultured with FK506. In a subset of samples in each group, the function of FKBP12 and/or FKBP52 was blocked by treating the DRG-ANA constructs with 100 ng/mL of antibodies for FKBP12 and/or FKBP52. Following 3 days of incubation at 37°C, the length and density of the extended neurites and Schwann Cell (SC) density in the ANA were measured in longitudinal histological sections of the constructs. The effect of local delivery of FK506 on neurite extension and SC proliferation with or without the blocking of FKBP12 and/or FKBP52 was evaluated.

**Results:** Local administration of FK506 to only the growing neurites significantly increased both neurite extension and neurite density in the ANA as compared with the negative control group. However, FK506 delivery to only the DRG or both the DRG and ANA significantly increased neurite extension without any effect on the neurite density as compared with the negative control group. A significantly higher SC density within the ANA was observed following administration of FK506 to only the growing neurites or the entire DRG-ANA construct. Blocking FKBP52, but not FKBP12 when FK506 was applied to the growing neurites, blocked its action of increasing neurite extension, neurite density, and SC density.

**Conclusion:** This *in vitro* study demonstrated that FKBP52 mediates the neuroregenerative effect of locally applied FK506 to the peripheral nerve. The findings provide new insights into the molecular and cellular mechanisms of the proregenerative action of FK506 on the peripheral nerve.
Introduction: Repair of nerve defects requires the use of a bridging material. There is a desire to transition to alternatives to autografts, such as acellular nerve allografts (ANAs). However, during regeneration in this context, the immune system plays a critical role. Previously, our studies showed that T cells are important for regeneration across ANAs. Now, we address how T cells promote regeneration.

Materials and Methods: ANAs were generated using a chemical detergent protocol. One (1) cm ANAs were used to repair sciatic nerve gaps in Rag1KO, IL-4GFP, IL-4KO, or wild-type (WT; control) mice. As well, in select mice CD4 antibodies were used to deplete CD4 T cells. Regeneration was quantified using qRT-PCR, histology, immunofluorescence analysis, and functional outcome metrics.

Results: Gene expression analysis of ANAs revealed that Th2 related cytokines, including IL-4, were reduced in Rag1KO vs WT ANAs. The source of endogenous IL-4 was determined using IL-4GFP reporter mice. These mice revealed a correlation between IL-4 expressing cells and T cell (CD3+) accumulation within ANAs. But, eosinophils (Siglec F+), rather than T cells, were the primary source of IL-4 within ANAs (Fig. 1A). Yet, depletion of CD4 T cells reduced accumulation of IL-4 expressing eosinophils in ANAs demonstrating that T cells regulate eosinophils and in turn IL-4 expression within ANAs (Fig. 1B). Finally, nerves repaired using ANAs in IL-4KO had reduced regeneration across the ANA and reduced functional recovery compared to WT establishing the importance of IL-4 signaling to regeneration across ANAs (Fig. 1C,D).

Conclusions: Our data suggest T cells regulate the expression of IL-4 within the ANA to promote regeneration of myelinated axons.
Fig 1 Assessment of the source of IL-4 expression, how it is regulated, and whether IL-4 has an impact on nerve regeneration ANA repair. Mean ± SD, n≥3/group; p values shown.

ASPN Scientific Paper Session I
#4 Conditioning Electrical Stimulation Improves Functional Recover in a Tibial to Peroneal Nerve Transfer
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**Background:** Injury to the common peroneal nerve (CP) results in foot drop, with major effects on patients’ function and quality of life. Attempts at reconstruction with distal nerve transfers (DNT) where the distal stump of the degenerated CP nerve is coapted with a branch of the tibial nerve have gained popularity; however, outcomes remain inconsistent due to poor regeneration, reinnervation, and cortical relearning. We hypothesize that delivering one hour of conditioning electrical stimulation (CES) 7 days prior to DNT surgery will significantly improve regeneration and functional outcomes.

**Methods:** Using a rat model, the CP nerve was crushed to replicate a traumatic CP nerve injury. CES was delivered to the tibial nerve in half the animals one-week post-injury. Fourteen days following CP nerve injury, a DNT was performed. A branch of the tibial nerve innervating the lateral gastrocnemius muscle was divided, and its proximal stump coapted to the distal stump of the injured CP nerve. Length of axonal regeneration was quantified 2 weeks following the injury. Motor reinnervation of the tibialis anterior muscle (neuromuscular junction analysis and muscle weight) and functional outcomes (kinetic/kinematic studies and skilled locomotion) were assessed 6-10 weeks following injury.
Results: Animals treated with CES prior to DNT had significantly greater regeneration and motor recovery compared to animals treated with surgery alone. The length of axon extension in CES-treated animals was 7.8 ±0.8 mm, significantly longer than 3.1 ± 0.5 mm in the non-conditioned controls (p<0.001). By 9 weeks, gait analysis of CES animals identified significant improvements in normalization of the vertical peak, braking, and propulsion forces, gait kinematics, and performance on the horizontal ladder test (p<0.05). The tibialis anterior of the affected limb had greater muscle mass, with significantly more reinnervated neuromuscular junctions on immunofluorescent analysis (p<0.01).

Conclusions: Delivery of CES one week prior to lower limb DNT from a tibial nerve branch to an injured CP nerve significantly improved muscle reinnervation in the tibialis anterior muscle, and is also reflected in superior functional recovery. Previous studies have established electrical stimulation as a clinically feasible method for conditioning, and this study demonstrates its application in potentially significantly improving outcomes in patients with CP nerve injuries undergoing DNT.

#5 Transdermal Application of 4-aminopyridine (4AP) Promotes Peripheral Nerve Injury Recovery: Improvements in Function, Axonal Degeneration, Myelination, Electrodiagnostic Parameters

 phára: Traumatic peripheral nerve injury (TPNI) represents a major health problem that often leads to significant functional impairment and permanent disability. Despite the available modern diagnostic procedures and advanced microsurgical techniques, most patients with TPNI do not regain full functional recovery. Therefore, there is an unmet need for new therapeutic strategies to promote functional recovery in TPNI patients. 4-aminopyridine (4AP), an FDA-approved drug for the treatment of multiple sclerosis, has been shown to improve neuromuscular function in patients with diverse demyelinating disorders. We demonstrated that systemic 4AP administration enhances global functional recovery of the affected limb, promotes remyelination and improves the nerve conduction velocity in a mouse model of TPNI. Although oral or injection routes are commonly used, the therapeutic benefits with transdermal delivery of drugs are well documented to provide a sustained circulating blood levels with enhanced patient compliance and importantly without the need for multiple daily oral dosing or injections. However, there is no data on the transdermal delivery of 4AP. Therefore, we asked whether 4AP could be used as a transdermal agent and what would be its effects on motor function and neuronal recovery after TPNI.

Materials & Methods: Mice were assigned to moderate sciatic nerve crush injury and the effects of acute and chronic treatments with transdermal 4AP (150 μg) and vehicle (DMSO) were investigated. Using Franz diffusion cells, the skin permeability of 4AP (40 mg/ml) in 0.5 mL water or DMSO was determined through mouse skin. Pharmacokinetic parameters of 4AP in
serum were determined by HPLC method at specified time points after applying 7.5µL of 4AP in DMSO (10 mg/ml or 20mg/ml) to the lower back skin of anesthetized mice.

**Results:** 4AP showed similar skin permeability coefficients in water and DMSO. Pharmacokinetic parameters of 4AP were linear, the maximum plasma 4AP concentrations were proportional to 4AP dose, and the time to maximum blood concentration was 60 min for both dosages. While a single dose of transdermal 4AP administration demonstrated rapid transient improvement in motor function, chronic transdermal 4AP treatment significantly improved motor function and nerve conduction and these effects were associated with fewer degenerating axons and thicker myelin sheaths than those from vehicle controls.

**Conclusions:** Topical 4AP, absorbed through the skin, enhances *in vivo* global motor function recovery with decreased axonal degeneration, increased myelination and faster nerve conduction in axons. These findings provide direct evidence for the potential therapeutic use of transdermal 4AP in TPNI.

ASPN Scientific Paper Session I

#6 Elucidating the Relative Impact of Muscle vs. Schwann Cell Denervation on Functional Recovery in a Novel Rodent Model

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**Background:** Functional recovery following peripheral nerve injury worsens with increasing duration of delay prior to nerve repair. Until reinnervation occurs, denervated muscle undergoes progressive atrophy that limits the extent to which motor function can be restored. Similarly, Schwann cells (SC) in the distal nerve undergo denervation atrophy that hinders their capacity to support regenerating axons. The relative contributions of these processes to diminished functional recovery is unclear.

**Methods:** We developed a novel rat model that isolates the effects of distal nerve vs. muscle denervation on functional recovery with four groups (Figure 1A) that underwent the following interventions for 12 weeks prior to nerve repair: 1) muscle denervation; 2) nerve/SC denervation; 3) muscle + nerve/SC denervation (negative control); 4) no denervation (positive control). Functional recovery was measured weekly using stimulated grip strength testing. Animals were sacrificed at 12 weeks for histological analyses.
Results: The muscle denervation group achieved greater functional recovery than the nerve/SC denervation group. Functional recovery in the muscle denervation group and nerve/SC denervation group mirrored the negative and positive control groups, respectively (p<0.05), Figure 1B. Neuromuscular junction analysis and muscle histology confirmed these results (Figure 1C and 1D).

Conclusions: The deleterious effects of muscle denervation are more consequential than the effects Schwann cell denervation on functional recovery. The effects of 12 weeks of Schwann cell denervation on functional outcome were negligible. Future studies are needed to determine if greater periods of Schwann cell denervation negatively impact functional recovery.
#7 Terminal Schwann Cells are Integral for Neuromuscular Junction Function and Reinnervation

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**Introduction:** Peripheral nerve injuries account for 5% of all hospital trauma patients in North America (Noble et al. 1998). Reinnervation capabilities are usually limited to 12-18 months following injury. Terminal Schwann cells (tSCs), are supportive glial cells that reside at the neuromuscular junction (NMJ), contribute to NMJ maintenance, and are active during reinnervation. The requirement of tSCs for NMJ function and reinnervation in mammalian species, however, is not known. In this study, we utilized immune-mediated tSC ablation to assess the contributions of these cells to NMJ maintenance and recovery. This knowledge will hopefully improve our ability to treat peripheral nerve injuries.

**Methods:** Adult *S100-GFP* mice underwent injection of the right extensor digitorum longus (EDL) with either GD3 anti-disialosyl antibodies (i.e. experimental group) or Lactated Ringer’s (i.e. control group), followed by normal human serum as a complement source. For nerve injury studies, mice underwent right peroneal nerve transection with repair simultaneously or 1 week prior to tSC ablation. Muscle force testing was performed at post-ablation days (PAD) 3 or 14 (no nerve injury) or 3 or 6 weeks after ablation and nerve injury (post-injury ablation, PIA). The EDL was harvested and stained with aBTX-Alexa 647 (selective for Acetylcholine Receptors) in order to identify and assess the NMJ.

**Results:** Morphologic testing showed the numbers of tSCs were reduced after ablation compared to controls at all time points (p<0.05). Even without nerve injury, there was a significant decrease in EDL force to 38% of control in the PAD14 group with 1-hour GD3 incubation (p<0.003). There were no differences in the PAD3 group compared to control. After nerve injury, motor endplate fragmentation was increased in mice that underwent tSC ablation versus control (PIA 3 wks: 22% vs 2%, p<0.01; PIA 6 wks: 27% vs 7%, p<0.01). EDL force was reduced at 64% of control at PIA 3 wks (p<0.05) and 50% of control at PIA 6 wks (p<0.001).

**Conclusion:** These data suggest tSC ablation affects both NMJ morphology and muscle function. tSCs are required for efficient NMJ function and reinnervation after nerve transection and repair. tSCs are a possible therapeutic target in patients with peripheral nerve injuries.

#8 RNA-driven Epigenetic Modulation of Neurotropic Factors Through CTDSP1 and REST Pathway As a Novel Strategy in Peripheral Nerve Regeneration After Traumatic Injury

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Modulation of CTDSP1 activity in mesenchymal progenitor cells as a novel strategy to promote peripheral nerve regeneration after traumatic injury

Introduction: Peripheral nerve injury is a frequent complication in high-energy musculoskeletal trauma. Current therapeutic interventions are hindered by limited regeneration of injured nerves and rarely lead to complete functional recovery. The regenerative ability of nerves depends in part on trophic support received from surrounding mesenchymal progenitor cells (MPCs) at the zone of injury. We have shown that MPCs that concentrate in traumatized tissue produce neurotrophic factors, which support neurite growth. The expression of neurotrophins such as BDNF, NGF and NT3, is regulated by repressor element-1 silencing transcription factor (REST), which is protected from degradation by C-terminal domain small phosphatase 1 (CTDSP1). While REST levels increase after central nervous system injury, the response in peripheral nerve injury is unclear. Here, expression profiles of REST, CTDSP1 and neurotrophins in traumatized muscle tissue and in MPCs are characterized, and effects of CTDSP1 knockdown on expression of neurotrophins in traumatized-tissue derived MPCs are examined as a potential way to promote peripheral nerve regeneration.

Methods: RNA and protein were isolated from traumatized muscle tissue and from traumatized muscle-derived MPCs at different time points post-injury. The expression of REST, CTDSP1 and neurotrophins were quantified by RT-PCR and Western Blot. MPCs were transfected with CTDSP1-targeting siRNA or non-targeting siRNA and cultured in either standard medium or neuroinduction medium. The effects of CTDSP1 knockdown on the expression levels of REST and neurotrophins were analyzed by RT-PCR. The levels of secreted BDNF were measured in cell culture supernatants by ELISA.

Results: REST, CTDSP1 and neurotrophin expression levels in traumatized tissue are altered in a time-dependent manner following traumatic injury. An inverse correlation between levels of CTDSP1 and neurotrophins was observed. Knockdown of CTDSP1 in MPCs resulted in higher expression levels of neurotrophins and increased secretion of BDNF.

Conclusions: The observed inverse correlation between CTDSP1 and neurotrophin levels in traumatized muscle tissue is consistent with CTDSP1 stabilization of REST and subsequent repression of neuronal genes expression, with decreased CTDSP1 leading to increased neurotrophin expression. Accordingly, knockdown of CTDSP1 in MPCs results in increased expression of neurotrophins, such as BDNF, which have been shown to promote neurite sprouting. Controlling the activity of CTDSP1 at the site of injury may therefore represent a novel strategy to promote nerve regeneration by increasing the availability of neurotrophins forming a favorable environment.
Introduction: Creating the optimal neuroma-in-continuity (NIC) model has been a long sought-after goal in experimental peripheral nerve surgery research. Our group has previously created a verified traumatic NIC model in rats. We present our current work of NIC in a mouse model to facilitate future drug targeted therapies to the regenerating nerve.

Materials & Methods: Unilateral sciatic nerves of nineteen C57BL/6 (N=8) and 129S6/SvEvTac (N=11) adult mice were surgically exposed and underwent a uniformly combined crush and stretch injury using a custom designed spacer. Injury specifications were done in preliminary work to achieve a Sunderland grade 4 injury and then replicated for consistency. Nerves were harvested at 6 days (+/- 1 day post-injury; N=14) for histological serial longitudinal evaluation of microarchitecture and compared to acutely (0 days post-injury; N=5) injured nerves and uninjured contralateral nerves.

Results: At one week following injury, histological studies confirmed both a grade 3 axonotmetic injury with endoneurium disruption but fascicular sparing and a grade 4 axonotmetic injury with a severe NIC while the epineurium remained intact (Fig.1). Ongoing work is evaluating the electrophysiological and behavioural consequences in a cohort of serially assessed mice receiving NIC versus simple crush injuries.

Conclusion: This is the first report of a reproducible crush-stretch injury in a mouse model. Graded controlled injury is achievable through different biomechanical applied forces. Ongoing work encompassing behavioral and functional studies of a larger group of animals will be available for presentation at the conference.

Fig.1

**Fig.1. Longitudinal immunohistochemistry sciatic nerve stretch-crush injury at day 6 neuroma-in-continuity (NIC)**

Top panel single nerve following stretch-crush injury. Left to right: proximal (Prox), injury site (NIC) and distal site (Dist). Lower panel proximal and NIC site of stretch-crushed nerve. Lower right is control- crush only injury site.

Markers key-code: Red- Neurofilament, Green- Glut1 to demonstrate perineurium and its disruption in NIC, Blue- DAPI
**Introduction:** The success of targeted muscle reinnervation (TMR) in preventing or relieving amputation-related neuropathic pain has been well shown in clinical studies. However, many questions remain about the changes in pain pathways and neuronal regeneration induced by TMR. These questions are best answered in an animal model. Pre-clinical studies using amputation are problematic as limb sensation and stimulation-related pain behaviors cannot be tested, precluding validation of pain. We hypothesize TMR can be used to treat neuropathic pain that follows peripheral nerve transection in the spared nerve injury model, a robust, well-validated model of neuropathic pain. This model retains the limb and thus, allows for standard rodent pain behavior testing.

**Materials and methods:** Spared nerve injury was performed in male rats by unilaterally ligating the common peroneal and tibial nerves. After 3 weeks, the rats developed a robust and consistent neuropathic pain phenotype and two interventions were studied: 1. TMR: the neuromas were excised and the animals underwent coaptation of the common peroneal and tibial nerves to nerve branches to the biceps femoris; and 2. Neuroma Excision (NE): the neuromas were excised and the muscle branches divided but no coaptations were performed. The plantar skin in the sural distribution was tested with von Frey threshold, pin touch hyperalgesia, and hypersensitivity to dynamic mechanical stimulation (brush), acetone (cold), and heat (Hargreaves) to characterize pain behavior prior to intervention and at 1 and 3 weeks following intervention. The healthy contralateral limb was tested as an internal control.

**Results:** The spared nerve injury caused a robust pain phenotype with increased hyperalgesic responses, reduced von Frey thresholds, and increased cold sensitivity. One week after TMR, pain behaviors were significantly changed compared to pre-intervention pain measures, while pain measures did not change in the NE group. In the TMR group, hyperalgesia responses to pin were reduced 80% within 1 week following TMR but did not change significantly following NE. Von Frey thresholds returned to the baseline following TMR but remained significantly elevated in the NE group. There was also a trend to reduced cold hypersensitivity in the TMR group.

**Conclusions:** This rodent model mirrors the clinical results found in TMR and allows for measurement of allodynia, hyperalgesia, and sympathetic-related pain behavior changes. This model will allow for determining the underlying mechanisms at the dorsal root ganglion and axonal levels responsible for the clinical outcomes of TMR surgery.
Introduction
Targeted muscle reinnervation (TMR) improves pain in patients with limb amputations. Most studies featured healthy patients who underwent amputations due to trauma or cancer. The success of TMR in patients who receive a lower extremity amputation due to uncompensated PVD or diabetes-related infections has not been studied. Since the inability to ambulate after amputation leads to a rapid decline in health and functional status, effective TMR may be critical in reducing post-amputation mortality in patients with uncompensated comorbidities. The purpose of this study is to evaluate the efficacy of TMR to reduce pain and improve ambulation in a comorbid patient population.

Materials and Methods
This study is a retrospective review of a single-surgeon experience with TMR in a patient population undergoing below-knee amputation from January to December 2018. Patient charts were reviewed for the comorbidities in the Charlson Comorbidity Index (CCI). Post-operative notes were assayed for patient’s residual limb pain from 0 to 10, presence of neuropathic pain, severity of phantom limb pain, ambulatory status, and mortality. A control group of patients undergoing below-knee amputation who did not receive TMR from January to December 2017 was used for comparison.

Results
54 patients were included in the TMR group with average age of 57, BMI of 29, and CCI of 5.4. 61% had PVD, 77% had DM, and 33% had ESRD. 46 patients were included in the non-TMR group with average age of 61, BMI of 31, and CCI of 5.4. Average time to follow-up for the TMR group was 3.1 months and 10.8 months for the non-TMR group. Relative to the non-TMR group, the TMR group demonstrated decreased residual limb pain (1.2 vs. 2.7, \( P = 0.02 \)), neuropathic pain (12.0% vs. 45.2%, \( P < 0.01 \)), and phantom limb pain (63.0% without pain vs. 47.8%; 1.9% with uncontrolled pain vs. 15.2%, \( P = 0.01 \)). Ambulation rates were higher with TMR (86.8% vs. 61.9%, \( P = 0.01 \)). Mortality rate for the TMR group at 1 year was 4.5% compared to 10.9% in the non-TMR group (\( P = 0.16 \)).

Conclusions
TMR effectively reduces overall, neuropathic, and phantom limb pain in patients undergoing below-knee amputation in the setting of uncompensated comorbidity. More significantly, performing TMR in these patients is associated with increased rates of ambulation and decreased mortality. Decreasing pain and improving ambulation may be critical in improving further morbidity and mortality rates in this very comorbid, high-mortality risk patient population.
**Introduction**

Acute Flaccid Myelitis (AFM) is a newly re-defined polio-like process affecting anterior horn cells, leading to lower motor neuron paralysis with preserved sensation. Persistent limb weakness in AFM presents as a brachial plexus-type deficit with few non-surgical interventions. Nerve, tendon, and free muscle transfers have been recognized as viable reconstructive options. We report our center’s experience with surgical management of AFM, which is the largest known series reported since the CDC’s new definition of the disease. Our hope is to help guide treatment going forward.

**Materials and Methods**

We performed a retrospective review for our tertiary Peripheral Nerve Center from 2009-2018, and identified patients who met diagnostic criteria for AFM. Data were collected for: demographics, motor deficits upon presentation, surgical interventions, and post-operative functional recovery. Our primary endpoint of interest was overall functional recovery.

**Results**

Of the 24 AFM patients referred to our Center, 9 were deemed surgical candidates and had 19 total operations. For surgical candidates, median age at disease onset was 6y (range, 10 months – 13 years); median time to presentation after onset was 8.2 months (range, 5.3 months – 11.7 years). Deficits of surgical candidates were in: upper extremity (n=6); lower extremity (n=2); and mixed upper/lower extremity (n=1). Unilateral deficits were present in 7, and bilateral deficits in 2. Functional deficits were noted with: intrinsic palsy (n=3), pronation (n=2), finger flexion at FDS/FDP (n=2), foot drop (n=2), elbow extension (n=1), elbow flexion (n=1), and shoulder abduction (n=1). The most commonly performed procedures were: nerve transfers (n=14), tendon transfers or re-routing (n=13), free functional muscle transfer (n=2) and nerve decompression (n=1). Operative intervention was generally delayed (median, 10.9 months after symptom onset; range, 7.9 months to 145.8 months). Median clinical follow-up time was 26.3 months post-operative (range, 3.7 months to 108.6 months). All 9 patients had significant improvement and regained function in their affected limbs.

**Conclusions**

AFM is a newly re-defined disease process which may leave patients with severe disability despite prompt non-surgical management. Nerve, tendon, and free muscle transfers can be excellent reconstructive options after AFM and are noted to improve functionality for these severely affected patients. Surgery is typically delayed to allow sufficient time for identification of persistent deficits following spontaneous but frequently incomplete recovery. Surgical decision-making including patient selection, surgery timing, procedure selection, and reconstructive staging will be discussed.
ASPN Scientific Paper Session I  
#13 4-Aminopyridine (4-AP): A Single-Dose Diagnostic Agent to Diagnose Peripheral Nerve Continuity  
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Introduction: Traumatic peripheral nerve injury (TPNI) represents a major health problem that often leads to significant functional impairment and permanent disability. Injured nerves may be stretched, crushed, or transected. Unfortunately, there is no early diagnostic test or tool available to differentiate a crushed nerve from a transected nerve. Advanced electro-diagnostic and imaging studies do not provide any diagnosis even after weeks or months of injury, and the treatment relies on ‘watchful waiting’ versus invasive exploration of the wound. Therefore, there is an unmet need for a diagnostic tool which could address these issues for early diagnosis and subsequent treatment strategies. 4-aminopyridine (4-AP) is a FDA-approved generic drug for symptomatic treatment of multiple sclerosis. We demonstrated that, at 1 day post-injury, a single dose of systemic 4-AP significantly can improve walking function in mice with crushed nerve injury but not in mice with transected nerves. While these findings indicate a role of 4-AP in identifying axonal continuity in awake/conscious animal, it is unknown whether this diagnostic criteria of 4-AP is applicable in unawake/sedated animal. We investigated the transient stimulatory effect of 4-AP on demyelinated axon for muscle contraction in fully sedated rats.

Materials & Methods: Sciatic nerve crush or transection injury was used to evaluate the effect of single dose 4-AP on muscle tension in anesthetized rats. A shielded stimulating electrode was placed under sciatic nerve and injury was performed distal to stimulating electrode. Sciatic nerve underwent a standardized crush injury or laceration injury. The triceps surae muscles were contracted for 10s at 40Hz, 0.1ms pulse, and a voltage two times above the motor threshold. The muscle tension was measured before injury, after crush or laceration, and at different time points after intraperitoneal injection of saline or 1mg/kg 4-AP.

Results: We found that both crush and transection injuries in sciatic nerve completely abolished muscle response to electrical stimulation. Single dose of 4-AP treatment significantly restored muscle responses to electrical stimulation in crush injury to a level 53% of pre-injury values within 30 min of 4-AP administration. However, 4AP treatment had no effect on muscle contraction after nerve transection.

Conclusion: We conclude that 4-AP could be a promising diagnostic agent in differentiating peripheral nerve injuries even in the unconscious patient, and this study provides the rationale for further investigation in the setting of peripheral neurotrauma where no alternative diagnostic tool is currently available.
INTRODUCTION: Re-creation of a spontaneous, emotional smile remains an overriding goal of smile reanimation surgery. However, the ideal innervation strategy remains unknown. An automated machine-learning tool was developed to compare spontaneous smiling in cross facial nerve graft, masseteric nerve, and dually-innervated free gracilis transfers.

MATERIALS AND METHODS: Validated humorous videos were used to elicit spontaneous smiles. Automated facial landmark recognition (Emotrics) and emotion detection software were used to analyze video clips of spontaneous smiling in nine normal subjects, and 43 facial palsy patients. Emotionality quotient (EmQ: probability of perceived joy / probability of perceived negative emotion) was used to quantify the ability of spontaneous smiles to express joy.

RESULTS: Spontaneous smiles of normal subjects exhibited median 100% joy and almost no negative emotion (EmQ score +100/0). Spontaneous smiles of facial palsy patients after smile reanimation, using cross facial nerve graft, masseteric nerve, and dual innervation, yielded EmQ scores of median +82/0, 0/-48, and +10/-24 respectively (joy p = 0.006, negative emotion p = 0.034). The main difference was found to be between the cross face and masseteric nerve driven gracilis FFMT (joy p = 0.001, negative emotion p = 0.008).

CONCLUSION: Computer vision software can objectively quantify differences between various reinnervation strategies in facial reanimation. Cross-facial nerve graft- and dually-innervated gracilis achieved greater degrees of improvement in emotionality during spontaneous smiling, whilst masseteric alone was significantly worse. This automated system for quantification of spontaneous smiling from standard video clips would facilitate blinded, multicenter comparisons of spontaneity outcomes.
Purpose: Patient selection for migraine surgery is the most important variable to ensure successful outcomes. From verbal and written descriptions alone it can be difficult to understand patients pain/trigger patterns. In our experience, a superior method to visualize pain is to ask patients to draw where the pain originates and where it radiates. We have found that there are pathognomonic pain patterns for all trigger sites that should be considered in patient selection. We typically do not operate on patients with atypical pain diagrams, as we believe they are poor candidates. There is a small subset of these atypical patients that undergo surgery based on other strong clinical findings. In this study we attempt to quantify this clinical experience.

Methods: One- hundred and six patients were prospectively enrolled in this study and asked to complete pain diagrams at screening. Diagrams were analyzed and categorized: 1) Typical- Pain over the distribution of a nerve with expected radiation 2) Intermediate- Pain over the distribution of the nerve with atypical radiation 3) Atypical- Pain outside of normal nerve distribution and atypical radiation. Surgical outcomes were documented using pre and postoperative Migraine Headache Index calculation. MHI between sub- categories was compared using unpaired T -tests.

Results: 74 patients demonstrated typical pain patterns, whereas 21 patients had intermediate and 11 patients had atypical pain patterns. Mean follow up was 14.12 months. MHI improved by 69.5% ± 38.7 in the typical pain pattern group compared to 69.35% ± 32.6 in the intermediate group and 39.04 ± 35.3 in the atypical group. There was no significant difference between the typical and intermediate group. However, there was a significant difference in MHI between the typical and atypical (p= 0.012), as well as the intermediate and atypical group (p= 0.017).

Conclusion: Patient self-created pain diagrams have become an important screening tool in our practice. We believe they represent a clear and easily interpreted test to screen candidates for surgery. This study suggests that surgical outcomes for patients with atypical pain patterns are significantly inferior when compared to normal or close to normal patterns. This tool and these findings should be taken into consideration when evaluating patients for migraine surgery.
#15 Axillary to Triceps Nerve Transfer for Restoration of Elbow Extension in Tetraplegia: A Cautionary Tale

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Introduction
Spinal cord injury (SCI) at the C7 level results in loss of triceps function, which hinders patient’s ability to transfer and position their hand in space. Traditional tendon transfer techniques to restore elbow extension provide functional improvement but require splinting and post-operative non-weight bearing, which can be challenging. Nerve transfer surgery obviates immobilization and others have reported promising clinical results. The authors provide a critical summary of their clinical experience with branches of the axillary to triceps nerve transfers in SCI, with the goal of elucidating what factors contribute to the success of this procedure.

Methods
A retrospective multi-centre cohort study of people with SCI who underwent axillary to triceps nerve transfer over a 5 year period (2013-2018) was performed. Pre-operative, intra-operative and post-operative clinical and electrodiagnostic (EDX) variables were recorded. The primary outcomes were triceps Medical Research Council (MRC) grade strength at final follow-up and time to innervation. Clinical features of successful and unsuccessful nerve transfers were assessed.

Results
Ten limbs in 6 individuals with SCI were included in this study (70% male, mean age 21 ± 11 years). Motor level at time of injury was C5 (20%) or C6 (80%), and 90% had ASIA A (motor complete) patterns of injury. Nerve transfer surgery was performed 9 ± 3 months after injury, with final post-operative follow up of 25 ± 7 months. In 2 of 10 limbs, MRC grade > 3 of elbow extension was achieved. Time to reinnervation was 23 ± 3 months. Factors that correlated with successful outcomes included an excellent quality donor (as determined by pre-operative exam and electrodiagnostic testing and intra-operative stimulation and frozen section), and the absence of scarring in the recipient nerve (as determined by intra-operative frozen section).

Conclusion
In the authors’ experience, the axillary to triceps nerve transfer to restore elbow extension in people with SCI needs to be approached cautiously. Careful pre and intraoperative exam of donor and recipient quality (including intraoperative frozen section testing) may optimize outcomes. Further work is needed to delineate other reasons for failure including investigation into the overlap in innervation between donor and recipients, timing of surgery and surgical technique.
Hypothesis: The transfer of the terminal branch of anterior interosseous nerve (AIN) into the deep ulnar motor nerve branch improves intrinsic hand function in patients with high ulnar nerve injuries by providing motor input closer to the motor end plates. We report outcome measurements of this nerve transfer in patients with compressive ulnar neuropathy and hypothesize that any improvement in intrinsic hand function is beneficial to patients.

Methods: A retrospective review was conducted of all AIN to ulnar motor nerve transfers, including both end-to-side (ETS) and end-to-end (ETE) transfers, from Jan 2011 to October 2018 performed by 2 surgeons. All adult patients that underwent the nerve transfer for compressive ulnar neuropathy (cubital tunnel syndrome), with >6 month follow-up and completed charts were included. Primary outcome measures were motor function using the British Medical Research Council (BMRC) grading system. Secondary outcome measures included complications and donor site deficits. Preoperative nerve conduction studies were also reviewed.

Results: Of sixty-five patients (mean age 56.1, 68% male) who underwent the nerve transfer, 32 patients met the inclusion criteria. The average follow-up was 12 months. The average time to surgery from initial injury or symptom onset was 14.1 months. The overall average BMRC was 2.94/5 with a statistically significant better recovery in patients who received earlier surgery (<12months =BMRC 3.73, >12months =BMRC 2.24, p-value <0.01). Patients with an ETS neurorrhaphy had a trend towards better results that those with an ETE neurorrhaphy (ETS = BMRC 3.24, ETE =2.6). All patients who underwent nerve transfer had severe compressive ulnar neuropathy with intrinsic wasting (McGowan 3). All patients at final follow-up, regardless of BMRC grade, had a positive Froment’s sign and some wasting of their first dorsal interosseous muscles. Therefore, recovery of intrinsic function was measured by the ability to abduct/adduct fingers and loss of Wartenburg’s sign. There were no donor deficits post-operation. One patient developed CRPS post-operation.

Conclusions: Patients with earlier surgery and receiving an ETS transfer showed improved recovery with a higher BMRC grade compared to those who underwent later surgery and/or and ETE transfer. Despite evidence of significant intrinsic wasting, patients that received surgery within 12 months of motor symptom onset had improved function with relatively higher BMRC scores. Even patients with low BMRC scores reported improved hand dexterity in follow up. We would recommend this surgery for patients with chronic compressive ulnar neuropathy, as any improvement is hand function is beneficial.
Concurrent AAHS/ASPN Scientific Abstract Session

#HSPN9 The Role of Micro-neurolysis for Hourglass Constrictions in Neuralgic Amyotrophy

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**Purpose:** Wide variability in recovery of patients affected by Neuralgic Amyotrophy (Parsonage Turner Syndrome) is recognized, with up to 60% experiencing residual motor deficits or pain. Using high-resolution MRI and ultrasound (US), we routinely identify hourglass constrictions (HGCs) in affected nerves of patients with NA. We hypothesized that patients with chronic NA and HGCs would experience motor recovery and functional improvement following microsurgical epi- and perineurolysis of the constrictions.

**Methods:** Ten patients (5 F), ages 21-61 years, with chronic, persistent motor palsy from NA were treated with microsurgical epi- and peri-neurolysis of HGCs. Average time from symptom onset to surgery was 12.2 ± 4.1 months. Preoperative electrodiagnostic (EDX) testing and manual motor testing confirmed complete muscle denervation in the distribution of affected nerve(s). HGCs were identified in one or more nerves in all patients using 3.0 T MRI and US. Microneurolysis was indicated for the following: failure to improve clinical and EDX function after 6 months with 3 successive exams, each at least 6 weeks apart (n = 3), or 12 months without improvement since symptom onset (n = 7). Recovery was assessed pre-and postoperatively using the modified Medical Research Council (MRC) scale and EDX. Changes in MRC and EDX classifications were assessed using a two-tailed Wilcoxon signed-rank test.

**Results:** Average postoperative clinical and EDX follow-up was 13.6 months (range, 4-29). Thirty-five HGCs in 14 nerves were identified on imaging and confirmed intra-operatively, involving the pronator teres and anterior interosseous fascicles of the median nerve and suprascapular, axillary and radial nerves proper. One patient presented initially with bilateral disease. 8/10 patients experienced functional recovery and 8/9 experienced electrical recovery in the majority of affected muscles. Average MRC increased from 0.0 to 3.6 ± 1.4 (p<0.01). EDX revealed significant motor unit recovery from axonal regeneration in 25/31 muscles (p<0.01).

**Conclusion:** High resolution MRI and US detected HGCs of peripheral nerves and nerve fascicles in NA patients with chronic, recalcitrant motor palsy. Microsurgical epi- and perineurolysis of HGCs in this small patient cohort was associated with significant electrical and clinical muscle recovery at an average follow-up of 13.7 months. We conclude that the HGC is unique to NA, and recommend microsurgical epi- and perineurolysis of HGCs for patients with NA who fail to improve with non-operative treatment.
Introduction: The sense of touch and proprioception permit meaningful interaction with the environment. Without meaningful and intuitive sensory feedback, even the most advanced prosthetic devices remain insensate, burdensome, and are associated with enormous cognitive demand and mental fatigue. We hypothesize that restoring sensory feedback to the user will not only improve functional prosthetic performance, but also improve prosthesis embodiment, or integration of the prosthesis to the user’s body imagery. Currently, direct peripheral nerve electrodes have been able to increase prosthesis embodiment, and reduce phantom limb pain through electrical stimulation. However, they have not been able to also produce high fidelity motor signals for prosthesis control. To provide a bidirectional motor and sensory neural interface, we have developed the Regenerative Peripheral Nerve Interface (RPNI). A muscle graft reinnervated by a transected peripheral nerve, RPNI’s have previously demonstrated stable high amplitude motor EMG signals with high signal to noise ratio in human studies.

Materials and Methods: In the current study, we tested if electrical stimulation of RPNI grafts produces proprioceptive and/or tactile sensations. Two distal transradial participants underwent surgical implantation of RPNIs for treatment of neuroma pain. The RPNIs were stimulated with a monopolar, charged balanced, biphasic square wave using intramuscular bipolar electrodes. Participants reported the location, and a description of the invoked sensation. A stepwise increment of 0.1 mA was used at a constant 20Hz frequency and 200 µS pulse-width to determine the sensory perception threshold.

Results: In both participants, proprioceptive sensation was reported in their referred phantom limb. In particular, stimulation of the median RPNI activated a “bending” sensation in the thumb or index finger of participant 1, while stimulation of the ulnar RPNI invoked a “bending” sensation of the ring or small finger. Likewise during stimulation of ulnar RPNI 1, participant 2 felt a “tugging” sensation at the ring distal interphalangeal joint, while stimulation of ulnar RPNI 2 and the median RPNI produced cutaneous sensations on the lateral side of the small finger and in the palm area below the thumb, respectively. Sensory stimulation thresholds remained stable across 7 and 11 months with an average amplitude of 1.27 ± 0.52 mA for participant 2, and 0.98 ± 0.04 mA for participant 1, respectively.

Conclusions: These results suggest that RPNIs have the potential to restore proprioceptive and cutaneous sensory feedback that could increase prosthesis embodiment and motor performance simultaneously, producing a stable bidirectional interface for advanced prosthesis control.
INTRODUCTION: An understanding of the injury features, patient factors, and patient goals are important in selecting the optimal treatment in the context of peripheral nerve injuries. While robust work has been done to elucidate prognostic features of nerve injuries there remains a paucity of evidence addressing the influence of patient factors such as comorbidities. The objective of this study was to evaluate the impact of body mass index (BMI) and comorbidities on the clinical outcomes of upper extremity nerve transfers.

MATERIALS & METHODS: The study design was a retrospective cohort of prospectively collected data. Medical records were reviewed for all patients undergoing nerve transfer surgery by a single surgeon (2012-2018). Patients were eligible for inclusion if they had undergone an upper extremity nerve transfer with a minimum of 12-months follow-up. Data was collected by two independent reviewers regarding demographics, comorbidities, injury etiology, nerve transfer, as well as preoperative and postoperative clinical assessments. The primary outcome measure was postoperative strength of the recipient nerve innervated musculature (Medical Research Council [MRC]). Statistical analysis used descriptive statistics and non-parametric tests.

RESULTS: Thirty-eight patients undergoing 43 nerve transfers were eligible for inclusion. Patients had a mean age of 48.8 years and mean BMI of 27.4 kg/m². Injuries involved the brachial plexus (32%) or its terminal branches (68%) and the most common etiologies were trauma (50%), compression (26%), and brachial neuritis (21%). Anterior interosseous nerve to ulnar motor nerve (35%) was the most common nerve transfer performed. Patients with acute etiologies underwent surgery a mean of 6.7 months following their injury and those with chronic compression underwent surgery a mean of 20.7 months following onset of symptoms. With a mean follow-up of 20.1 months following nerve transfer surgery, the mean MRC increased significantly (p=0.000) from 1.0 to 3.3. Increased BMI was significantly associated with poorer postoperative strength in the recipient nerve innervated musculature (r=-0.320, p=0.036). Mean postoperative MRC amongst active smokers (2.6) was significantly lower (p= 0.021) than non-smokers (3.6). There were no significant differences in outcomes based on the presence of other comorbidities.

CONCLUSIONS: This retrospective cohort study demonstrated that increased BMI and smoking may be associated with worse outcomes in upper extremity nerve transfers. To facilitate patient selection and guide expectations regarding prognosis, further experimental and clinical work is warranted to understand the potential influence of BMI and smoking on recovery following nerve injury and nerve transfer surgery.
Introduction: Enhanced Recovery After Surgery (ERAS) protocols have recently emerged as patient-care pathways to improve surgical outcomes and reduce length of stay. A novel ERAS protocol has been put into place for all patients undergoing elective spine and peripheral nerve surgery at a single academic institution since April 2017. The authors sought to assess the efficacy of their neurosurgical ERAS protocol in patients undergoing common peripheral nerve procedures.

Methods: Patients who underwent elective peripheral nerve surgery at a single academic institution between April 2017 and December 2018 were prospectively enrolled in a unique ERAS protocol. The control group was a historical cohort of patients who underwent elective peripheral nerve surgery at either the same hospital or a sister institution between September 2016 and December 2016. Surgeries included upper and lower extremity nerve decompression/transfer, nerve biopsy, peripheral nerve tumor resection, and brachial plexus surgery. The primary outcomes were length of stay and discharge disposition. The secondary outcomes included pain score at discharge and self-reported opioid use at one month after surgery.

Results: A total of 50 peripheral nerve surgery patients were enrolled into the ERAS protocol. The historical control group comprised 15 peripheral nerve surgery patients. The two groups were similar in baseline demographics and surgical type (p=0.07). In compliance with the ERAS protocol, the use of a PCA postoperatively was completely eliminated (ERAS 0.0% vs. control 46.7%, p<0.001) and that of IV narcotics was significantly reduced (ERAS 30.0% vs. control 73.3%, p=0.006). The ERAS group demonstrated decreased length of stay in the hospital compared to the control group (1.3 vs. 2.3 days; p=0.018). Although most patients in both groups were discharged home (ERAS 96.0% vs. control 93.4%), 0.0% of the ERAS patients, compared to 47.6% of the control patients, needed to be discharged with health services. Interestingly, the average pain score reported at discharge was lower for the ERAS group compared to the control group (2.1 vs. 6.0, p<0.001). Lastly, a smaller proportion of the ERAS group reported opioid use at one month postoperatively compared to the control group (ERAS 31.7% vs. control 73.3%, p=0.007).

Conclusion: This study suggests that implementation of the ERAS protocol in the peripheral nerve surgery population facilitates expeditious discharge out of the hospital, without the need for postoperative health services, and reduces opiate use at one month postoperatively.
#HSPN13 Range of Independence with Feeding, Bladder Management and Transfers by Motor Level in Cervical-Level Spinal Cord Injury

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**Background:** The advent of upper limb nerve transfer surgery to improve function may transform management of cervical spinal cord injury (SCI). Surgery can restore elbow and wrist extension and finger flexion and extension. Information on the implications of having these movements on activities of daily living (ADL’s) is limited. The objective of this study was to assess the degree of gains in independence for a given level of upper extremity motor function.

**Methods:** Using the European Multi-center Study about Spinal Cord Injury (EMSCI) data set*, analysis was undertaken of eligible individuals with traumatic C5-C8 SCI to ascertain motor function recovery (6 and 12 months after injury, n = 388). Data on feeding, bladder management and transfers (bed to chair) were compared at 6 months and 12 months post-injury for each motor level. Subgroup analyses were performed: symmetric vs. asymmetric SCI; complete vs. incomplete SCI. The impact of age, gender, and degree of asymmetry on functional independence were analyzed.

**Results:** Independent feeding with or without assistive devices was noted in individuals with strong wrist extension (C6); feeding independently required strong finger flexion (C8). With bladder management, strong finger flexion (C8) was required for independence. Individuals that were younger, male or had trunk control (asymmetric SCI) had greater independence with bladder management. With transfers (bed to chair), elbow extension (C7) did not uniformly result in transfer independence, whereas finger flexion (C8) did. Subgroup analysis showed that people with younger age and/or trunk control also had improved ability to transfer. There was no significant increase in independence between 6 and 12 months with any activities, though a trend towards gain in function was seen.

**Conclusion:** Although independence with transfers might be expected in individuals with intact elbow extension movement, this was not seen. The presence of finger flexion had the most profound effect on independence with transfers, feeding and bladder function. This information that will be useful when counseling people with SCI who are considering surgical treatment for restoration of upper extremity motion.

*The EMSCI database includes rigorously and prospectively collected neurological and functional independence measurements.
INTRODUCTION: Treatment outcomes and quality of life for traumatic brachial plexus injury (BPI) patients vary widely. These outcomes have not been shown to depend strictly on severity of the injury, extent of surgical treatment, or other easily quantifiable measure. Our objective was to build a greater understanding of each patient’s experiences in order to identify common qualitative factors and themes in patients’ lives that affect outcomes.

MATERIALS AND METHODS: Qualitative interviews were conducted with 10 BPI patients who were 6+ months after reconstructive surgery. The interview guide contained questions regarding patients’ experience with their BPI, from the initial injury to the interview date. Inductive thematic analysis was used for the qualitative data to identify themes and knowledge gaps.

RESULTS: Patients described varying levels of understanding about their condition and prospects for recovery (Figure 1). Their knowledge was based on a variety of sources, with widely varying levels of accuracy. Many patients wanted a detailed explanation of what their ultimate injury outcome would be, or expressed a desire to have received “realistic” descriptions of their outcomes earlier in their treatment timeline. Some felt that having that information would allow them to make plans for their future, or come to terms with their “new normal”. Patients also described conversations with their surgeons regarding their expectations and estimated odds of recovery, which did not necessarily align with providers’ estimates of recovered function or degrees of recovery.

CONCLUSIONS: These results indicate substantial and highly variable knowledge gaps for BPI patients, and a communication disconnect that can lead to higher levels of patient frustration, decreased compliance with surgeon recommendations, and ultimately a detrimental effect on patient outcomes. Educating patients about their condition, the potential degrees of recovery they could see, and how their compliance with recommendations can specifically affect their recovery earlier in the patient’s treatment timeline has the potential to lead to more positive patient outcomes. Based on these findings, we are developing a patient-centered education tool specific to BPI that covers the knowledge gaps brought forward by patients.
#JOP PN2 A Nerve Wrap for Localized FK506 Delivery to Enhance Peripheral Nerve Regeneration

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Introduction: FK506, an FDA-approved drug, encapsulated in biodegradable microspheres and hydrogel, enhances peripheral nerve regeneration in rats. Though effective, this process is not yet user-friendly enough for human surgical use. We hypothesize that incorporating FK506 within a nerve wrap will be simpler and more clinically-feasible to deliver FK506 locally and improve regeneration following microsurgical repair. We aim 1) to develop an implantable FK506 delivery nerve wrap with properties for clinical application, 2) to sustain bioactive FK506 release, and 3) that the wrap be biocompatible and biodegradable.

Material & Methods: 1) Coaxial electrospinning, a one-step process to create core-shell fibrous mats, was used to fabricate the wrap with the biodegradable synthetic polymer polycarbonate urethane (PCNU) which encapsulated FK506 in the inner shell to protect it from burst release within an outer polymer shell. Scanning electron microscopy determined fiber diameter and porosity. Tensile tests measured the dry elastic modulus. Thermogravimetric analysis and differential scanning calorimetry analyzed thermal properties. 2) \textit{In vitro} incubation and mass spectrometry determined the encapsulation efficiency of FK506 and the release profile. 3) Nerve wraps were implanted around rat sciatic nerves for 7 and 60 days. Hematoxylin & eosin staining was used to determine the \textit{in vivo} inflammatory response and to quantify biodegradation and nerve compression.

Results: 1) The fiber diameter and porosity were $320 \pm 70$ nm and $40 \pm 10\%$ (means ± standard deviation) respectively, and the dry elastic modulus was $2.38 \pm 1.05$ MPa. Physical properties of the nerve wrap indicated a longer degradation rate to prolong FK506 delivery and high tensile strength to withstand surgical forces. Onset of degradation for PCNU-FK506 nerve wraps occurred at $260^\circ$C with a melt temperature of $43.16^\circ$C, indicating thermal stability at physiological temperature ($37^\circ$C). FK506 encapsulation efficiency was $92 \pm 14\%$, indicating complete availability of FK506 to encourage nerve regeneration following implantation of the wrap. 2) Burst release was observed within 24 hours, but ~4.8ng FK506 remained after 28 days of incubation, still sufficient to enact a neurotrophic effect. 3) Major inflammation was not observed after 7 and 60 days of implantation and the PCNU-FK506 nerve wraps degraded by 58% over 60 days without causing morphological nerve compression.

Conclusions: The electrospun PCNU and FK506 fibers have the potential to form clinically useful and feasible nerve wraps that enhance peripheral nerve regeneration due to their simplicity, ideal physical properties, high FK506 loading, and general biocompatibility and biodegradability.
Background: Despite curative intents of treatment in localized malignant peripheral nerve sheath tumors (MPNST), local recurrences and distant metastases are very common and survival remains poor. A better understanding of the clinical prognostic factors of this rare sarcoma is necessary to ameliorate clinical decision-making. This study set out to investigate overall survival, treatment modalities, and factors associated with survival in non-retroperitoneal and retroperitoneal MPNSTs using a Dutch nationwide cohort of patients.

Methods: Data were obtained from the Netherlands Cancer Registry (NCR) and the Dutch Pathology Database (PALGA). All pathologically confirmed primary MPNSTs were collected. Patient and tumor characteristics, treatment modalities, and survival were extracted from the database and pathology reports. Pediatric cases (age ≤18 years) and patients with synchronous metastases were excluded from analyses. Separate Cox proportional hazard models were made for localized non-retroperitoneal (Figure 1) and retroperitoneal MPNSTs (Figure 2). A conditional inference tree was constructed for non-retroperitoneal localized MPNSTs (Figure 3).

Results: A total of 784 patients (26.8% neurofibromatosis type 1, NF1) had a final pathological diagnosis of MPNST. In 72 (9.2%) cases, MPNSTs arose within neurofibromas. Most MPNSTs arose in truncal sites (n=355, 45.2%) of which 43 (5.5%) were situated retroperitoneal. Most tumors were large (>5cm, 67.9%) and deep-seated (under the fascia, 75.2%). In 11.5%, patients presented with synchronous metastases. In localized MPNST surgical resection was performed in 88.1%. In surgically treated patients, radiotherapy was administered in 44.2% and less commonly in retroperitoneal tumors (29.6%, p<0.05). Chemotherapy was used in 6.7% of resected MPNSTs and more commonly in retroperitoneal tumors (18.5%, p<0.05). In non-retroperitoneal MPNST, older age (≥60), NF1 patients, large, and deep-seated tumors were independently associated with worse survival (p<0.05). MPNSTs arising within neurofibromas tended to do better (p>0.05). In retroperitoneal MPNST male sex and age ≥60 years old were independently associated with worse survival. R1 resections were not associated with worse survival in both non-retroperitoneal and retroperitoneal MPNST. Chemotherapy and radiotherapy use were not associated with survival in both models.

Conclusion: In localized MPNST, risk stratification for survival can be done using several patient- and tumor specific characteristics. Although MPNSTs can present diversely, achieving at least an R1 resection is the most important predictor for survival. Controlling for several factors, no significant difference in survival is seen between R0 and R1 resections. This is true for both retroperitoneal and non-retroperitoneal MPNSTs.
Introduction: There is a fundamental gap in understanding how to provide prosthetic limbs with intuitive afferent somatosensory feedback essential for interaction with the environment, while simultaneously acquiring efferent motor signals for prosthetic control. The composite regenerative peripheral nerve interface (C-RPNI), created by surgically implanting the distal end of a transected peripheral nerve in between an autogenous free muscle and dermal skin graft, is a novel construct designed to overcome this problem. The long-term goal of this research is to develop a single biologic interface where we can record from C-RPNIs to provide high fidelity motor control of a prosthetic limb, while simultaneously stimulating the dermal component of the C-RPNI to provide sensory feedback.

Materials & Methods: C-RPNIs were surgically implanted on the end of the transected peroneal nerves of 16 rats, using free muscle grafts obtained from the animal’s contralateral limb, and de-epithelialized dermal grafts harvested bilaterally from the glabrous skin of 8 donor rat hindpaws. At three and six months post-surgery, the C-RPNI constructs were electrophysiologically evaluated ex-vivo by stimulating: 1) nerve, 2) skin, and 3) muscle, while simultaneously recording signals from: a) muscle and skin, b) nerve and muscle, and c) nerve and skin, respectively. After six months, eight C-RPNI constructs were harvested, and labeled with fluorescent antibodies targeting nerve fibers, neuromuscular junctions, and motor axons using the iDISCO tissue clearing method.

Results: Electrophysiological evaluations at 3 and 6 months revealed muscle, skin and nerve capable of generating peak CMAP amplitudes and conduction velocities of 7.9±2.2 mV and 9.8 ±1.3 m/s, and CSNAPs with 133±30 µV peaks at 9.8 ±1.3 m/s velocity [Fig. 1]. End-point histology displayed healthy vascularized muscle maintaining 73±9% original muscle mass, and preferential migration of sensory and motor fibers into the skin and muscle portions of the construct, respectively [Fig. 2].

Conclusions: Mixed sensorimotor nerves demonstrated preferential motor reinnervation of muscle and sensory reinnervation of skin in the C-RPNI. Electrophysiological evaluations of these constructs revealed stable and appropriate afferent and efferent signals over a six month period, confirming the potential of C-RPNIs to provide closed-loop neural control of prosthetic devices.
Figure 1. C-RPNI construct and ex-vivo CMAP and CSNAP recordings. A. In-situ rat C-RPNI: De-epithelialized dermal graft surgically sutured to muscle graft and reinnervated with peroneal nerve. B. Stimulation and recording schematic for a C-RPNI ex-vivo. Skin electrodes are used as stimulators. Muscle electrodes record EMG. The neural cuff electrode can act as both stimulator (for CMAP recordings from muscle) and signal recorder (for CSNAPs resulting from an electrical stimulus on skin). C. CMAP recordings obtained from muscle component of C-RPNI. D. CSNAP recordings in response to a biphasic electrical stimulus delivered to the skin graft component of the C-RPNI, using a platinum wire electrode.

Figure 2. Histological Assessment of C-RPNI constructs A,B. Representative H&E sections showing vascularization, nerve regeneration and a clear distinction between skin and motor C-RPNI construct components. C. C-RPNI construct immunohistochemically prepared using the DISCO whole tissue clearing method, and imaged using confocal microscopy. The image shows neural reinnervation to both the muscle portion (bottom of section) and skin portion (top of section). D. Planar C-RPNI section within the construct imaged at the interface between the skin (light gray) and muscle (brown) portions of the C-RPNI. Neurofilament stained axons (white) are innervating the skin component of the C-RPNI; double stained motor axons for Choline Acetyl-Transferase (orange) are exclusively innervating muscle.
**Introduction:** After a nerve defect is bridged with an allograft nerve, neovascularization precedes neural changes to produce a supportive microenvironment for nerve regeneration to occur. Little is known about the revascularization patterns after such an injury. The aim of this study was to explore the revascularization patterns after surgical angiogenesis of a nerve allograft at various time points.

**Materials and Methods:** In 51 Lewis rats, 10 mm sciatic nerve gaps were repaired with a (i) nerve autograft, (ii) nerve allograft and (iii) surgically revascularized nerve allograft using a pedicled superficial inferior epigastric artery fascia flap (SIEF). At two, 12 and 16 weeks, the rats were sacrificed and Microfil® compound was injected to preserve vascularization. With micro computed tomography (micro CT), the vascular volume was measured in harvested nerve samples and compared to the unoperated side. Cross-sectional images were obtained over the length of the nerve. These images were divided into three concentric rings and the number of vessels in each ring was counted in the proximal, mid and distal sections of the nerve samples.

**Results:** At two weeks, the vascular volume in control nerves was superior to autograft and allograft, and similar to revascularized allograft nerves (SIEF group). At 12 weeks, the vascular volume in SIEF nerves was statistically higher than allografts (P<0.05) and at 16 weeks the volume in SIEF nerves was superior compared to other groups (P<0.0001). The cross-sectional images showed that the number of vessels in surgically revascularized allografts was statistically increased compared to allografts alone in all rings in the proximal section of the graft. In the mid-section of the graft, the number was statistically superior in the middle and central ring. No significant differences between groups were seen in the distal section of the nerve grafts. At sixteen weeks, these patterns were more evidently significant.

**Conclusions:** Surgical angiogenesis of nerve allografts greatly improves revascularization. In this study revascularization occurred primarily from proximal to distal (proximal inosculation) and not from both ends as previously believed. It also confirms the theory of centripetal revascularization.
Scientific Paper Session II
#18 Development of novel microelectrode arrays for intraneural recording of autonomic neural activity
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Introduction: Vagus nerve stimulation (VNS) was first approved by the FDA to treat epilepsy in 1997. It has since been approved to treat depression, with substantial research demonstrating benefits in treating tinnitus, diabetes, and rheumatoid arthritis. As this field of bioelectronic medicine grows, so does the demand for advanced electrical interfaces that can provide highly selective stimulation and monitoring to improve clinical therapy. Unfortunately, current cuff and intrafascicular electrodes for autonomic nerves lack spatial resolution for extracting detailed neural activity. We are developing two microneedle arrays for interfacing with small autonomic nerves such as the vagus.

Materials and Methods: We hypothesized that axon-sized needles will minimize tissue reactivity around implanted electrodes, and thus improve the recording and stimulation characteristics in both acute and chronic applications. Using a custom 3D-printed nerve hook, we implanted non-functional microneedle nerve arrays (MINA) of 24 electrodes of 140 mm length into the left cervical vagus nerve of rodents (Figure 1). Micro-CT and histomorphometry of the electrode needles were assessed at both 1 and 6 weeks post-implantation. We also implanted functional 200 mm length carbon fiber microelectrode arrays (CFMA) in rodent cervical vagus and peroneal nerves for acute recordings.

Results: MINA arrays remained intact in the vagus nerve at 1 week and adjacent at 6 weeks. We have observed neural recordings with CFMA in the vagus nerve in response to glucose infusions and KCl application on the nerve, and in the peroneal nerve due to cutaneous brushing. Development of functional MINA and chronic CFMA are on-going.

Conclusions: Both microelectrode arrays show potential for high-fidelity interfacing in the vagus nerve and other autonomic nerves across acute and chronic timeframes. These advanced neural interfaces may lead to more efficient neural stimulation therapies for targeting a broad range of autonomic nerve disorders.
#19 Comparison of Amniotic Membrane and Collagen Nerve Wraps Around Sciatic Nerve Reverse Autografts in a Rat Model

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Introduction: Clinically, repair of peripheral nerve injuries is problematic due to the slow rate of axon regeneration, irreversible muscle fibrosis, and axonal misrouting. Nerve wraps provide a protective encasement around peripheral nerves following neurorrhaphy, which mitigates epineural scarring and adhesions, facilitates axonal regeneration, and improves functional recovery. Various types of nerve wraps are available for use in clinical practice. Human amniotic membrane (hAM) and collagen matrix are easily obtainable FDA approved biomaterials with no donor site morbidity and minimal inflammatory response. However, their efficacy has not been compared. Collagen nerve wraps are semi-permeable and allow for diffusion of neurotrophic factors. hAM nerve wraps provide a neurotrophic effect, containing mesenchymal stem cells (MSCs) which can secrete neurotrophic factors, differentiate into neural phenotypes and enhance Schwann cell proliferation. Here we compared the efficacy of collagen and hAM nerve wraps in a rodent sciatic nerve reverse autograft model, with the hypothesis that the use of hAM nerve wraps would result in improved nerve regeneration and functional recovery compared to collagen or control groups.

Materials & Methods: Lewis rats (n = 29) underwent sciatic nerve injury and repair in which a 10-mm gap was bridged with reverse autograft combined with either no nerve wrap (control), collagen nerve wrap or hAM nerve wrap. Behavioral analyses were performed at baseline, 4, 8 and 12 weeks. Electrophysiological studies were conducted at 8, 10 and 12 weeks. Gastrocnemius muscle weights were evaluated at 12 weeks, and fibrosis, inflammation and axonal regeneration were investigated via histological and immunohistochemical analyses.

Results: Application of hAM nerve wraps in a rodent sciatic nerve reverse autograft model results in improved functional and histological outcomes compared to control and collagen groups at 12 weeks. We identified significantly greater numbers of axons and reduced adhesions in hAM-treated rats compared to collagen-treated rats and controls at 12 weeks (p< 0.05). This correlated with significantly improved functional outcomes and ratios of experimental to control gastrocnemius muscle weights in the hAM group compared to the collagen and control groups (p< 0.05).

Conclusions: Comparative analysis of controls and collagen and hAM-treated groups suggests that the use of hAM nerve wraps results in improved nerve regeneration and functional recovery following peripheral nerve injury and repair. The superior outcomes of hAM-treated groups indicate that hAM mitigates fibrosis and may enhance nerve regeneration due to its anti-inflammatory and pro-regenerative effects, making it a promising biomaterial for clinical applications in peripheral nerve repair.
#20 Wrapping Nerves with Conduits Results in Worse Histologic and Functional Outcomes
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Introduction: The concept of utilizing a nerve conduit for augmentation of a primary nerve repair has been advocated as a method to prevent neural scarring and decrease adhesions. Despite clinical use, little is known about the effects of a nerve conduit wrapped around a primary repair. To better understand this, we investigated the histologic and functional effects of use of a nerve conduit wrapped around a rat sciatic nerve repair without tension.

Methods: Twenty Lewis rats were divided into two groups of 10 rats each. In each group, unilateral sciatic nerve transection and repair was performed, with the opposite limb utilized as a matched control. In Group 1, direct repair alone was performed, and in Group 2, this repair was augmented with a porcine submucosa conduit wrapped around the repair site. Sciatic functional index was measured at 6 weeks with walking track analysis in both groups. Non-survival surgeries were then performed in all animals to harvest both the experimental and control nerves to measure histomorphometric parameters of recovery. Histomorphometric parameters assessed included total # of neurons, nerve fiber density, nerve fiber width, G-ratio, and % debris. Unpaired t-test was used to compare outcomes between the two groups.

Results: Representative photomicrographs from the second experiment, in which sharply transected nerves were repaired directly with or without a conduit wrap, are seen in Figure 1. The density of nerve fibers was significantly lower in the group with the conduit wrap (p<0.001) (Figure 2) while the fiber width was significantly higher (p<0.001) (Figure 3). The total number of neurons in each did not differ (p=0.52) (Figure 4), but the G-ratio was significantly different between the groups, with the conduit wrap group having a higher G-ratio (p=0.002) consistent with less myelin regeneration (Figure 5). The normalized % debris was significantly higher (p < 0.001) (Figure 6) and the SFI significantly worse (p < 0.001) when a conduit wrap was applied (Figure 7).

Conclusions: Utilization of a conduit wrap after a direct nerve repair was associated with worse remyelination, increased debris, as well as decreased fiber density and worse functional outcomes. More investigations should be performed before routinely recommending the use of a conduit nerve wrap after a primary nerve repair.
Scientific Paper Session II

#21 Differential gene expression changes between motor and sensory components of Composite Regenerative Peripheral Nerve Interfaces (C-RPNIs)

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Introduction: Critical to the design of an ideal bioprosthetic device is the development of a single interface between human and machine that allows for transmission of both afferent somatosensory information and efferent motor signals for device control. The Composite Regenerative Peripheral Nerve Interface (C-RPNI) is a novel biologic interface that demonstrates promise in this role. The C-RPNI is a surgical construct composed of a transected, mixed peripheral nerve implanted between a composite free graft consisting of de-epithelialized glabrous skin and skeletal muscle. We have previously shown that C-RPNI constructs remain viable for at least 6 months, and are capable of producing both afferent and efferent electrophysiological activity. Previous research has shown that motor and sensory Schwann cells (SC) express different phenotypes which regulate axonal regeneration. In the current study, we performed in-depth molecular analysis to assess potential differences in factors such as cytokines, regeneration associated genes (RAGs), and growth factors between the motor and sensory components of C-RPNIs.

Materials and Methods: C-RPNIs were implanted in 8 animals and allowed to mature for 3 months. They were subsequently harvested and separated into separate muscle and skin components. Competitive reverse transcriptase-PCR (RT-PCR), 10x Genomics, and RNAseq bioinformatics analysis was performed. A total of ~500 million reads were generated from the 10X Genomics sequencing analysis for each of the C-RPNI replicates. Sequencing data was processed with Cell Ranger software, with further downstream analysis performed using the Seurat R package. All cells with less than 500 genes per cell and more than 25% mitochondrial read content were excluded.

Results: Substantial differences were found for both up and down regulated factors between the motor and sensory C-RPNI components. Briefly, mRNA for factors like nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), Oct6, and vascular endothelial growth factor (VEGF) were expressed vigorously by the dermal sensory component. In contrast, mRNA for pleiotrophin (PTN), glial cell-line derived neurotrophic factor (GDNF), c-maf, and connexin-43 was upregulated to a greater degree in the C-RPNI motor component.

Conclusions: These findings suggest that both the muscle and dermal components of C-RPNIs upregulate factors in a manner similar to motor and sensory axons during axonal regeneration following nerve injury. These results provide additional support that C-RPNI constructs are appropriate surgical interventions to establish closed loop neural control of prosthetic systems.
#22 Conditioning electrical stimulation promotes sensory and motor nerve regeneration without inducing inflammation

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**Conditioning electrical stimulation promotes sensory and motor nerve regeneration without inducing inflammation**

**Background:** Conditioning electrical stimulation (CES) delivered prior to a nerve repair promotes functional nerve regeneration beyond that of a conditioning crush lesion (CCL). Forty years of research has established that the inflammatory response evoked by the CCL injury prior to the cut and coaptation is essential to elicit the conditioning response. It is not known how CES promotes nerve regeneration.

**Hypothesis:** CES promotes functional nerve regeneration in a non-injurious, and non-inflammatory manner.

**Methods:** Sprague Dawley rats were equally divided into three cohorts: i) CES, ii) CCL (positive control) and iv) sham-ES (negative control). CES, CCL and sham-ES conditioning were delivered one week prior to nerve cut/coaptation. The conditioning site and the corresponding dorsal root ganglion neurons were harvested at 1 day, 3 days, and 14 days post-repair (n=10/cohort). Immunocytochemistry for macrophages and neurofilament to investigate inflammation and Wallerian degeneration respectively was performed. Data was corroborated by western blot analysis. CCR2-/- and RAG1-/- mice (n=3/cohort) were conditioned with CES to determine if monocytes or lymphocytes, respectively, are necessary to mount a conditioning effect and upregulate regeneration associated genes at the DRG.

**Results:** Rats treated with CCL alone at all time points evoked an inflammatory response. Unlike CES and sham-ES, there was significant macrophage infiltration at 1, 3, and 14 days following CCL at the conditioning site (p<0.001). Furthermore, although CCL induced Wallerian degeneration distal to the conditioning site, CES and sham-ES did not cause degeneration of the distal axons. The CES treated CCR2-/- and RAG1-/- mice both showed the same upregulation of pCREB (p<0.001) and ATF3 (p<0.001) as their corresponding C57/Bl wildtype mice, suggesting CES does not require the immune response to evoke the conditioning effect.

**Conclusion:** CES significantly improves regeneration and reinnervation beyond that attainable with current clinical standards. CES is a clinically feasible method of improving outcomes for patients with peripheral nerve injury as it does not evoke an inflammatory response or Wallerian degeneration. Further investigation is required to delineate the molecular and cellular mechanisms underlying the ability of CES to promote regeneration without evoking an immune response.
#23 Microparticles Releasing GDNF and NGF Improve Axonal Growth and Functional Recovery after Sciatic Nerve Injury in Rats

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Introduction: Autologous nerve grafts and end-to-end neurorrhaphy are considered the gold standard for large nerve gaps (i.e. >5 mm gaps) for peripheral nerve injury repair. During nerve regeneration, the local presence of growth factors plays an important role. In order to aid in delivery of growth factors, we have modified standard surgical micro-sutures with calcium phosphate (CaP) coatings, which we have designed to deliver cytokines in a temporally modulated manner, specifically GDNF (glial derived neurotrophic factor) and NGF (nerve growth factor). In this study, rat sciatic nerve injury is repaired with autograft with sutures coated with GDNF and NGF with the goal of promoting more axonal growth and improved functional outcomes compared to control rats repaired with non-coated suture.

Methods: To create the injury, all rats had 6 mm of the right sciatic nerve removed, and then a 10 mm isograft from a donor rat microsutured end-to-end with two standard 9-0 Nylon sutures on the proximal end and two sutures of the correct treatment on the distal end. There were 5 groups (control, CaP only, GDNF, NGF, GDNF + NGF), each group consisted of 6-8 rats. Functional testing was done weekly for each rat from week 5 to 12 post repair. The experiment was terminated at 12 weeks and axonal growth was assessed with electron microscopy

Results: Rats treated with MCM sutures coated with GDNF + NGF had significantly increased (p=0.0014) axonal counts (mean: 8511) in the sciatic nerve distal to neurorrhaphy compared to controls mean: 5123). Functional testing as measured by ankle contracture, showed statistically significant improvement (p = 0.0014) starting at week 7 and continuing to week 12 compared to control groups.

Conclusions: Mineral coated microparticle (MCM) sutures releasing GDNF increase the amount of axons in the graft and distal to the graft. MCMs releasing NGF and GDNF increase functional recovery after week 7.
Macrophages and Schwann cells play a key role in the orchestration of early events after peripheral nerve injury. One opportunity for functional improvement after nerve reconstruction is manipulation of the microenvironment at the site of nerve repair to promote modulation of the host inflammatory response and promote Schwann cell (SC) migration and axon extension across the repair site. We have recently shown that this is an effective approach. Here we determined the effects of a decellularized peripheral nerve matrix (PNM) hydrogel on macrophage gene expression, phenotype and migration using a combination of FACS, Nanostring technology and immunohistochemistry. We also evaluate effects on SC migration, numbers of motor neurons reaching their target, axon extension, neuromuscular junction formation, compound motor action potential and peak tetanic force using a combination of mouse and rat models.

Finally, functional recovery was determined after both sciatic crush injury and common peroneal nerve transection associated with a short gap. Control groups including uninjured animals, exposure of the nerve without injury, transection and ligation without repair and delivery of hydrogel to uninjured nerves were also performed (n=8/group). Animals were followed for 12 weeks and assessed longitudinally using multiple measures of sensory and functional recovery. Metrics included von Frey nociception assay, sciatic functional index, and kinematic analysis. At 12 weeks animals were subjected to electrophysiologic assessment of evoked compound motor action potential prior to euthanasia for tissue collection and subsequent histologic analysis.

Macrophage, particularly M2 (regenerative phenotype), recruitment to the injury site was increased in both mice and rats (2 fold increase, p<0.05). PMN promoted SC migration both in vitro and in vivo (p<0.05). Axon extension determined by extension of GFP+ axons across an 8mm gap was increased in the presence of PNM compared with an empty conduit (>5 fold increase, p<0.05). PNM increased the number of retrograde labelled MN at 12 weeks after injury compared to empty conduit (20%, p<0.05).

The amplitude of Compound Motor Action Potentials across the sciatic transection injury site, demonstrating axonal regrowth to the terminal muscle was increased by 50% compared to control following subepineural delivery of PNM 8 weeks after nerve transection. Peak Tetanic force was also increased by 30% at 4 and 8 week time points.

These results demonstrate that an injectable, peripheral nerve matrix hydrogel derived from porcine sciatic nerve can modulate the response of two key cell types which conduct the early response to nerve injury.
Introduction:

Following denervating injury of the sciatic nerve, several proteins are modulated in the distal region but not in the proximal injured nerve. Using next-generation proteomics analyses and biomaterial stimulated regenerative response in rat critical gap (15 mm) injury model, we have identified Erythropoietin (EPO) protein to be associated in the activation of PI3K/Akt pathway. Therefore, it is hypothesized that overexpression of EPO in conjunction with Nerve Growth Factor (NGF) will act as upstream regulator of the PI3K/Akt pathway in peripheral nerve injury model inducing tissue regeneration. To reveal the regenerative effect of EPO and NGF proteins on sciatic nerve injury, functional and biological response of neurons and schwann cells were assessed in vitro.

Materials and Methods:

Protein expression of EPO and NGF non-viral vector (Sino Biological) transfected neurons (E14 rat DRGs) and primary schwann cells were analysed using Operetta High Content Analysis System (PerkinElmer). Myelination of schwann cells as functional response to EPO/NGF modulated expression was assessed by immunocytochemistry, western blotting and qPCR. Also, functional response of neurons to EPO/NGF transfection was evaluated using live calcium imaging, microelectrode arrays and qPCR techniques.

Results:

Immunocytochemistry results showed that DRG neurons and schwann cells express EPO protein in their cytoplasm. The highest axonal elongation in neurons (Figure 1 A,B) and myelination rate in schwann cells (Figure 1 E) were observed if EPO/NGF ratio is increased. In vitro electrophysiology and calcium imaging results showed that the DRG neurons are more active in the culture if EPO/NGF ratio reduced (Figure 1 C,D). qPCR results showed that different ratio of EPO and NGF transfection has differential effect on expression of Akt and mTOR genes (Figure 1 F,G,H).

Conclusion:

Real-time PCR results confirmed that expression of EPO and NGF is related to the PI3K/Akt pathway. Functional and biological responses of neurons and schwann cells showed that EPO and NGF genes are working synergistically in cells. Effect of EPO and NGF gene overexpression in peripheral nerve injury target cells will give us insight into peripheral nerve regeneration process on molecular level.
INTRODUCTION:
Standard-of-care large nerve gap (>3cm) reconstruction requires donor autograft. Alternatives are sought in clinical scenarios where donor limb is unavailable (e.g. multiple extremity trauma) or donor site morbidity (e.g. painful neuroma formation, paresthesia) makes autograft use suboptimal. A variety of alternatives have been proposed (e.g. allografts, conduits). Photochemical tissue bonding (PTB) as an alternative to traditional suture neurorrhaphy has been extensively studied in the rodent sciatic nerve model. PTB reduces needle trauma, prevents axonal escape, and creates a water-tight seal. We report a non-human primate study which recapitulates human anatomy, allows for objective quantitative functional outcomes testing, and electromyography (EMG). The purpose of this study is to evaluate whether PTB can elevate the performance of acellular nerve allograft (ANA) to that of standard-of-care autograft/suture.

MATERIALS & METHODS:
Nineteen rhesus macaques underwent 4cm proximal radial nerve defect creation in the right upper extremity, the radial nerve transected proximally at the spiral groove, distally at the branch to brachioradialis. The radial nerve was selected as it performs an isolated function with no input from other nerves which may confound recovery data. Three repair techniques were evaluated: n=6 autograft/suture, n=6 ANA/suture, n=7 ANA photosealed with light-activated human amnion wraps (PTB). An objective functional outcome test was conceived using an apparatus that accurately measures degree of wrist extension longitudinally (monthly) [Figure 1A-B].

RESULTS:
Average loss of wrist extension was 95.0±9.4° after radial nerve defect creation [Figure 1C-D]. At 6 months, autograft group animals recovered 75.5° (n=1 failure), ANA/PTB=35.1° (n=3 failures), ANA/suture=15.1° (n=2 failures) [Figure 1E-F]. At 4 months percent recovery of baseline EMG amplitude: autograft=35.2%, PTB/ANA=26.7%, ANA/suture=0%, at 8 months: autograft=59.4%, PTB/ANA=66.8%, ANA/suture=24.3%. Muscle mass retention (ECRL/ECRB muscle complex) at euthanasia results to date: no significant difference between autograft (76.1±18.3%) and ANA/PTB (61.9±35.7%), p=0.51.

CONCLUSION:
This radial nerve defect model improves upon existing animal models by allowing for large nerve gap testing in a primate model more analogous to the clinical large nerve gap injury in
humans. As expected, there was variability in functional outcomes in the ANA groups. ANA/PTB resulted in earlier electromyographic reinnervation and functional recovery than ANA/suture. Muscle mass retention was similar with autograft/suture and ANA/PTB. In the ANA group animals demonstrating functional recovery, outcomes were similar to autograft group animals. This preliminary data confirms PTB as a promising technique to improve outcomes of large nerve gap reconstruction in combination with autograft (previously demonstrated) and with ANA.
#27 Neuronal Plasticity: Daily High Electrical Activity Of Cat Hindlimb Motor Nerves And Silencing Of Their Activity By Spinal Isolation and Deafferentation, Decrease And Increase Their Size, Respectively

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Introduction: The effects of neuromuscular activity on nerve caliber are conflicting due to the wide variability in levels of neuromuscular activity from study to study which, in turn, vary with the models of hyper- and hypo-activities employed (reviewed by Gordon & Pattullo Exerc. Sport Sci Rev 21,331-302. 1993). In this study, we addressed the hypotheses that 1) high levels of daily activity of 50%, typical of motoneurons innervating slow motor units, reduce the size of their motor nerves and 2) activities of <1% of daily activity of motoneurons, typical of those of the largest fast glycolytic (FG) motor units (MUs), increase motor nerve size.

Methods: In adult cats, 1) the nerve to medial gastrocnemius (MG) muscle was maximally stimulated with chronically implanted cuff or intramuscular wire electrodes for daily electrical stimulation (ES) at 20Hz in a 50% duty cycle -3 minutes on and 3 minutes off. Conduction velocities (CVs) of single motor nerves were recorded in control cats and chronically for up to 240 days in experimental cats in a final experiment under deep anesthesia, and 2) the spinal cord was transected at L5 and the L5-L7 and S1-S3 dorsal roots transected bilaterally proximal to the root ganglia to isolate and silence motoneurons to MG and soleus muscles. Three weeks and 8 months later, the MG and soleus nerves (n) were removed and post-fixed in osmium tetroxide for measurements of fiber diameters (Dn) and comparison with those from age-matched cats.

Results: 1) The mean MG nerve CVs declined as a function of time after 50% ES, to a plateau that equaled that of isolated slow nerves and was significantly different from control CVs (p<0.01) (Fig. 1A). 2) The cumulative distributions of the Dn of the silenced MG and the soleus motor nerves were shifted to the right with significant increase (Kolmogorov-Smirnov test, p<0.01) in the size of the motor nerve fibers (Fig, 1B, C), the Ds of the 1a sensory fibers <10µm not changing.

Conclusions: Motor nerves display the same plasticity as the muscles in their adaptation to levels of neuromuscular activity with the size of the nerves decreasing with high levels of activity of the slow MUs and increasing with low or absence activities levels in the fast MUs. This neuronal plasticity is consistent with the known recruitment order of MUs from the smallest most active to the largest and least active FG MUs during movement.
Introduction: Currently available techniques to evaluate axonotmetic nerve injuries are imperfect, making it difficult to localize the zone of injury and severity of injury. Development of intra-operative techniques to better assess these parameters would guide treatment and prognosis. Increased neural vascularity has been found to correlate with chronic nerve compression, but it is unknown whether there is a similar response after acute traumatic nerve injury.

Methods: To address this, we utilized laser doppler flowmetry to assess changes in flux of a rat sciatic nerve at varying time points after a crush injury (Figure 1). Eight rats in each group underwent an initial survival surgery and baseline imaging of red blood cell flux with a laser doppler flowmeter. A graded crush model was created utilizing three distinct vascular clamps with differing degrees of applied force. One clamp per group was applied to the sciatic nerve for 30 seconds. Baseline flux was also measured in the contralateral sciatic nerve to use as a control. After recovering for varying time periods (6 hours, 2 weeks, 6 weeks), laser doppler flow measurements were obtained again at non-survival surgery along with nerve conduction studies and maximal tetanic force measurements. ELISA was used to assay for MMP-9 expression in the control and crushed nerves.

Results: 72 rats underwent the above procedure with varying degrees of crush. Persistent hyperemia was noted in the zone of injury compared to baseline at 2 weeks (Figures 2 & 3) and 6 weeks with incomplete nerve recovery (as evidenced by tibialis anterior muscle weakness) (Figure 4). Gastrocnemius weakness resolved in all animals by 6 weeks and latency of the compound nerve action potential (CNAP) returned to normal. CNAP amplitudes remained diminished when compared to baseline at 6 weeks in all groups. Greater elevation of MMP-9 at 6 hours corresponded to poorer functional recovery of tibialis anterior muscle force, but MMP-9 was not elevated at 2 weeks or 6 weeks (Figure 5). Proximal to the zone of injury, initial hyperemia appeared but then resolved by 6 weeks, suggesting a possible link between location of nerve injury and location of increased blood flow.

Conclusion: These findings suggest that nerve hyperemia and MMP-9 expression may be markers of severe crush injury and resolution of hyperemia may correlate with nerve recovery from trauma. CNAP amplitudes fail to recover to baseline, even at 6 weeks, and may be a more sensitive measure of persistent nerve dysfunction.
Figure 1: Diagram of experimental set-up, 8 rats crushed by each of 3 clamps were sacrificed at one of three time points.
INTRODUCTION

Peripheral nerve allografts are a strategy of nerve gap reconstruction with advantages including native nerve microstructure, donor Schwann cells that maintain a pro-regenerative milieu, expendable supply, and avoidance of donor site morbidity. Despite good outcomes, clinical use of unprocessed, fresh nerve allografts (FNAs) is limited due to requirement for transient systemic immunosuppression. To circumvent the systemic effects of FK506, an FDA-approved immunosuppressant, our laboratory developed a local FK506 drug delivery system that provides sustained, targeted drug release over 28 days. The objective of this study was to investigate if local FK506 delivery enhances nerve regeneration in a rodent model of nerve gap defect reconstruction with unprocessed FNAs.

MATERIALS & METHODS

In male Lewis rats, a 10 mm hindlimb common peroneal (CP) nerve gap was reconstructed with 20 mm long nerve isografts from donor Lewis rats or FNAs from donor ACI rats. The latter rats received either systemic FK506 (2 mg/kg/d intraperitoneal injections), local FK506 (420 µg FK506 encapsulated in poly(lactic-co-glycolic acid) microspheres suspended in fibrin hydrogel), or no treatment. After 4 weeks, nerve regeneration was evaluated using retrograde labeling to enumerate motor and sensory neurons that regenerated axons through the graft, quantitative histomorphometry of the midgraft and distal CP nerve, and serum cytokine profile.

RESULTS

Rats with untreated FNAs demonstrated very poor nerve regeneration compared to isografts or FNAs treated with systemic FK506 (p<0.001). Rats with FNAs treated with local FK506 demonstrated robust motor and sensory neuron regeneration significantly better than rats with untreated FNAs (p<0.001) and comparable to rats with nerve isografts and rats with FNAs treated with systemic FK506. These findings were consistent on histomorphometric analysis in the midgraft and in the distal nerve with local FK506-treated FNAs having comparable numbers of myelinated axons as isografts and FNAs treated with systemic FK506. Serum concentrations of the pro-inflammatory cytokine IL-12 were significantly lower 7 days after surgery in rats with FNAs treated with local FK506 (p<0.05) or systemic FK506 (p<0.001); however, rats with local FK506 had undetectable serum levels of FK506.
CONCLUSION

A local FK506 drug delivery system enhances motor and sensory nerve regeneration through fresh nerve allografts to a level comparable to that with systemic immunosuppression or with nerve isografts. Local FK506 delivery may have clinical application in transplantation of peripheral nerve or vascularized composite allografts. Future directions include evaluating functional outcomes and using an immunodeficient rat to distinguish the neurotrophic and immunosuppressive effects of local FK506.

Motor neurons that regenerated axons 5 mm distal to nerve graft

![Graph showing motor neurons that regenerated axons 5 mm distal to nerve graft.](image-url)
Introduction: Neuropathic pain is the most severely disabling type of chronic pain resulting from amputation, with associated US health care costs estimated to be greater than $600 billion annually. This type of pain is almost always resistant to opioid treatment. Currently, there is a fundamental gap in knowledge in understanding how to effectively treat neuropathic pain with non-opioid based strategies. Until this is solved, it will continue to lead to debilitating pain, an inability to perform daily activities of living, lack of meaningful employment, and an inability to wear prosthetic devices resulting in continued disability and poor quality of life. We have developed the Regenerative Peripheral Nerve Interface (RPNI) as a novel treatment strategy for the alleviation of neuropathic pain. RPNIIs are constructed by surgically implanting the distal end of a transected nerve into an autogenous muscle graft. We assessed the ability of RPNIs to alleviate established chronic pain in both male and female rats.

Materials and Methods: 12 male and 12 female rats were randomly assigned into either a neuroma only group, or a neuroma + RPNI treatment group. At baseline, all animals were assessed in the following pain tests: (1) thigh von Frey (neuroma tenderness); (2) paw von Frey (tactile allodynia); (3) acetone test (cold allodynia), and; (4) Hargreaves test (thermal allodynia). All animals then underwent tibial neuroma surgery, and were serially assessed in behavioral pain measures for 2 months. At the 2 month timepoint, half of the animals received RPNI surgery, and the other half received sham surgery. Animals were then serially followed for an additional 2 months. At study endpoint, both spinal cord and dorsal root ganglion (DRG; L4/L5) were harvested for immunohistochemistry.

Results: All animals developed chronic pain hypersensitivity following neuroma creation. Following RPNI surgery, neuroma tenderness decreased drastically in both sexes. However, tactile allodynia (a surrogate marker of phantom limb pain) was attenuated in males to a much greater degree than in females. Female rats were also more sensitive to both cold and thermal allodynia. Reactive microgliosis was similar in both sexes, but the purinergic receptor P2X4 was upregulated exclusively in male rats.

Conclusions: Results show that RPNI surgery attenuates chronic neuroma pain in both sexes, although to a greater degree in male rats. Purinergic receptor upregulation is also specific to males. Taken together, these results demonstrate the existence of sexually dimorphic pain signaling in rats following neuroma and RPNI treatment.
Scientific Paper Session II
#31 Neurofibromatosis-Associated Malignant Peripheral Nerve Sheath Tumors in Children Have a Worse Prognosis: a Nationwide Cohort Study
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Background: Malignant peripheral nerve sheath tumors (MPNST) are rare and aggressive non-rhabdomyoblastic soft tissue sarcomas (NRSTS) in children. More can be learned about prognostic factors on survival in pediatric MPNSTs as it may help tailoring clinical decision-making. This study set out to investigate differences in clinical presentation between adult and pediatric MPNSTs as well as neurofibromatosis type 1 (NF1) and non-NF1 pediatric patients. Overall survival, treatment modalities, and factors associated with survival were evaluated using a Dutch nationwide cohort of patients.

Methods: Data were obtained from the Netherlands Cancer Registry (NCR) and the Dutch Pathology Database (PALGA). All pathologically confirmed primary MPNSTs were collected. Patient and tumor characteristics, time period (based on the 2006 publication of chemotherapy trials in pediatric MPNST), treatment modalities, and survival were extracted from the database and pathology reports. Demographical differences were analyzed between adult and pediatric (age ≤18 years) MPNST. Demographical and treatment differences between NF1 and non-NF1 were also analyzed. A Cox proportional hazard model was constructed for localized pediatric MPNSTs.

Results: A total of 784 MPNST patients were registered of which 70 were children (37.1% NF1). No significant demographical differences were present between children and adult cases. In children, most tumors were large (>5cm, 71.4%), and deep-seated (under the fascia, 86.0%). Six non-NF1 patients presented with synchronous metastases (8.6%). Tumor size were more commonly large in NF1 patients (92.3% vs. 59.1%, p = 0.05). No statistically significant differences were observed for treatment modality between localized NF1 and non-NF1 patients. Localized disease was resected in 90.6%; R0 resection was achieved in 66.7%. Radiotherapy and chemotherapy were administered in 37.5% and 34.4% respectively. Non-NF1 patients tended to receive chemotherapy more commonly compared to NF1 patients (39.5% vs. 26.9%, p>0.05). Overall, estimated 5-year survival rates of localized pediatric MPNST was 66.3% (SE: 6.0%), which was lower in NF1 (52.4%, SE: 10.1%) compared to non-NF1 patients (75.8%, SE: 7.1%, p<0.05). The multivariate model showed worse survival in NF1 patients and increased survival in patients diagnosed after 2005 (both p<0.05). No treatment factors were independently associated with survival.

Conclusion: Pediatric MPNST present similarly compared to adult MPNST. In children, NF1 patients will generally present with larger tumors, but are treated similarly compared to non-NF1 MPNSTs. In localized pediatric MPNST, NF1 status is independently associated with poor survival. No treatment related factor was independently associated with survival. Also, life expectancy has increased for pediatric MPNSTs after 2005.
**Figure 1**

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**Background:**

Double fascicular transfer is argued to result in improved elbow flexion compared to the traditional, single transfer Oberlin procedure because it reinnervates both the biceps and the brachialis. This study seeks to determine if double fascicular transfer should be preferred over Oberlin transfer to restore elbow flexion in patients with upper trunk brachial plexus injuries by analyzing the current database of literature on the topic.

**Methods:**

A systematic review was conducted according to PRISMA guidelines. Inclusion criteria were studies reporting Medical Research Council (MRC) scores on individual patients undergoing Oberlin’s transfer (ulnar nerve fascicle donor) and double fascicular transfer (ulnar and median nerve fascicle donors). Patients were excluded if: age <18 years old and follow-up <12 months. Demographics obtained include age, sex, extent of injury (C5-C6/C5-C7), preoperative interval, procedure type, and follow-up time. Outcomes collected include absolute MRC score, ability to achieve MRC score ≥3 and ability to achieve MRC score ≥4. Univariate and multivariate regression analyses were completed to evaluate predictors of postoperative outcomes.

**Results:**

19 studies with 188 patients were included for pooled analysis. Patients that underwent double fascicular transfer had a higher percentage of patients attain a MRC score ≥4 compared to Oberlin subjects (84.7% vs. 63.0%, p=0.003). Double fascicular transfer was a predictor of achieving high MRC scores (OR=3.264, p=0.004). Time before procedure (OR to ≥3: 0.893, p=0.005; OR to ≥4: 0.933, p=0.035) and extent of brachial plexus injury (OR to ≥3: 0.254, p=0.015; OR to ≥4: 0.512, p=0.039) were also predictors of ability to achieve MRC scores ≥3 and ≥4, respectively. Multivariate analysis showed that procedure type was the only significant predictor of ability to obtain MRC ≥4 (OR: 2.889, p=0.011).

**Conclusions:**

This analysis demonstrates that double fascicular transfer is associated with superior postoperative outcomes. Double fascicular transfer should be performed for restoring elbow flexion when possible.
Scientific Paper Session II
#33 Sensorimotor Connections between CN V and CN VII May Provide a Possible Anatomical and Histologic Basis for Synkinetic Buccinator Hypertonicity
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Buccinator muscle hypertonicity, a primarily masticatory muscle that may contribute to resting facial tone, is a disfiguring sequela often observed in patients with post-paretic synkinesia. The buccinator muscle is uniquely described as a dually innervated muscle through plexus-like connections between the trigeminal and facial nerves- receiving motor innervation from the facial nerve (CN VII) and sensory innervation from the buccal branch of the trigeminal nerve (V3). However, it is currently unknown why this specific muscle receives its innervation through this sensorimotor network. Therefore, this study aims to elucidate histologically the innervation of this muscle and provide suppositions on how its dysregulation may underlie buccinator hypertonicity observed in post-paretic synkinesia.

5 formalin-fixed and fresh-frozen hemifaces were dissected for this study. After exposing distal branches of the facial nerve, the mandible was resected in order to expose branches of the mandibular nerve and the foramen ovale. CN V/CN VII anastomoses were meticulously dissected under loupe magnification, and verified by histology using S100 (myelin), H&E, VAchT (motor axons), and TH (autonomic axons) staining. Biopsies of the buccinator, masseter, and orbicularis oculi muscle were also analyzed using the same stains. Histologic images were objectively quantified using ImageJ.

Plexiform anastomoses between the buccal nerve (V3) and buccal branches of the facial nerve were reliably found in all hemifaces superficial to the buccinator. Histologic analysis of these anastomoses revealed a fusion of CN V3 and CN VII fibers into a single nerve characterized by a single continuous epineurium. Quantification of VAchT staining showed a linear decrease in mean staining intensity along the length of the anastomosis ($R^2=0.67$) toward the V3 connection point, indicating fusion of sensory and motor fibers. A higher concentration of sensory fibers and a co-localization of sensory nerve endings with motor fibers were also observed in buccinator muscle biopsies, but notably absent in those of the masseter and orbicularis oculi.

The close coupling of sensory and motor innervation was found to be a unique feature of the buccinator muscle. Anatomical and histologic findings seem to suggest CN V and CN VII are involved in a concerted feedback system with the buccal nerve possibly contributing proprioceptive information from the buccinator muscle while the facial nerve contributes motor response. Proprioceptive feedback seems to be essential for the maintenance of resting tone. An imbalance between motor and proprioceptive feedback may underlie buccinator hypertonicity, however, further studies are needed to elucidate this concept.
#34 Sciatic Fascicular Transfers to Restore Leg Function in Patients with Acute Flaccid Myelitis

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**Introduction:** Acute Flaccid Myelitis (AFM) is a devastating paralytic condition affecting previously healthy children. Although much remains unknown about its pathology, the disease attacks the anterior horn cell causing lower motor neuron injury in unpredictable patterns, with proximal muscles more severely affected. It often leaves children with mixed upper and lower extremity paralysis and ventilator-dependent. Recovery patterns are also highly varied. Here, we present results of sciatic fascicular nerve transfers to restore knee flexion, knee extension and hip abduction and stabilization.

**Materials & Methods:** A retrospective case series of 6 consecutive patients with AFM diagnosed in 2018 treated with sciatic fascicular nerve transfers from April to July 2019 are presented. History, physical examination and electrodiagnostics determined suitability for nerve transfers. Sciatic fascicles were identified intra-operatively, and redundant donor fascicles confirmed using intra-operative nerve stimulation. Each patient had a unique pattern of deficit requiring tailored transfers, often with final fascicular transfer plan determined based on intra-operative stimulation.

**Results:** Patients undergoing transfers ranged 1 to 10 years of age. Time of nerve transfer from initial illness ranged from 5 to 10 months. 14 sciatic fascicular nerve transfers were undertaken in 8 limbs of 6 patients. Intra-operative stimulation confirmed functioning donor fascicles. Of the 14 sciatic donor fascicles, 7 were peroneal fascicles for toe extension, 2 were peroneal fascicles for foot eversion, and 6 were tibial fascicles for toe flexion. The most suitable target for transfer was also identified using intra-operative stimulation. Of the 14 recipient nerves transferred, 4 went to biceps femoris, 2 to semitendinosus, 4 to vastus medialis, 3 to vastus lateralis, and 2 to superior gluteal nerves. Of the 8 limbs that were operated on, all underwent concomitant superior and inferior gluteal nerve decompression, sciatic nerve decompression at the sciatic notch, and femoral nerve decompression. No patients had downgraded function post-operatively, 3 have early improvement in motor function, and the remainder have inadequate follow up at the present time. Outcome data collection is ongoing and will be presented.

**Conclusions:** Sciatic fascicular nerve transfers are an exciting new option for children afflicted with AFM, with early outcomes extremely promising. Ongoing clinical and basic science research is needed to optimize treatment for this complex disorder.
#35 Incidence of Nerve Injury After Extremity Trauma in the United States

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**Background:** Traumatic peripheral nerve injuries cause chronic pain, disability, and long-term reductions in quality of life. Depending on trauma location and severity, 1 to 3% of patients are diagnosed with nerve injuries during the first few months after trauma. However, establishing the long-term prevalence and burden of nerve injuries has proven difficult, as they are often diagnosed months to years after trauma and in a variety of care settings.

**Methods:** The Truven MarketScan Commercial Claims and Encounters database from 2010 to 2015 was used to identify patients aged 18 to 64 who presented to emergency departments for upper and/or lower extremity traumas in the United States. Cumulative incidences were calculated for nerve injuries diagnosed within two years of trauma. Cox regression models were developed to examine associations between upper extremity nerve injury and chronic pain, disability, and use of physical therapy or occupational therapy.

**Results:** The final cohort consisted of 1,230,362 patients. By two years after emergency department presentation, nerve injuries were diagnosed in 2.6% of upper extremity traumas and 1.2% of lower extremity traumas. Among patients who required hospitalization for their injuries, nerve injuries were eventually diagnosed in 9.2% of upper extremity traumas and 2.0% of lower extremity traumas. Moreover, only 9% and 38% of nerve injuries were diagnosed by the time of emergency department and hospital discharge, respectively. In general, upper extremity nerve injuries were diagnosed earlier than lower extremity nerve injuries. Nonetheless, 12.2% of brachial plexus injuries and 21.3% of median nerve injuries were identified more than 1 year after extremity trauma. Patients with nerve injuries were more likely to be diagnosed with chronic pain (HR 5.9, 95% CI 4.3 to 8.2), use physical therapy services (HR 10.7, 95% CI 8.8 to 13.1), and use occupational therapy services (HR 19.2, 95% CI 15.4 to 24.0) more than 90 days after injury.

**Conclusion:** The incidence of nerve injury in this large national cohort was higher than has been previously reported. Despite recent advancements in the treatment of nerve injuries, only a minority were diagnosed by the time of emergency department or hospital discharge. These findings may improve practitioner awareness and inform public health interventions for injury prevention.
Introduction: In a recent cross-sectional study of 202 children aged 8-19 years, we demonstrated that point prevalence of pain in brachial plexus birth injury (BPBI) was 42% and that a key predictor of this pain was cervical root avulsion. The purpose of this study was to evaluate self-report pain characteristics and pain interference in children with BPBI.

Materials & Methods. All children 8-19 years reporting pain in the initial survey were invited to participate in a subsequent cross-sectional, descriptive study of pain characteristics (intensity, distribution, quality, frequency, onset) and pain interference in BPBI. Pain assessments were completed in-person or through video teleconference, using two validated self-report pediatric measures, the Adolescent Pediatric Pain Tool (APPT) and the pain interference scale of the Pediatric Pain Questionnaire (PI-PPQ).

Results. 83 of 123 eligible children (mean age 14.6 ± 3.2 years, 63% female) have completed the pain assessments thus far. Mean age of pain onset was 8.1 ± 1.7 years, with 2 patients reporting complete resolution of their symptoms within 1 year. Most commonly, participants reported occasional pain (range, “never” to “often”) with a mean pain intensity of 42.0 ± 22.3 mm (range, 0 to 80.5 mm) on the APPT 100mm visual analogue scale. Children with total plexus palsies had more severe pain than children with upper plexus palsies (64.3 ± 20.1 vs 34.5 ± 17.5, P <0.0001). The shoulder was the most frequently circled pain location, endorsed by 70% of children, followed by the elbow by 27%, and the hand by 25%. Hand pain was reported by children with both upper and total plexus palsies. The median total number of pain descriptors circled on the APPT was 17 (range 0-36 out of 67 possible words), with 92% of children selecting a mixture of neuropathic and nociceptive descriptors. The median ratio of neuropathic to nociceptive words selected was 0.63 (range, 0 to 8). Sixty-nine children (83%) reported that their pain sometimes, often or always impacted at least one of 11 different activities on the PI-PPQ; “sports”, “sleeping” and “seeing friends” were the most commonly affected activities.

Conclusions. Pain in BPBI is typically spontaneous and intermittent, ranging in intensity from mild to severe. The etiology of this pain remains unclear, with pain location and descriptor data suggesting both musculoskeletal and neuropathic origins as plausible. Importantly, pain in BPBI negatively impacts the child’s function.
Background: Targeted muscle reinnervation (TMR) is an emerging surgical technique for the treatment of neuroma pain. Sensory and mixed motor nerves are transferred to nearby redundant motor nerve branches. TMR was recently shown in a randomized-controlled trial (RCT) to provide significant reductions in post amputation pain relative to conventional neuroma excision and muscle burying. This study analyzes patient-reported outcomes for a cohort of TMR patients screened but not randomized into the RCT.

Methods: Patients who were ineligible for randomization, or refused to be randomized, and underwent TMR for pain or prostheses control were assembled for the present analysis. Data were collected prospectively from 2013 to 2017. The primary outcomes measured were the difference in residual limb pain (RLP) and phantom limb pain (PLP) before and one year after surgery, assessed by an 11-point Numerical Rating Scale (NRS). Secondary outcomes measured were change in Patient Reported Outcome Measurement System (PROMIS) pain intensity and pain interference scores with respect to RLP and PLP (p≤0.01 for all comparisons). On functional assessment, OPUS Rasch scores improved by a mean of 2.7 points and Neuro-QOL improved by 2.2 points.

Results: Thirty-three patients comprising 35 limbs were included in the study. NRS scores for RLP decreased by a mean of 2.6, and PLP decreased by 2.2 1-year post TMR. Patients experiencing low pain at baseline (NRS 0-3) were more likely to experience worsening of both RLP and PLP after TMR compared to those with higher baseline pain (RLP: +0.5 vs -3.2, p<0.01; PLP: +0.7 vs -3.0, p<0.01). PROMIS pain intensity and pain interference scores both saw statistically significant improvements with respect to RLP and PLP (p≤0.01 for all comparisons). On functional assessment, OPUS Rasch scores improved by a mean of 2.7 points and Neuro-QOL improved by 2.2 points.

Conclusions: This prospective cohort of thirty-three amputees corroborates the results of a recently published RCT and additionally demonstrates improvement in RLP, PLP, and functional status.
Introduction:

Over 17,500 traumatic peripheral nerve injuries (TPNI) to the extremities occur annually in the United States. This study seeks to develop a novel strategy for nerve recovery monitoring based on Diffusion Tensor Imaging (DTI) using Diffusion-Weighted Magnetic Resonance Imaging (DW-MRI). We hypothesize that data acquired via DTI will improve our ability to monitor and predict nerve regrowth following surgical repair of cut or severe crush injuries.

Materials and methods:

DW-MRI studies were performed on 13 patients using a 3.0 T Philips Achieva magnetic resonance scanner. We compared injured median nerves to their correspondent ulnar nerves. One set of data was acquired after initial repair for all 13 patients. Longitudinal data over two timepoints were completed for 3 patients after repair. Qualitative comparison was done using tractography. Quantitative analysis of nerve regrowth was performed using multiple DTI parameters such as: fractional anisotropy (FA), radial diffusivity (RD), axial diffusivity (AD), and mean diffusivity (MD).

Results:

Qualitative comparison on DTI shows axonal regrowth beyond the coaptation site. With a FA closer to 1 correlating with diffusion occurring in a single axis, the quantitative analysis of the data shows that the difference in FA between injured and unaffected nerves is predominantly related to an increased in Radial Diffusivity (RD) which translates into an additional radial regrowth of nerve ending instead of a sole axial growth paralleling the axonal direction (Figure 1). An increase in RD has been correlated historically in the literature with an increase in size of the extracellular compartment surrounding axons. FA improved progressively with time in patients 1 and 2 suggesting a regrowth tendency toward a single axis. With patient 3, we noticed a decrease in FA in the second time period after surgery, but some improvement in FA was witnessed with additional recovery time (Figure 2).

Conclusion:

Early results indicate that DTI using DW-MRI can aid in tracking nerve recovery and potentially guide clinical management either toward or away from surgical re-intervention. The objective of this study is to evaluate the ability of DTI to monitor and to predict nerve regrowth following crush or repaired cut nerve injuries.
Figure 1

Figure 2
INTRODUCTION

The trapezius muscle plays an important role in normal subjects, yet the spinal accessory nerve is an often-used donor nerve in patients with obstetrical brachial plexus birth palsy (OBPBP). This study examined the role of the trapezius in children with upper trunk OBPBP during movements of the shoulder, forearm and elbow. As denervation of the lower portion of the trapezius alone is possible, the upper and lower trapezius (UT and LT) were examined separately.

METHODS

Five patients, mean age 11.2 years (7.6 to 18.1 years), with OBPBP underwent bilateral upper extremity motion analysis. Subjects were evaluated with 3-dimensional motion analysis, 16-channel electromyography, and video monitoring. Data was recorded for shoulder abduction, external rotation, internal rotation, elbow flexion, forearm supination, and forearm pronation. Analysis was performed using MOTIONBROWSER, a novel analytic workflow, to compare heterogeneous muscle bundles and extract significant muscle activities through semi-automated comparisons with comprehensive visualization techniques and an interactive user interface.

RESULTS

In unaffected side shoulder abduction and external rotation, LT was primarily activated with secondary UT activation in shoulder abduction. Affected side PT and PQ were primarily activated. In unaffected side shoulder internal rotation, variable activation of PQ and UT were seen; on the affected side, variable activation of EDC, PT, and LT were seen. In unaffected side elbow flexion, BIC and LT were primarily activated; on the affected side, PT was primarily activated. In unaffected side forearm pronation, variable combinations of PT, PQ, and UT were seen; on the affected side, PT was primarily activated. In unaffected side forearm supination, BIC was primarily activated; on the affected side, PT was the primary. Individual subjects had disproportionate UT or LT activation: for shoulder internal rotation, one patient demonstrated 0.92% vs. 53% LT activation on the unaffected vs. affected sides. For elbow flexion for two patients, LT activation was 17% and 4.7% in the unaffected sides vs. 35% and 23% on the affected sides. For forearm pronation, two patients did not activate UT in their unaffected side, but LT was strongly activated (57% and 36%) in their affected sides.

CONCLUSIONS

Both UT and LT have important compensatory roles during motion in patients with OBPBP. Future evaluation of the spinal accessory as a donor nerve transfer during obstetrical brachial plexus is necessary.
Introduction: The timing of nerve transfer or graft after peripheral nerve injury is critical, and accepted to be approximately 3 to 6 months. However, in practice, patients often present in a delayed manner for surgical intervention. This study describes the timing of surgery after peripheral nerve injury for adult patients in Alberta, and explores patient perspectives regarding barriers to care for peripheral nerve injury.

Design and Methods: A retrospective analysis of adult patients undergoing peripheral nerve transfer or grafting in Alberta from 2005 to 2017 was completed. One hundred and sixty-six patients who underwent distal nerve transfers or grafts for either upper or lower limb peripheral nerve injuries were included in the analysis of time to surgery. One hundred and twenty-nine patients with a minimum of one year follow up, after peripheral nerve surgery, were included in the analysis of factors affecting clinical outcomes.

Outcome Measures: A Cox Proportional Hazard Regression was completed to determine correlation of patient, injury and systemic factors with time to surgical intervention after injury. Additionally, a clustered multivariable logistic regression analysis was completed to examine the association of time to surgery, patient, injury and operative characteristics on MRC strength outcomes.

Results: The mean (SD) time to surgery was 221 (118.1) days. A referral made by a surgeon approximately doubled the hazard of earlier surgery as compared to a general practitioner (p=0.006). An increase in one comorbidity resulted in the adjusted hazard of earlier surgery decreasing by 16% (p=0.014).

Numerous factors are associated with post-operative strength outcomes including: time to operative intervention, operative procedure, and injury. For every week increase from injury to time of surgery, the adjusted odds of the patient achieving a MRC strength grade ≥ 3 decreases by 3% (p=0.02). If a patient received a nerve transfer instead of a nerve graft the adjusted odds of the patient achieving a MRC strength grade ≥ 3 was 388% (p=0.003). The adjusted odds of achieving a MRC ≥ 3 decreased 65% if the injury sustained had a component of pre-ganglionic injury (p=0.05).

Conclusions: The timing of operative intervention after peripheral nerve injury is critical, and delays in surgical intervention are best explained by both patient and systemic factors. These areas of deficiency in the peripheral nerve injury service pathway require further exploration and improvement in order to optimize patient care.