3D Magnetic Hyaluronic Acid Hydrogels to Modulate Expressions of Mechano-Sensitive Ion Channels for Pain Relief

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'*Summer's coming! Come join our fitness classes and get a beach-ready bod!*' reads the advertisement from the school gym. For many, including myself, gyms are facilities where we train and evolve our bodies to better withstand environmental stresses such as squeezing into a rush-hour bus. Cells respond to external mechanical stimuli just like us! Cancer cells, for instance, can evolve to tolerate higher mechanical stresses to squeeze through tiny capillaries during metastasis.

In this talk, I will present the 3D magnetic hyaluronic acid (**HA**) hydrogels we have developed to condition primary dorsal root ganglion (**DRG**) neurons to become more mechanically robust through modulating their expressions of mechano-sensitive PIEZO2 and TRPV4 channels (**MSCs**). As MSCs are typically over-expressed in patients suffering from spinal cord injuries and concomitant pain, we believe this neuro-modulatory tool can also help promote tissue regeneration and pain relief.

Our hydrogel was synthesized by reacting 4-arm-polyethylene-glycol-vinyl-sulfone (PEG-VS) with HA-thiol, the main component of the brain/spinal cord extracellular matrix (ECM) and 1 µm fluorescent thiol-functionalized-magnetic microparticles. HA was also used as it is anti-inflammatory and can promote tissue regeneration. The biomechanical properties of the hydrogel were optimized to have similar storage modulus (~150 Pa) as the brain/spinal cord ECM. We could also apply forces between 0.15-1 µN with the gel.

We found no significant difference in cytotoxicity and metabolic activities of neurons grown on 2D PLL-coated coverslips, 3D HA gels and 3D magnetic HA gels with or without acute/chronic mechanical stimulation. Neurite outgrowth/branching and calcium levels were similar across different conditions.

PIEZO1, PIEZO2, TRPV4 and N-type Ca2+ are the only MSCs that have been shown to be activated by mechanical forces. After verifying the specificity of antibodies, we performed immuno-labeling and found that DRG neurons express high density of PIEZO2 and TRPV4 channels but not PIEZO1 and N-type Ca2+.

Acute stimulation induced calcium influx in DRG neurons with 50% increase in Ca2+ fluorescence. Ca2+ influx was unaffected by ω-conotoxin, a specific inhibitor of N-type Ca2+ or Yoda1, a specific agonist of PIEZO1. However, there was dramatic quenching of Ca2+ influx with Ruthenium red that inhibits PIEZO2 and TRPV4. Next, we combined micropillars and electrophysiology to verify that magnetic HA hydrogels mechanically stimulated the DRG neurons mainly through PIEZO2 and TRPV4 channels.

Using the magnetic HA gel, we also showed that chronic mechanical stimulation of DRG neural networks with increasing forces daily (up to 4 days) reduced expressions of mechano-sensitive PIEZO2 and TRPV4 channels, consistent with previous findings that neural networks actively maintain homeostasis in the presence of continual excitation by downregulating their expressions of MSCs.

In conclusion, we fabricated magnetic HA hydrogels with similar biochemical/physical properties to brain/spinal cord ECM. Acute mechanical stimulation induced calcium influx *via* PIEZO2 and TRPV4 and chronic stimulation modulated the expression of mechano-sensitive PIEZO2 channels. We believe that the magnetic HA gel has potential for use in mechano-transduction and pain modulation where treatments with addictive opioids, invasive toxins injection and poorly understood electrical stimulations are ineffective.

For the next step, we are mechanically conditioning neural stem cells (NSCs) with our magnetic hydrogels. NSCs are useful for tissue regeneration but they experience excessive mechanical stress during injection and mechanical incompatibility with *in vivo* diseased tissues. We hope to train an army of mechanically-robust NSCs for more effective tissue regeneration.

Title: A Novel Model of Soft Tissue Manipulation in Rodents with Induced Low Back Pain Attenuates Inflammation and Improves Function

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Purpose/Hypothesis: Soft tissue manipulation is often used to treat musculoskeletal pain disorders. Instrument-assist soft tissue manipulation (IASTM) uses rigid devices to deliver targeted treatment forces through the intact surface of the body to underlying tissues. This study aimed to explore the effects of IASTM on pain sensitivity, functional measures, and biomarkers using a novel model of IASTM in sentient rodents with chemically-induced low back pain (LBP).

Subjects: 44 Sprague-Dawley rats (307.4±7.3g).

Materials/Methods: Unilateral, chronic inflammatory LBP was induced by injecting complete Freund's adjuvant into the backs of anesthetized rats. IASTM treatment (5min/session) was delivered to the back of conscious animals under light restraint. Animals were randomized into groups: A) cage control; B) 3d post-injured (inj), untreated; C) 3d inj, <30min post-IASTM; D) 3d inj, 2h post-IASTM; E) 14d inj, untreated; F) 14d inj, <30min post-IASTM; G) 14d, 2h post-IASTM. A single IASTM session was given at 3d post-injury, or 6 IASTM sessions over 14d. An electronic Von Frey monofilament was used to determine back pain pressure threshold (PPT); the TreadScanTM3.0 with a BCamCap program to capture gait patterns; and a TSE Grip Strength Meter to assess forepaw grip strength. Mesenchymal stem cells (MSCs) were characterized using flow cytometry. Biomarkers (NPY, TNFα, IL-10, IL-6, RANTES) were assessed using ELISA kits. Data was analyzed using repeated measures ANOVA with Bonferroni adjustment for multiple comparisons and paired t-tests as appropriate ($p<0.05$).

Results: A significant drop in back PPT occurred bilaterally at 3d post-injury prior to IASTM ($p<0.05$), and was further lowered on the injured side post-IASTM ($p<0.05$). However, no difference was found in back PPT between sides at 14d, although both remained lower than pre-injury levels. Immediate improvements in gait patterns were found post-IASTM, including homolateral coupling and homologous coupling, and over time in diagonal coupling ($p<0.05$). Significant alterations in grip strength were not detected. No significant differences were found in circulating MSC levels. NPY increased 3d post-inj at <30min post-IASTM and remained elevated at 14d. RANTES levels decreased ($p<0.05$) and IL-10 increased ($p=.086$) at 14d.

Conclusions: Back PPT decreased bilaterally post-injury, and was lowered even more on the IASTM-treated side initially, but later equalized with repeated IASTM applications. Gait improved to near pre-injury values, indicating improved movement patterns and spinal elongation. Grip strength performance was highly variable, requiring further testing. MSC circulation levels did not vary, but additional stem/progenitor cell populations should be considered. Elevated NPY levels in response to IASTM may serve to modulate pain. IASTM may have an anti-inflammatory effect as seen by lowered RANTES and increased IL-10 levels.

Clinical Relevance: LBP sensitized subjects to painful stimuli, whether applied to the injured or uninjured side. IASTM amplified this effect initially, but eventually had a therapeutic impact with repeated sessions. Functional gait also improved. Positive findings may be related to pain modulation and an anti-inflammatory treatment effect. Further research is needed in humans.

References:

1. Loghmani MT, Bane S. Instrument-assisted soft tissue manipulation: evidence for its emerging efficacy. J Nov Physiother. 2016, S3:012. DOI: 10.4172/2165-7025.S3-012.
2. Electronic Von Frey Monofilament. www.iitcinc.com.
3. TSE Grip Strength Meter. www.TSE-Systems.com.
4. Hoheisel U, Mense S. Inflammation of the thoracolumbar fascia excites and sensitized rat dorsal horn neurons. Eur J Pain 19. 2015;419-428.
5. Langevin HM, Stevens-Tuttle D, Fox JR, Badger GJ, et al. Ultrasound evidence of altered lumbar connective tissue structure in human subjects with chronic low back pain. BMC Musculoskeletal Disorders. 2009;10:151. doi:10.1186/1471-2474-10-15.
6. Corey SM, Vizzard MA, Bouffard NA, Badger GJ, Langevin HM. Stretching of the Back Improves Gait, Mechanical Sensitivity and Connective Tissue Inflammation in a Rodent Model. PLoS ONE. 2012;7(1):www.plosone.org.
7. Rapaport MH, Schettler P, Bresee C. A preliminary study of the effects of repeated massage on hypothalamic-pituitary-adrenal and immune function in healthy individuals: a study of mechanisms of action and dosage. J Altern Complement Med. 2012;18:789-797.

A Role for Endogenous Neural Precursors in the Vascular Response to Focal Cortical Infarcts

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Focal ischemic brain injury instigates proliferation and migration of neural precursor cells (NPCs) and remodeling of peri-infarct vasculature. The functional relevance of, and interactions between, these two processes has been unclear. Past work suggests that NPCs closely associate with remodeling blood vessels surrounding the infarct. With an inducible cre/reporter mouse system, we labeled NPCs and repeatedly imaged them in cortex surrounding a photothrombotic ischemic lesion with two photon microcopy. After the long-distance migration from subventricular zone to peri-infarct tissue (>2 mm), NPCs assumed positions close to vessels, where they remained for months. This positions NPCs to influence post-ischemic vascular responses. Next, we characterized time-dependent structural changes in the vasculature after cortical infarction. Peri-infarct vascular density increased within days of injury, an increase that was largely maintained for at least 2 months. Finally, we examined the relevance of NPCs for vascular remodeling using a pharmacogenetic method to conditionally ablate NPCs prior to cortical infarcts. Mice with ablated NPCs had exaggerated increases in peri-infarct vascular density, more disordered vessel networks, and significantly impaired behavioral recovery compared to control mice. These findings support a functional role for NPCs in regulating vascular remodeling after focal ischemic brain injury. Future work will examine interactions between rehabilitative training and NPCs on vascular and neural remodeling after focal infarcts.

AAV delivery of α-Klotho: gene therapy as a strategy to counteract sarcopenia

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ABSTRACT

Beyond the role of skeletal muscle as a mechanical motor, the endocrine function of muscle and its critical role in maintaining organismal metabolic health is increasingly being appreciated. With aging, however, there is a steady decline in both motor and endocrine function of skeletal muscle, resulting in organism-wide pathologies, including sarcopenia. In the elderly, the loss of skeletal muscle mass and accompanying muscle strength translates to increased morbidity. Recently, the anti-aging hormone, α-Klotho, has been associated with increased activity levels and has been shown to play a critical role in skeletal muscle regeneration after acute injury. In addition, we have shown circulating levels of α-Klotho are inversely proportional with age, and studies report mice genetically deficient for α-Klotho exhibit declines in forelimb muscle strength, similar to that observed in their aged counterparts. While the functional declines are apparent in aged mice, we show that the sarcopenic profile is truly evident in geriatric (>26 months) mice, corresponding to the timpoint when α-Klotho is significantly diminished. In this study, we investigated the ability of AAV-vector mediated delivery of α-Klotho to reverse the effect of sarcopenia in aged and geriatric mice. We found that AAV-mediated delivery of α-Klotho to aged mice resulted in a significantly improved functional capacity; however, the improvement was diminished in geriatric mice. These findings are consistent with previous reports of anabolic resistance with advanced age, and suggest that the therapeutic window for α-Klotho administration may be dependent on age of the host.

Administration of α-Klotho systemically enhances skeletal muscle regeneration

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ABSTRACT

One consequence typical of the aging process is an impaired ability of myofibers to regenerate and restore their original architecture following an acute injury. This reduced regenerative capacity can result in loss of muscle mass and mobility, leading to declining function and increased morbidity for an elderly population. Coinciding with this decline in regenerative capacity, studies have shown a gradual systemic decline in the anti-gerontic protein α-Klotho. This age-related decrease in α-Klotho has been shown to have deleterious effects on cognition, muscle strength, and endurance. Our findings have also demonstrated an important role for α-Klotho in the skeletal muscle regenerative cascade. Following acute injury, we have found that young mice exhibit a marked upregulation of Klotho within skeletal muscle. However, this upregulation diminishes with age. In this study, we evaluate the restoration of myofiber regenerative capacity in old mice through the administration of α-Klotho via two methods: intraperitoneal injection (IP) and adeno-associated virus injection (AAV). IP administration of the recombinant α-Klotho protein increased both local and systemic Klotho, and showed beneficial effects on myofiber regeneration and muscle function. However, the timing of administration relative to time of acute injury plays an important role in determining the response. Similarly, mice injected with AAV-Klotho showed upregulation of Klotho as well as beneficial effects on regeneration. These results suggest that α-Klotho delivery represents a novel therapeutic target to enhance skeletal muscle healing in an elderly population.

Autologous Micro-Fragmented Adipose Tissue Injection under Ultrasound Guidance for Chronic, Recalcitrant Shoulder Pain in Manual Wheelchair Users with Spinal Cord Injury

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Background/Objective: *Rotator cuff disease* is the most common cause of shoulder pain in persons with chronic spinal cord injury (SCI). It usually resolves with non-operative treatments such as pharmacological agents and physical therapy; however, when these fail, surgery may be the only option. Injection of autologous, micro-fragmented adipose tissue has shown promise in treatment of shoulder injuries in non-SCI populations; however, its use in those with SCI and shoulder pain has not been reported. The objective of this pilot study is to determine the safety and efficacy of autologous, micro-fragmented adipose tissue injection under ultrasound guidance for chronic, recalcitrant shoulder pain due to rotator cuff disease in persons with chronic SCI.

Methods: Twelve (12) persons with chronic SCI (≥12 months) who have chronic, recalcitrant shoulder pain in-spite of completing conservative treatment and are diagnosed with rotator cuff disease on examination will be enrolled in this study. Autologous, micro-fragmented adipose tissue will be obtained using a minimal manipulation technique in a closed system (Lipogems® system), without the addition of enzymes or additives. Between 5.0 and 10.0 mL will be injected into the tendon(s) and surrounding structures under continuous ultrasound guidance. After 24 hours of relative rest, subjects will begin a standardized stretching protocol for 1 month, followed by a formal strengthening program for the duration of the study. Subjects will be followed for adverse events and changes in shoulder pain intensity on an 11-point numerical rating scale (NRS), the Wheelchair User's Shoulder Pain Index (WUSPI), and a 5-point subject global impression of change (SGIC) scale at 1, 2, 3, and 6 months. Functional impact of pain will be measured using the Brief Pain Inventory (BPI) Pain Interference items.

Results: We report results on the first 6 subjects to complete this study (5 male, 1 female; age = 58.2±8.1 years; 2 with tetraplegia, 4 with paraplegia; duration of injury = 21.8±10.0 years). Significant reductions were observed in WUSPI, BPI, and NRS scores after 6 months post-treatment ($p<.05$), with average WUSPI, BPI, and NRS scores decreasing 82%, 86%, and 88%, respectively. All subjects surpassed the minimal clinically-important difference (MCID) in NRS scores of 2 (median = 5.0), and 5 surpassed the BPI MCID of 1 (median = 4.7). Two subjects reported to be "very much improved" on the SGIC, and 4 reported "much improved."

Conclusions: Preliminary results from this ongoing study suggest that a single injection of autologous, micro-fragmented adipose tissue injection, coupled with a standardized shoulder exercise protocol, greatly reduced chronic, recalcitrant shoulder pain in manual wheelchair users with SCI, thus possibly avoiding surgery. Lack of blinding and a suitable control group may have influenced results. Longer-term follow-up and a randomized controlled trial are warranted.

Support**:** Derfner Foundation

Changes in supraspinatus tendon signal intensity after micro-fragmented, autologous adipose tissue treatment in a wheelchair user with paraplegia: A case study.

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Background: Wheelchair users with spinal cord injury (SCI) are at a greater risk of developing rotator cuff tendinopathy from upper extremity overuse during activities of daily living. Conservative treatments, such as physical therapy or pharmacological agents, are eventually inadequate when the pain and dysfunction becomes severe. Surgery is generally the next option after such treatments fail. However, surgical outcomes are inconclusive and unloading of the upper extremity is required to facilitate healing, which means power wheelchair use, caregiver assistance, and possible hospitalization during the rehabilitative process. Biologics have shown promise for treatment of shoulder pain and rotator cuff tendinopathy in able-bodied individuals without the need for surgery. However, this has not been tested in wheelchair users with SCI. The following case study tracks MRI changes in the supraspinatus tendon after an autologous, micro-fragmented adipose tissue (MFAT) treatment followed by a standardized rotator cuff stretching and exercise program.

Methods: The participant was recruited because of severe, refractory shoulder pain (7/10 on a Numerical Rating Scale [NRS]). He was successfully enrolled after diagnosis with rotator cuff disease using physical and ultrasound examinations. Adipose tissue was harvested from the abdomen and a Lipogems® processing kit was used to prepare the MFAT for injection. Lipogems® uses mechanical stimuli to separate MFAT from pro-inflammatory blood and oils and process the adipose tissue while keeping intact the stromal vascular niche, which contains the regenerative factors potentially responsible for tissue healing. Serial proton-density (fat suppressed) MRI images of the supraspinatus tendon in the oblique-coronal plane were collected prior to treatment, and at two, three, six, and twelve months post-treatment. Greyscale properties of the tendon were quantified using a MATLAB algorithm. The perimeter of the tendon was outlined, with care not to include the bursa or muscle. Greyscale values of each pixel within the tendon were calculated and averaged. The tendon greyscale value was normalized to a point outside the tendon. Signal intensity of proton-density images indicates the density of water within the tissue, which is a marker for tendon degeneration. Thus, a higher greyscale value would theoretically indicate a more pathological tendon.

Results: Baseline greyscale ratio (tendon to reference) was 1.68. The ratio increased to 1.91 two months post-treatment, then dropped to 1.51 at three months, 1.35 at six months, and 1.42 at twelve months.

Conclusion: A single MFAT treatment initially increased tendon greyscale values, which could be reflective of an acute, pro-inflammatory response initiated by the needling procedure. Sustained reductions in tendon greyscale beyond three months may indicate that the regenerative properties of the adipose tissue reduce inflammation for up to one year. Further testing is needed to better understand the relationship between shoulder pain and MRI-derived changes in tendon abnormalities. Validity and reliability of the MRI analysis must be established to establish the utility of this technique to measure changes in tendons over time.

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Combining a Peripheral Nerve Matrix Derived Hydrogel and Post-Surgical Therapy for Improving Functional Recovery Following Nerve Reconstruction

In the US, peripheral nerve injury (PNI) affects an estimated 20 million people, totaling nearly $150 billion yearly in health-care costs. Without intervention, peripheral nerves show a slow and lacking regenerative response following injury, making surgical intervention an imperative. Creating a therapy to both increase the rate of regeneration as well as the extent of function regained is of great clinical interest. A novel peripheral nerve-specific extracellular matrix (PNM) hydrogel has been shown to increase constructive remodeling of injured peripheral nerves, and this study primarily aims to observe the PNM hydrogel's efficacy in a rat sciatic transection model. Also, this study aims to observe potential changes in the PNM hydrogel's efficacy by incorporating post-surgical therapy. This rationale is based on electrical stimulation therapies having shown to increase nerve recovery when used in conjunction with standard nerve repair techniques. Data will be collected up to 24 weeks post-surgery using sciatic functional index, end-study electrophysiology metrics, and histological analysis to assess differences in nerve regeneration with varied treatments and age of the animal. Initial data from a rat sciatic crush model shows that the nerve gel increases the rate of recovery, matching positive controls quicker than groups treated without the gel. Up through 12 weeks of the 24 total weeks have been completed by all rats entered into the study. Kinematic metrics have shown recovery up through 10 weeks post-surgery with no significant differences observed between repair groups with and without the PNM hydrogel. While this study focuses on a transection injury model, we are also conducting parallel studies to observe the PNM hydrogel's efficacy in crush and gap models. Furthermore, we are conducting this study to create a multi-faceted therapy to regenerate peripheral nerves more effectively by inclusion of post-surgical therapy.

TITLE: “Combining Traditional Medicine, Regenerative Treatment and Current Technology to Optimize Care of Patients with Chronic Obstructive Pulmonary Disease (COPD)”

Chronic obstructive pulmonary disease (COPD) is characterized by chronic airflow obstruction, impaired gas exchange and destruction of alveoli. It is a progressive and eventually fatal disease. Worldwide, more than 210 million people suffer from COPD and is predicted that it will be third leading cause of death globally by 2020. According to 2010 economic analyses, indirect and direct costs related to COPD treatment, missed work and disability totaled over $35 billion. COPD is an often-overlooked disease and many COPD patients are marginalized once conventional treatment has failed.

Current management of COPD may alleviate symptoms but no conventional treatment can modify the progressive course of the disease except lung transplant which has a 50% mortality rate at 5 years. Inhalers, steroids and lung reduction surgery may temporarily reduce symptoms but COPD continues to have a significant negative impact on quality of life. Recent advances in the field of Regenerative Medicine for lung disease have shown that autologous cellular therapy along with activated platelets may directly affect chronic airflow obstruction by reducing inflammation and promoting the repair of damaged lung tissue. The long-term mechanism of action is not yet completely known and remains under investigation.

400 patients underwent cellular therapy with their own cells and platelet rich plasma (PRP-PC) harvested from peripheral blood and/or bone marrow. Patients underwent pre-treatment spirometry for baseline Forced Expiratory Volume in 1 second (FEV1) %. FEV1 is a direct reflection of the severity of obstruction and is the standard for determining a patient’s COPD GOLD Stage (stages I-IV with I being mild and IV being very severe obstruction). As COPD worsens, FEV1 decreases. After 3 months, patients underwent post-treatment spirometry to detect any change after treatment. Informed consent was obtained from all patients prior to collecting any data.

The mean age of the sample was 71 years. 59.8% were men, 40.2% women. 87% of the sample were former smokers. No patients were smoking at the time of treatment. 3 months post-cellular therapy, 56% of patients improved their FEV1. The mean improvement was 6.2%, 35% had a decline in FEV1 with an average decline of 3%, and 9% had no change from baseline. The Clinical COPD Questionnaire (CCQ) was administered to all patients at pre-treatment and at 3 months post-treatment. 77% reported an improved quality of life at 3 months. 13% reported a decline in quality of life, and 10% were unchanged.

Although encouraging it is but one avenue of treatment. It is known that Pulmonary Rehabilitation programs are also very useful to these patients with studies showing physical activity through metabolic, mechanical and hormonal stimuli can increase activation, mobilization and differentiation of stem cells resulting in positive impacts on endurance, shortness of breath, quality of life and FEV1 decline. But, although instructed to do so, documentation showed only 19/400 started a program with 7 completing it.

The Lift Pulmonary Rehabilitation Program is an online program for patients with COPD available via smartphone, tablet or computer. In initial studies, patients participating in Lift saw a 150% increase in self-reported exercise capacity (n=92) and a 37% improvement in CCQ scores (n=8).

We would like to show that the combination of conventional medical therapy with Regenerative Medicine based treatment and the application of current interactive communication technology to improve patient compliance with Pulmonary Rehabilitation will result in more effective control of COPD and improvement in patients’ quality of life.

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Title: Early Increase in Voluntary Running Activity Was Associated with a Slower Rate of Cartilage Degeneration in Rats following Medial Meniscal Transection

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BACKGROUND: Despite extensive rehabilitation, reduced activity level is often observed in patients following traumatic knee injuries. Such behavior change may result in insufficient mechanical joint loading required to maintain knee cartilage health. While reduced knee loading post-injury has been associated with post-traumatic osteoarthritis (PTOA) in patients with knee injuries, the relationship between post-injury change in activity level and degrees of subsequent cartilage degeneration remains unknown.

PURPOSE: Using a rodent knee OA model induced surgically, this study aimed to establish the relationship between post-injury changes in voluntary activity and knee PTOA development.

METHODS: Thirteen adult male Lewis rats received medial meniscal transection (MMT) surgery in their left hind limbs. These samples were part of a larger experiment comparing different rehabilitative interventions post-MMT. Specifically, 6 of the 13 rats also received 30 mins/day treadmill running (4 days/week) for 2 weeks before surgery and for the entire 8-week follow-up post-MMT. Changes in voluntary activity were assessed by placing each rat inside a cage mounted with a running wheel for 7 consecutive days 1 week before surgery and at 2, 5, and 8 weeks post-surgery. To allow time for acclimation to the running wheel, only data from day 3-7 (i.e. 5 consecutive days) at each week of wheel running activity assessment were used for analysis. Rats were euthanized at the end of the 8th week, and the 3D microstructure and composition of the tibial cartilage was quantified using contrast enhanced μCT. Multiple linear regression analyses were used to examine the associations between post-MMT changes in voluntary daily running distance and cartilage microstructure/composition while controlling for the potential effects of different interventions.

Results: While on average the rats demonstrated a small change in daily running distance at 2, 5, and 8 weeks post-MMT (mean: +6.9, -7.7, and -8.3 % of pre-MMT, respectively), a big variation was observed among the rats (SD: 49.0, 41.6, and 62.6 %, respectively). Decreased voluntary daily running distance 2-week post-MMT was associated with a greater exposed bone area ($P = 0.013$) as well as increased thickness ($P = 0.010$) and decreased proteoglycan composition ($P = 0.042$) of the remaining tibia cartilage.

Conclusions: Our preliminary findings suggest that higher levels of voluntary activity following traumatic knee injuries may be associated with a slower rate of PTOA progression in rats with MMT. Future studies implementing a more stable pre-MMT baseline activity level as well as continuous monitoring of post-MMT changes in activity may provide further insight into the benefits of facilitating early weight-bearing activities on maintaining cartilage health after knee injuries. A better understanding of how post-injury changes in activity affect joint health is also essential on determining the efficacy of regenerative therapies (e.g., stem cells) that are sensitive to joint loading environment.

Effectiveness of Mesenchymal Stem Cells for Treating Patients with Knee Osteoarthritis: A Meta-analysis Toward the Establishment of Effective Regenerative Rehabilitation

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ABSTRACT:

INTRODUCTION: Physical factors regulate mesenchymal stem cell (MSC) differentiation and tissue development, pointing to a potential therapeutic strategy for enhancing the MSCs injected or implanted into the knee joint, such as the recently proposed new field *regenerative rehabilitation*. This systematic review with a meta-analysis aimed to summarize the current evidence of the effectiveness of MSC treatment for knee osteoarthritis (OA) and to examine whether rehabilitation is an effect modifier of the effect estimate of MSC treatment.

METHODS: A literature search was conducted in PubMed, PEDro, CINAHL, and Cochrane CENTRAL until August 2017 were searched. Where possible, data were combined into a meta-analysis; the pooled standardized mean differences (SMD) of between individuals with knee OA and healthy adults were calculated using the random-effect model. If study heterogeneity was confirmed using the I^2 statistic ($I^2 \geq 50\%$ indicates heterogeneity), random effects meta-regression was performed using the certain parameters including the presence of rehabilitation defined when patients were treated using physical therapy modalities, range of motion exercise, or muscle strength exercise at least one time after MSC treatment.

RESULTS: A literature search yielded 659 studies, of which 35 studies met the inclusion criteria (n = 2385 patients; mean age: 36.0–74.5 years). The meta-analysis results suggested that MSC treatment through intra-articular injection or arthroscopic implantation significantly improved knee pain (standardized mean difference [SMD]: −1.45, 95% confidence interval [CI]: −1.94, −0.96), self-reported physical function (SMD: 1.50, 95% CI: 1.09, 1.92), and cartilage quality (SMD: −1.99; 95% CI: −3.51, −0.47). However, the MSC treatment efficacy on cartilage volume was limited (SMD: 0.49; 95% CI: −0.19, 1.16). Minor adverse events (knee pain or swelling) were reported with a wide-ranging prevalence of 2–60%; however, no severe adverse events occurred. The evidence for these outcomes was “very low” to “low” according to the Grades of Recommendation, Assessment, Development and Evaluation (GRADE) system because of the poor study design, high risk of bias, large heterogeneity, and wide 95% CI of the effects estimate. Performing rehabilitation was significantly associated with better SMD for self-reported physical function (regression coefficient: 0.881, 95% CI: 0.049, 1.712; P = 0.039).

DISCUSSION: MSC treatment improves knee pain, physical function, and cartilage quality, without any severe adverse events. However, evidence for these outcomes that are considered critical for clinical decision making was “very low” to “low” according to the GRADE system because of the poor study design, high risk of bias, large heterogeneity, and wide 95% CI of the effects estimate. Detail information about rehabilitation is lacking; therefore, the role of rehabilitation in MSC treatment in patients with knee OA is unclear. However, rehabilitation was a significant effect modifier of better MSC treatment on self-reported physical function, supporting a concept of the newly born field, *regenerative rehabilitation*. Integration of rehabilitation into MSC-based therapy may be beneficial at least in improving self-reported physical function. These findings would help researchers and clinicians in designing future high quality clinical trials.

Effectiveness of rehabilitation after cell transplantation for peripheral nerve regeneration: protocol for a systematic review

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Introduction
Autograft is a gold standard for regeneration after peripheral nerve defect but leads to donor-site dysfunction. Cell transplantation offers an alternative treatment for peripheral nerve injuries. Rehabilitation, such as ultrasound stimulation, could accelerate peripheral nerve regeneration. However, it is uncertain whether rehabilitation enhances the effects of cell therapies or not. The aim of this systematic review is to evaluate the effectiveness of rehabilitation after cell transplantation in animal models of peripheral nerve injuries.
Methods
This systematic review was conducted according to PRISMA protocols. We developed a search strategy to investigate studies which combined cell transplantation and rehabilitation after peripheral nerve injuries. The electronic databases of PubMed, Physiotherapy Evidence Database (PEDro), and Cumulative Index to Nursing and Allied Health Literature (CINAHL) were used.
Results
Ten studies on the combination of cell transplantation and rehabilitation were identified. They transplanted Schwann or stem cells and conducted physical or exercise therapies as methods of rehabilitation. Either cell transplantation or rehabilitation could increase neurotrophic factors and promote nerve regeneration and functional recovery, which are better enhanced by their combination. However, compared with autograft, the effect of combination therapy is inadequate.
Conclusion
Rehabilitation after cell transplantation is effective for peripheral nerve regeneration, however, the effect is insufficient. For further advances of cell therapy to facilitate peripheral nerve regeneration, a study on optimal intensities of rehabilitation is required.

Effects of the combined therapy of rehabilitation with cell therapy on motor functional recovery after cerebral infarction: A systematic review.

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Introduction

Cerebral infarction is known as a top cause of indispensable nursing care in Japan because of its severe motor dysfunction, as a common complication. Cell therapy strategy is a noted approach for neural repair, and the rehabilitation is known as a conventional strategy for functional recovery after cerebral injury, however it is unclear whether rehabilitation enhance effects of cell therapy. The aim of this study is to systematically and comprehensively collect papers reporting effects on motor functional recovery under the combined therapy in the brain ischemic animal model, and clarify the current situation.

Methods

The research design was systematic review and was carried out according to the PRISMA statement. The electronic data bases of PubMed, Physiotherapy Evidence Database (PEDro), Cumulative Index to Nursing and Allied Health Literature (CINAHL), and Cochrane Central Register of Controlled Trials were used, using search expression; stroke or brain ischemia, and stem cell or cell transplantation, and rehabilitation or environment or recovery of function. All papers were published in English by May 2018, and medical diseases and genetic diseases were excluded.

Results

The ten studies referring to the combined therapy were extracted, and most of studies indicate that rehabilitation enhance the effect of cell therapy, however there were no consistent results or unified views.

Discussion/Conclusions

The combined therapy may enhance these independent effects of each single therapy, but the evidence for the benefits are lacking, and mechanisms are unclear. The number of studies with respect to the combined therapy is few, and their interventions are not sufficiently verified. Further evidences of rehabilitation effects in the cell therapy are expected. We consider that these evidences demonstrated by physiotherapists themselves, who can explain the importance of physical therapy intervention in regenerative medicine even better, is much more potent.

Exosomes carrying Klotho: a potential biomarker for developing customized rehabilitation protocols

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ABSTRACT

Epidemiologically, low systemic level of an anti-geronic protein, α- Klotho, has been associated with low activity levels, poor grip-strength, lower muscle function and declining cognition in humans. It has also been found that high circulating levels of Klotho can delay the onset of age-related declines in skeletal muscle regeneration and cognition. Studies in rodents have indicated that contractile muscle activity boosts circulating α-Klotho levels as well as upregulates *Klotho* in the hippocampus. In order to establish a molecular mechanism correlating the exercise-mediated boost in serum α-Klotho levels and enhanced *Klotho* in the hippocampus, we proposed that exosomes are the means for crosstalk of α-Klotho between muscle and the brain. We used Surface Plasmon Resonance (SPR) technique to evaluate α-Klotho on the membrane of exosomes in mouse serum. Here we show that, serum from control young mice (3 months, C57/BL6) express α-Klotho in CD63 and CD81 positive exosomes. However, this expression in exosomes is enhanced with muscle contractile activity via Neuro-Muscular Electrical Stimulation (NMES). We also used Raman Spectroscopy to obtain the Raman fingerprint of the circulating exosomes in both groups. The spectroscopy indicated the presence of fewer exosomes in the control group which significantly increased with NMES. Such fingerprints can be used as a tool for easy monitoring of progression of rehabilitation protocols. Taken together, these findings suggest that an exercise-induced boost in circulating α-Klotho, is in part, due to an increase in release of exosomes that carry α-Klotho into the circulation. This novel mechanism could help the field of skeletal muscle rehabilitation, implement customized rehabilitation techniques that would depend on the serum exosome-Klotho levels; thereby using exosomes that carry α-Klotho as a biomarker for predicting various age-related declines in muscle vitality and cognition.

Exposure of muscle stem cells to a stiff microenvironment drives an "aged" mitochondrial phenotype

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Aging is typically associated with increased tissue stiffness due to fibrosis and abnormal extracellular matrix (ECM) deposition. Although it is widely appreciated that extrinsic mechanical properties have the potential to impact stem cell fate, we know little about how tissue stiffening associated with increased age affects stem cell function. Our previous study demonstrated that aging causes alterations in ECM mechanical properties in skeletal muscle, and that these changes drive fibrogenic conversion of muscle stem cells (MuSC) (Stearns-Reider, et. al., Aging Cell, 2017.).

Here, we tested the hypothesis that aberrant ECM stiffness impacts muscle stem cell mitochondrial structure and bioenergetics. We focused on mitochondrial phenotype and function given its potential role in fibrosis. We also focused on the effect of substrate stiffness on regulation of the longevity gene, *Klotho,* given our recent findings that Klotho plays a critical role for MuSC bioenergetics. Klotho also has previously reported role in the inhibition of signaling pathways associated with fibrosis, including Wnt/β-catenin and TGF-β1, both of which have been reported to drive a muscle stem cell fibrogenic conversion with age (Zhou et. al. 2013).

First, to quantify how aging affects muscle stiffness, we performed biaxial testing on young and aged muscle, followed by finite element analysis. Our analysis reveals the Young's Modulus (E) of aged muscle to be approximately four-fold higher than young muscle.

Next, to study the effect of static muscle mechanical properties on MuSC mitochondrial structure and function, we engineered PDMS substrates that mimics the stiffness of young and aged muscle based on our finite element analysis. We then seeded the constructs with young MuSCs. Consistent with our finding that aged MuSCs display a significantly reduced mitochondrial network size and bioenergetics, we find that young MuSCs cultured on the more rigid (aged) PDMS display decreased mitochondrial network size and bioenergetics. Moreover, MuSCs cultured on a more rigid PDMS also exhibited Klotho repression, which is also consistent with our previous finding that Klotho expression of aged MuSCs is attenuated (Sahu et al., Nat. Commun., accepted).

Taken together, these findings suggest that age-related increases in muscle stiffness may drive an age-like phenotype of mitochondria and mitochondrial dysfunction, as determined by mitochondrial morphology and bioenergetics. These declines may be attributed to declines in Klotho expression. Our studies underscore the importance of skeletal muscle ECM rigidity on MuSC function and provide potential mechanism of myogenic-to-fibrogenic conversion of MuSC due to increased age.

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Poster title:
Functional Rehabilitation of neural networks following Moderate-to-Severe Traumatic Brain Injury using combination of biomaterials and electric stimulation methods

Abstract:
Traumatic Brain Injuries (TBI) are complex injuries ranging from mild to severe physical trauma, and that result in devastating neurological consequences and disability depending on the extent of primary brain tissue damage and secondary neuro-inflammatory and cytotoxic damage sustained. There are currently no treatments to facilitate the regeneration and functional recovery of damaged neural tissue after TBI. Using a combination of both in vitro and in vivo models of TBI we have begun to understand the underlying mechanisms that govern molecular, cellular, network and cognitive changes following TBI.

We have previously demonstrated that the injection of neural stem cells encapsulated in a brain extra-cellular matrix (ECM) mimicking chondroitin-sulfated glycosaminoglycan matrix significantly enhances neural stem cell maintenance and neuroprotection following severe TBI [1, 2] . In recently published studies, we used embryonic stem cell-derived neuron and glial co-cultures exposed to inhibitory doses of the excitotoxic agent glutamate, and investigated the effects of three separate functional electrical stimulation (FES) paradigms on network activity using high-density microelectrode arrays. Results from these studies suggest that electric stimulation, when administered acutely after an injury, can enhance neuronal network excitability and synchrony, by mechanisms involving the enhanced expression of plasticity genes -NMDA receptor NR2A, brain-derived neurotrophic factor (BDNF) and Ras-related protein (RAB3A) [3]. Based on these results, we hypothesize that the application of electrical stimulation alone and in combination with neurointegrative glycomaterial implants would support the increased repair and re-functionalization of damaged brain tissue in an animal model of severe TBI.

Our preliminary results demonstrate that the application low current, low-frequency stimulation for one week following a controlled cortical impact injury of the motor cortex (Forelimb M1 cortex) resulted in the partially enhanced rehabilitation of both balance related behavior as well as skilled motor control in rats. These promising results open up possibilities for the application of passive/adaptive stimulation techniques in combination with regenerative glycomaterial constructs containing pre-functionalized neural networks for the functional repair of damaged brain tissue. These approaches could facilitate the rehabilitation of the subject without compensatory or loss of tertiary cognitive functions in the future.

[1] Betancur, Martha I., et al. "Chondroitin Sulfate Glycosaminoglycan Matrices Promote Neural Stem Cell Maintenance and Neuroprotection Post-Traumatic Brain Injury." *ACS biomaterials science & engineering* 3.3 (2017): 420-430.
[2] Karumbaiah, Lohitash, et al. " 'Brain glue' for the acute treatment of severe traumatic brain injuries." *Brain Injury*. Vol. 31. No. 6-7. 530 Walnut street, ste 850, Philadelphia, PA 19106 USA: Taylor & Francis Inc, 2017.
[3] Latchoumane, Charles-Francois V., et al. "Chronic Electrical Stimulation Promotes the Excitability and Plasticity of ESC-derived Neurons following Glutamate-induced Inhibition In vitro." *Scientific reports* 8 (2018).

Functional Seated Vertical Traction for Rehydration Promotion in Lumbar Intervertebral Discs: a three-phase pilot study

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INTRODUCTION: Prolonged workplace sitting at a computer is nearly ubiquitous, with more than 80% of a workday spent sitting for certain jobs. Sitting statically may have pernicious effects on the worker's spinal health. Limited research exists for *in vivo* changes when human lumbar intervertebral discs (LIVDs) undergo repetitive prolonged creep deformation. Under sitting compression, healthy LIVD cells can be deprived of vital nutrients, even lag in recovery, promoting avenues of structural weaknesses and degeneration. Furthermore, injured LIVDs lack pressure and integrity to withstand even minimal creep loading, making sitting difficult. Reducing gravitation forces allows disc hydration to resume. Lying supine or using traction devices has long been implemented to do this; often used in rehabilitation. In many cases, however, horizontal traction or supine lying (SL) prevents typical participation in computer work. A three-phase pilot project was designed to explore how vertical traction (VT) may reduce LIVD compression and promote hydration, all while sitting and allowing computer work performance. The overarching aims were as follows: 1) find an effective, safe and comfortable VT dosage, 2) determine LIVD rehydration capabilities (akin to SL) by comparing seated spinal height changes (SHC) in VT/computer use, SL, and sitting (S)/computer use, and 3) compare SHC in healthy subjects to subjects from prolonged sitting occupations with possible low back pain and age-related LIVD degeneration.

METHODS: A repeat measures-crossover design was used in all phases. A sitting-based stadiometer was used for all spinal height measurements. Institutional review board approved subjects consented and passed a stadiometer inclusion criterion of 1.3 mm SD over five consecutive measurements, out of a maximum of 10 tries in Phase 1, and out of 30 in Phases 2 and 3. In Phase 1, each of four 10-min positions was tested with 48 h in between: 1) SL (no tasks), 2) 20%, 3) 35%, and 4) 50% body weight removed (BWR) (with computer tasks) via VT apparatus. A custom-built belt with vertical harness pulley system was used to remove body weight. Hydration standardization methods were added in Phase 2 and Phase 3 to normalize individuals' LIVD hydration prior to testing. Phase 2 tested healthy subjects in triplicate in VT, SL, and S, and required a criteria-based stadiometer review before each test. The same criteria-based stadiometer review and variables were tested singularly in Phase 3 (including visual analog pain ratings) on a population from prolonged seated occupations and possible age-related spine degeneration. An optional 3-question, qualitative survey was offered at the end of each phase. The overall SHC means were statistically analyzed with a repeat measures ANOVA with post hoc analyses.

RESULTS AND CONCLUSION: Phase 1 (N = 24; 12 F, 12 M, $\acute{x}_{age}$ = 22.6 yrs (±2.9); $\acute{x}_{weight}$ = 72.8 kg (±12.4)) determined VT dosage of 35% BWR (P=.300) was most equivalent to SL in Phase 1, and most comfortable and effective. Phase 2 used 35% BWR VT (N = 15; 8 F, 7 M, $\acute{x}_{age}$ = 22.3 yrs (±3.0); $\acute{x}_{weight}$ =79.2 kg (±12.5); $\acute{x}_{height}$ = 174.9 cm (±12.1); $\acute{x}_{BMI}$ = 26.1 kg/m^2 (±4.6)) and showed significant SHC difference between both VT-S and SL-S (P=.001 and P=.000 respectively) and VT-SL were equivalent (P=.935). In Phase 3 (N = 27; 21 F, 6 M, $\acute{x}_{age}$ = 41.1 yrs (±12.1); $\acute{x}_{weight}$ =76.3 kg (±18.5); $\acute{x}_{height}$ = 169.8 cm (±9.9); $\acute{x}_{BMI}$ = 26.3 kg/m^2 (±5.1)), significant differences between the same variables were also found (P=.045), however, they were undetectable via post hoc analyses. Phase 2 and 3 VT means were not significantly different (P=.228) due to sample size limitations

in Phase 3. This project sheds light on an alternative to providing *in vivo* LIVD hydration promotion externally, and the potential to impact rehabilitation, occupational, regenerative and prevention medicine approaches for LIVD health. A future study involving diffusion weighted MRI is currently under negotiation and collaboration.

Hoxa10 regulates skeletal muscle regeneration in a body-region-specific manner.

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Muscle regeneration depends on muscle stem cells, named satellite cells. Satellite cells have prominent regenerative capacity, and thus satellite cell-based regenerative therapy would be a promising option for intractable muscular diseases such as muscular dystrophy. Although not all muscles throughout the body are equally affected in muscular diseases, little is known about the underlying mechanisms. In this study, genome-wide gene expression analysis revealed that *Hoxa10*, one of the homeobox genes, was highly expressed in limb but not in head muscles of adult mice. Genetic inactivation of Hoxa10 in satellite cells isolated from limb muscles resulted in a decrease in phosphorylation levels of Rb protein, leading to a reduction in proliferation ability in culture ex vivo. Satellite cell-specific deletion of *Hoxa10* in mice caused remarkable defect in regeneration of limb muscles after cardiotoxin-induce muscle injury. We further showed that gene expression pattern of *Hoxa10* in adult muscles almost mirrored the embryonic origin: *Hoxa10* was detected in only somite-derived muscles and their associated satellite cells in adults. The regional-specific expression of *HOXA10* was also observed in human skeletal muscle, with its knockdown affecting proliferation ability of satellite cells. Taken together, our data indicated that Hoxa10 plays an indispensable role in muscle regeneration by regulating satellite cell function in somite-derived muscles, contributing to the body-region specificity in muscle diseases.

Immmuno-regulatory roles of cyclic loading that promotes skeletal muscle regeneration

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Skeletal muscle repair and regeneration are governed by inflammation. Physical manipulation of tissue including massage therapy has been utilized to aid skeletal muscle recovery from injury, yet the link between mechanical stimulation (MS) and inflammation for tissue repair and regeneration is unknown. Here, we investigated the impact of cyclic loading on interstitial inflammation and the functional consequences on muscle regeneration following severe injury. To this end, we utilized a soft robotic device to externally apply cyclic loading on the severely injured tibialis anterior muscle of mice. First, injured muscle was found to exhibit significant improvements in contraction force and histological features (i.e. reduction in fibrosis and calcification) after 14 day-MS as compared to a group without MS. Interestingly, the group treated with MS showed a significant reduction in pro-inflammatory immune cell population relative to its control counterpart after 3 days. Cytokine array analysis indicated that the majority of cytokines were reduced with MS, and significantly decreased cytokines were specifically associated with pro-inflammatory cytokines. Lastly, *in vitro* studies indicated that the altered pro-inflammatory factors stimulated the proliferation of satellite cells while diminished their myogenic differentiation. This data implies that prolonged residence of specific pro-inflammatory factors in damaged tissues may delay the commitment of satellite cells into myogenic differentiation, which is a key player in the regenerative process. In summary, cyclic loading can modulate the immune cell population and inflammatory cytokine profile in injured tissue, thereby stimulating muscle regeneration, and this finding can be broadly applicable to tissue regenerative therapy.

Immunomodulation of the eye microenvironment using a corneal wound model

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Ocular injuries remain a significant cause of morbidity for military service members. While 85% of surviving wounded can return to active duty, only 25% of those with eye injuries can return to combat status.[1] To address the challenge of vision loss and fibrosis due to corneal injury, we sought to define the immune profile in corneal injury associated with fibrosis and vision loss and develop immunomodulatory biological scaffolds for corneal regeneration and fibrosis reduction. Biological scaffolds, also termed extracellular matrix (ECM) scaffolds, are used clinically in a variety of applications to facilitate repair, however the way in which this repair is done is poorly understood.[2345] Utilizing a murine model, we aimed to characterize and quantify the immune populations associated with corneal damage and ECM treatment. We devised three experimental wound groups, including a control group, an incisional wound model, and a debridement wound model. Each group was then either given sub-conjunctival injections of PBS or ECM. The study consisted of two-day and one-week experimental groups. At the experimental time point, the ipsilateral and contralateral eyes and draining lymph node were harvested and the corneas were surgically removed. The immune populations were then analyzed using flow cytometry and RT-PCR. The pathology of the cornea was confirmed using H&E staining. At the 48-hour timepoint, we see neutrophil infiltration and increased IL-4 expression in the ECM treated mice, suggesting a pro-regenerative state. At one-week, expression of SiglecF, an eosinophil marker, was higher in the ECM treated mice. Expression of IFNγ, an inflammatory cytokine, was lower in the ECM treated mice when compared with the control. In conclusion, we have shown that ECM nanoparticles have an immune modulatory capacity at early time points. In order to further study the immune modulatory capacity of ECM, a four-week fibrosis study will ensue.

1 Aslam S, Griffiths MF (2005) Eye casualties during Operation Telic. J R Army Med Corps 151: 34-36.

2 Dreifuss SE, Wollstein R, Badylak SF, Rubin PJ (2015) Acellular micronized extracellular matrix and occlusive dressings for open fingertip injuries. Plastic and Aesthetic Research 2: 282.

3 Valerio IL, Campbell P, Sabino J, Dearth CL, Fleming M (2015) The use of urinary bladder matrix in the treatment of trauma and combat casualty wound care. Regenerative medicine 10: 611-622.

4 Afaneh C, Abelson J, Schattner M, Janjigian YY, Ilson D, et al. (2015) Esophageal reinforcement with an extracellular scaffold during total gastrectomy for gastric cancer. Annals of surgical oncology 22: 1252-1257.

5 Beachley VZ, Wolf MT, Sadtler K, Manda SS, Jacobs H, et al. (2015) Tissue matrix arrays for high-throughput screening and systems analysis of cell function. Nat Methods 12: 1197-1204.

In Vitro Trauma Induces TDP-43 Proteopathy and Exacerbates Motor Neuron Degeneration in ALS Patient iPSC-derived Motor Neurons

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Background: A history of neurotrauma, such as mild traumatic brain injury, appears to be an epidemiological risk factor for Amyotrophic Lateral Sclerosis (ALS). To help address the clinical association of neurotrauma with ALS, we applied an *in vitro* stretch trauma model to the study of patient-specific motor neurons (MNs) generated from induced pluripotent stem cells (iPSCs). This recently developed trauma system has now been validated to deliver a tunable injury ranging from severe neurodegeneration to milder forms of axonal and synaptic injury.

Materials and Methods: Briefly, day 4 iPSC-MNs derived from healthy controls, SOD1A4V and C9orf72 mutants underwent stretch injury. A 96-well multi-electrode array (MEA), custom built, polydimethylsiloxane (PDMS) bottomed plate was lowered, stretching the well membrane over the rims of static metal posts, causing mechanical stress to the cells in the well. Posts were omitted from the post array to create unstretched control wells within the same plate. The cultures were then fixed 4-72 hours after injury and stained for analysis.

Results and Discussion: We found that MNs from C9orf72, but not healthy controls or SOD1A4V, had abnormally increased TDP-43 in the cytoplasm. This phenomenon is referred to as TDP-43 mislocalization. Interestingly, these results are congruent with the clinical observation that C9orf72, but not SOD1, patients exhibit TDP-43 proteopathy. Additionally, all ALS mutant cell lines exhibited greater vulnerability to degeneration after mild trauma as compared to healthy control MNs.

Future Work: Further studies to elucidate the molecular mechanisms that mediate ALS-specific pathology after stretch injury are currently underway. To functionally characterize trauma models, flexible serpentine electronics with capabilities to withstand the trauma system described above are being implemented in plates with PDMS substrate. Current work has shown feasibility to grow MN-iPSCs before and after injury. The interface with Maestro Axion Biosystems to record functional electrical activity from flexible MEAs is in progress.

Title: Inducing robust forelimb recovery with optogenetic spinal stimulation in a rat model of chronic cervical spinal cord injury

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Abstract: Spinal cord injury (SCI) disrupts spinal circuitry causing movement, sensation, and proprioception deficits. When located in the cervical spinal cord, hand and arm function are diminished, which severely limits autonomy and quality-of-life. To date, there are approximately 288,000 individuals in the US living with chronic SCI with 47.2% of those individuals having a cervical incomplete injury. Restoring function in these individuals requires the formation of new circuitry that bypasses the lesion site. Both axonal plasticity and synaptic reorganization are critical components of new circuit formation, but are primarily inhibited in the adult central nervous system (CNS), which is exacerbated in the chronically injured spinal cord. Fortunately, a variety of studies have found neuronal activation via electrical stimulation to enhance plasticity through the upregulation of growth factors, neuroprotective factors, and increased perfusion. Further, electrical stimulation of the spinal cord after injury has been shown to improve locomotion and hand/arm function. Optogenetic spinal stimulation (Opto-Stim) is an alternative method of neuronal activation that utilizes optogenetic technology. Specifically, spinally injured rats are transduced with an optogenetic vector in the region of the cord caudal to the lesion site, then are implanted with a blue micro-LED on the surface of the spinal cord for stimulation. Optogenetic stimulation may be more effective at improving recovery after chronic injury compared to electrical stimulation as studies suggest it induces a more natural recruitment pattern compared to electrical stimulation.

The current study investigates the effects of Opto-Stim in rats with a cervical hemicontusion at C4. Opto-Stim is targeted caudal to the lesion site at C6. Stimulation is delivered at 4Hz, 5ms pulse width, 5 seconds on/15 seconds off at movement threshold for 1hr/ day for four days during the first week of stimulation (induction phase), then 1hr/week for the remaining weeks of stimulation (maintenance phase). Stimulation is always completely during rehabilitation in order to guide plasticity to the appropriate targets. Intriguingly, our preliminary results indicate Opto-Stim has robust effects on functional recovery even in these chronically injured rats. Specifically, 5 weeks of Opto-Stim beginning 6 weeks post-injury led to a 77% improvement on a skilled forelimb-reaching task with an exciting 32% increase in performance on the first day of stimulation. Additionally, we saw a 33% increase in skilled forelimb reaching performance after 1 week of stimulation beginning 10 weeks post-injury. Immunoreactivity against GAP-43, a marker of plasticity, was also enhanced in rats that received Opto-Stim compared to controls. These findings indicate Opto-Stim has the potential to be a powerful treatment option for those with chronic SCI.

Low Intensity Vibrations Augment Proliferation and Differentiation in Aging Mesenchymal Stem Cells

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Poor musculoskeletal health is one of the primary contributors to disability among aged individuals. Stem cell aging, typified by reduced proliferative and differentiative capacity, is in-part driven by decreased mechanosensory capabilities of cells –observed as attenuated exercise efficacy in older individuals. A principal source of mechanical signals that are universally recognized to maintain a healthy musculoskeletal system is exercise. A primary component of exercise, mechanical signals, when applied in the form of low intensity vibration (LIV), increases MSC osteogenesis and decreases adipogenesis. Our team has shown that MSC mechanotransduction in response to LIV relies on LINC (Linker of Nucleoskeleton and Cytoskeleton) complexes that connect the cytoskeleton to nucleus. Not only is LINC required for a fully-executed mechanoresponse but it promotes mechanoadaptation: application of LIV increases expression of LINC elements in MSCs. We have found that inducing LINC deficiency accelerates MSC adipogenesis and halts osteogenesis, suggesting that LINC-mediated connectivity may be a key aspect of MSC health – such that its loss during aging contributes to a degraded mechanoresponse. Therefore we asked if improving LINC expression via LIV can restore MSC mechanoresponse in an *in vitro* aging model.

In vitro aging model, while not entirely consistent with “real” aging, has previously been used to model aspects of aging in vitro. Two identical set of MSCs were kept sub-confluent (<60%) and passaged twice weekly, while one set was LIV treated (Indicated as P5L, P7L etc…) twice daily (20min at 90Hz, 0.7g) the other set was not vibrated (indicated as P5, P7 etc...). At P17, we observed a decrease in LaminA/C (73%, $p<0.05$) and LINC elements Sun-1 (70%, $p<0.05$), Sun-2 (86%, $p<0.5$), Nesprin-2 (82%, $p<0.5$) while application of LIV increased LaminA/C, Sun-2 and Nesprin-2 by two-fold ($p<0.05$). Comparing p-FAK (Tyr397) phosphorylation, indicative of integrin engagement, between young P6 and older P40 MSCs showed a 87% decrease ($p<0.05$) while p-FAK levels were improved 3-fold ($p<0.05$) in P40L MSCs. LIV further promoted proliferation rates, increasing cell-doubling 81% ($p<0.05$) over 40 passages which resulted in 47% shorter telomeres ($p<0.05$). Osteogenic capacity measured by ALP (Alkaline Phosphatase) expression at P30 against young P6 MSCs remained 58% higher in LIV-treated P30L MSCs ($p<0.05$). An LIV regimen, added to test mechanoresponse, increased ALP expression of non-treated (P30) and LIV-treated (P30L) MSCs 2.8-fold and 4.8-fold, respectively.

In summary our findings that despite having shorter telomeres, long term LIV-treated MSCs remain proliferative, pro-osteogenic and more mechanoresponsive may lead to a strategies to improve MSC mechanosignaling and health for non-pharmacologic regenerative medicine strategies.

Markerless Mobility Assessment Techniques Toward an Establishment of Outcome Measures of Regenerative Rehabilitation for Knee Osteoarthritis

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ABSTRACT:
INTRODUCTION: Measurement of treatment outcomes and mobility limitation over time is a critical component of clinical practice and research in regenerative rehabilitation for people with knee osteoarthritis (OA). A recently published meta-analysis demonstrated that rehabilitation may enhance therapeutic effect of mesenchymal stem cell treatment for self-reported physical function in people with knee OA (Iijima H, npj Regen Med 2018). However, objectively measured mobility assessment was lacking in these clinical studies, which could limit development of *Regenerative Rehabilitation* in the clinical setting. We have proposed markerless mobility assessment systems during Timed Up and Go (TUG) test "Laser-TUG" (Yorozu A, Sensors 2015), which enable us to calculate the spatiotemporal gait parameter of TUG subtask (sit-to-walk [STW], walking a short distance, and turning). To further progress establishing markerless mobility assessment systems, we proposed an inertial sensor unit (IMU)-based gait asymmetry assessment which could offer complementary information to the spatiotemporal parameters in people with knee OA. This study aimed to apply these markerless measurement techniques to determine whether these systems can capture knee OA-related mobility limitation.

METHODS: Community dwelling adults participants in the Japanese early osteoArthritis Cohort Knee (JACK) study. Each participant underwent 1) Laser-TUG measurement to calculate spatiotemporal parameters in TUG; and 2) gait analysis at self-selected speed for IMU-based gait asymmetry assessment (harmonic ratio [HR] and improved HR [iHR]). Isometric muscle strength of quadriceps was measured using a hand-held dynamometer. Knee pain severity was evaluated using self-reported questionnaire. Multiple linear regression analysis was performed to examine the relationship between knee pain or quadriceps muscle strengths and spatiotemporal TUG parameter or HR/iHR.

RESULTS: Laser-TUG (n = 165; mean age, 68.6 [50–85] years; mean BMI: 22.8 kg/m^2, 70.3% female) and IMU-based gait asymmetry assessment (n = 131; mean age, 74.2 [65–88] years; mean BMI: 21.7 kg/m^2, 71.8% female) found that subjects with severe knee pain demonstrated slower STW and greater gait asymmetry, respectively. While Laser-TUG revealed that subjects with weaker quadriceps muscle strength demonstrated slower STW, IMU-based gait asymmetry assessment revealed that subjects with greater inter-limb quadriceps muscle strength demonstrated greater gait asymmetry, rather than quadriceps muscle weakness itself.

DISCUSSION: Practical relevant of this study is that both Laser-TUG and IMU-based gait asymmetry assessment identified knee pain-related mobility limitation in people with knee OA. Interestingly, these measurements systems offer complementary information about quadriceps muscle strength; while Laser-TUG captured strength-related mobility limitation, IMU-based gait asymmetry capture inter-limb strength asymmetry. Since mobility limitation can be easily assessed by these markerless assessment systems in both inpatient and outpatient clinics, these assessment systems may be a candidate of outcome measures to assess therapeutic effects of *Regenerative Rehabilitation* for people with knee OA and musculoskeletal disease.

Minimally-Invasive Muscle Embedding (MIME) Generates Donor-Derived Muscle Fibers that have Multiple Sarcomeres and Nuclear Domains in Series

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BACKGROUND. Minimally-Invasive Muscle Embedding (MIME), is a technique developed in our laboratory, to facilitate the development of donor-cell-derived muscle fibers in a host muscle. MIME involves passing a sterile needle through a host muscle, and then implanting a segment of donor muscle tissue along with its myogenic satellite cells, in the needle track within the host muscle.
METHODS. We performed MIME on the left tibialis anterior (TA) muscle of immunodeficient host mice that expressed a green fluorescent protein (GFP; NSG-GFP mice, JAX Stk# 021937; N = 4). We implanted a single extensor digitorum longus (EDL) muscle from donor mice that expressed a red fluorescent protein (RFP; DsRed.T3 mice, JAX Stk# 006051; N = 2). After implanting donor muscle tissue by MIME, we sealed the needle wounds in host mice with veterinary tissue adhesive. As a SHAM procedure, we created a needle track in the right TA of the host mouse, but did not implant donor tissue. Several minutes after MIME (or SHAM), we injected barium chloride (BACL, myotoxin) into the left and right TA muscles, to induce degeneration of the host TA muscle along with the implanted donor EDL muscle. At 14 days post-MIME and BACL-injection, we euthanized the host mice, and collected their MIME- and SHAM-treated muscles. We weighed the muscles, snap-froze them in liquid nitrogen, and stored them at -80 °C. We made cross sections of MIME- and SHAM-treated muscles to quantify donor-cell-derived myogenesis, by counting the numbers of donor RFP+ and host GFP+ muscle fibers. On serial cross sections, we also performed immunofluorescent labeling of desmin (Z-disk protein, muscle marker) and dystrophin (sarcolemma-associated protein), and co-stained the sections with DAPI to visualize nuclei. We additionally labeled RFP, desmin, dystrophin, and nuclei in longitudinal sections, to ascertain if donor-derived fibers have multiple sarcomeres and nuclear domains in series, as would be expected of mature muscle fibers. We analyzed muscle weights, and GFP+ and RFP+ fiber counts, by Student's t-tests; and analyzed the percentage of RFP+ fibers that were positive for desmin, dystrophin, and central nucleation (marker for myogenesis), by one-way ANOVA. P-values less than 0.05 were considered significant. All cell counts were performed by blinded evaluators.
RESULTS. There was no difference in muscle weight between MIME- and SHAM-treated muscles. In MIME-treated muscles, 22 ± 4 % and 78 ± 4 % muscle fibers were RFP+ and GFP+, respectively. There were no RFP+ fibers in SHAM-treated muscles. All RFP+ fibers were positive for desmin and dystrophin, and 65 ± 4 % fibers were centrally nucleated. Donor-derived fibers had multiple sarcomeres and nuclear domains in series. Results are reported as Mean +/- S.E.M.
CONCLUSION. MIME helps generate donor-cell-derived muscle fibers in host muscle. These donor-derived muscle fibers are mature and viable since they express desmin and dystrophin, and have multiple sarcomeres and nuclear domains in series. Central nucleation of the donor-cell-derived fibers, suggests that, they have originated from myogenesis rather than from *en bloc* muscle tissue engraftment. Future experiments will assess if MIME together with progressive-resistance training, can promote regeneration in the context of volumetric muscle loss caused by muscle disease or trauma. In future studies, exercise will be used to stimulate controlled muscle degeneration and regeneration instead of BACL.

Patellofemoral osteoarthritis progression related with gait kinematics changes in rat with destabilized medial meniscus

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<Introduction>

Osteoarthritis (OA) is a progressive disease involving cartilage damage and bone deformation. It is well known that the meniscus injury induces OA on the tibiofemoral (TF) joint. However, it is unclear whether the meniscus instability promotes OA progression on patellofemoral (PF) joint, as well. In addition, the relationship between PF cartilage damage and gait kinematics remains unclear. The objective of this study was to investigate the PF OA change and the relationship between the cartilage damage and gait kinematics in the rat OA model with destabilized medial meniscus (DMM).

<Methods>

Fifty-four 12-week-old Wistar male rats were randomly divided into three groups: DMM, sham and naïve control groups (n = 18 per group). DMM rats were operated on the medial meniscotibial ligament transection in the right knees. Sham rats were received only capsule incisions in the right knees. We sacrificed and removed knee joints to assess the OA progression in histology at 2, 4 and 8 weeks (n = 6 in each time point) postoperatively. The PF OA progression was evaluated by OARSI score. We analyzed gait kinematics before sacrificed, using the three dimentional motion capture system (Kinematracer; KISSEI COMTEC). The target parameters of gait analysis were knee flexion angle and pelvic tilt angle. We performed the correlation analysis between the OARSI score and gait kinematics in all data.

<Results>

The PF OARSI score in DMM group was significantly higher than control and sham group in 2 weeks, and significantly higher than control group in 4 weeks. The PF OARSI score in sham group was significantly higher than control group in 2 and 4 weeks. But in 8 weeks, the PF OARSI score had decreased in DMM and sham groups, and there was no significant difference among three groups. The PF OARSI score had significant positive correlations with knee flexion angle ($\rho = 0.457$) on foot contact (FC), maximum knee flexion angle on initial swing ($\rho = 0.274$) and pelvic tilt angle on FC ($\rho = 0.406$).

<Conclusion>

Medial meniscus destabilization affected PF OA, which correlated with knee flexion and pelvic tilt angle during gait. To observe the kinematics changes may be important to elucidate the pathomechanics in early PF OA.

Rapid Musculoskeletal Assessment: An Application to the Sit-to-Stand Action

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Introduction:

Quantitative functional assessment measures offer the ability to track patient progress during recovery and determine the efficacy of interventions. While several quantitative measures, such as knee strength and horizontal body momentum, have been shown to be different in subjects with Parkinson's disease, it is difficult to obtain these measures without access to a specialized biomechanical facility. Furthermore, the time and expertise required to run these experiments limits the broader application of these biomechanical measures. This work investigates the potential of using a recently developed depth camera system for tracking subject motion and estimating changes in key clinical metrics in subjects performing a standard clinical assessment, the sit-to-stand action.

Methods:

Ten non-clinical subjects (3F, 7M, 1.76 ± 0.12m, 67.4 ± 11.2 kg) were recruited under informed consent (UCSF IRB 16-21015). A single Kinect 2 depth camera was used to track key body landmarks. Subjects were asked to perform three sets of three sit-to-stand actions. An allometrically scaled sagittal-plane rigid-body-model (Figure 1) was combined with an unscented Kalman filter to obtain filtered estimates of joint angles and torques in the ankles, knees, and hips, and shear and compressive forces in the low-back [2]. These values were evaluated against full body motion capture, and sensors under measures of absolute error and concordance coefficients.

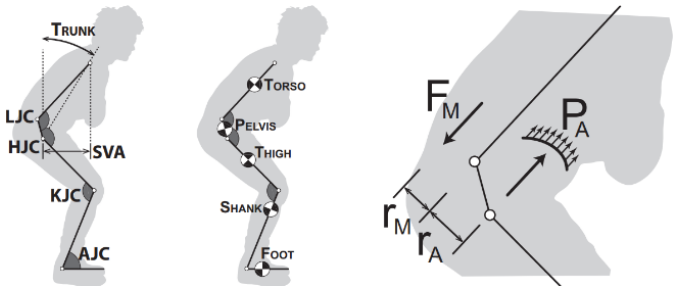

Figure 1: Sagittal plane kinematic, dynamic, and musculoskeletal models used in this work.

Results/Discussion:

The proposed method was found to provide excellent concordance (CCC≥0.75) for measures of SVA, torso centre of mass, torso momenta, and estimates of sacral load at L5S1. The Mean Absolute Errors (MAE) for the joint angles were 6 deg for all joints, with associated joint velocity errors of under 14 deg/s. Dynamic validation against the force platform data showed comparable performance to the motion capture system with force and torque MAEs of under 35 N and 33 Nm. The high concordance, low error, and low complexity of the proposed system support its use as a clinical system for quantifying patient biomarkers throughout the recovery process. As the system does not require any markers, we estimate a total setup and experiment time under five minutes.

Future Work:

This system is currently being used to collect sit-to-stand motion in subjects before spinal fusion, and throughout the recovery process. Preliminary results show variation in these biomarkers pre- and post- surgery, as well as improvement during the recovery process.

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Reduction of Magnesium Degradation-Induced Biomineralization using Matrix GLA Protein

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Abstract: The research for implant biomaterials has gradually shifted toward bioabsorbable magnesium (Mg) and its alloys. Many clinical trials investigating the efficacy of Mg stents for endovascular therapy have reported promising results. However, they also have shown both *in vivo* and *in vitro*, that Mg degrades spontaneously in the biological environment, triggering the deposition of biominerals on the metal. Upon complete absorption of the metal, the minerals remain in the tissue, which ultimately could lead to pathogenic calcification. Hence, the goal of this study is to test the feasibility of matrix GLA protein (MGP) to locally inhibit the biomineralization of Mg that is induced by the metal degradation process. MGP is a small secretory protein that has been shown to inhibit soft tissue calcification, especially in the vascular environment. In the present study, we exposed Mg to MGP stably transfected into mammalian cells. Results showed that less calcium and phosphorous were deposited on the Mg surface when MGP was present relative to when it was not. In the *in vivo* mouse intramuscular model conducted for 4 and 6 weeks, Mg rods were embedded in collagen scaffolds that had been seeded with cells overexpressing MGP. As confirmed by electron dispersive x-ray spectroscopy and histological assessments, microtomography analyses revealed a significantly lower deposited mineral volume around Mg rods from the MGP group. Compared to other groups, higher volume loss after implantation was observed from the MGP group at both time points, indicating a higher metal corrosion rate without the protection from the outer mineral layer. The present study is the first to demonstrate that local exposure to a biomolecule, such as MGP, can effectively improve the function of Mg-based implants. These findings may have important implications for the fabrication not only of endovascular stents but for devices used in orthopedic and craniofacial treatments as well. The relation between degradation product and the metal corrosion rate was also explored in this study to better understand the role of Mg in implant applications.

Rehabilitation After Osteochondral Autograft Transplantation: A Single Case Report

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ABSTRACT:

INTRODUCTION: Osteochondral autograft transplantation (OAT) is one of the repair techniques for cartilage defects of knee with promising knee functional recovery. Postoperative rehabilitation is a critical component of better functional recovery and structural outcome. Dynamic malalignment during postoperative period could influence clinical and structural outcome *via* altering cartilage stress of implanted and host tissues. However, we are not aware of any studies, even in case report, which adequately consider dynamic alignment during postoperative rehabilitation of OAT. This is a crucial research gap to develop a recently suggested new born field "*Regenerative Rehabilitation*." This study is a retrospective analysis of a single case who underwent OAT and postoperative rehabilitation.

METHODS: Case: A 18-year-old Japanese man presented with the knee pain in his right knee joint 2 years prior to surgery and diagnosed as osteochondritis dissecans (OCD) by medical doctor. Magnetic resonance imaging (MRI) revealed an osteochondral defect of approximately 14.3 mm in diameter on the posterolateral face of the medial femoral condyle with osteochondral loose bodies. Autograft osteochondral plugs were transplanted into the cartilage defected area and subsequent postoperative rehabilitation was started. Range of motion (ROM) exercise was gradually started at 2-week after the surgery. The patient walked with the assistance of crutches and knee braces after the surgery. Partial weight-bearing was started at 8-week after the surgery, and full weight-bearing was permitted at 16-weeks after the surgery. During rehabilitation process after full weight-bearing, dynamic rotational alignment during knee flexion was visually checked by physical therapist and be corrected by neuromuscular training and corrective exercise with the aim to improve dynamic malalignment for preventing excessive cartilage share forces generated at the time of internal tibial rotation and flexion.

RESULTS: Overall, this case showed good clinical (ROM and muscle strength) and MRI outcomes even after 18 months follow-up. Knee ROM after the surgery was gradually improved; 0-80°, 0-140°, and 0-150° at 2-, 4-, and 9-weeks after the surgery. Inter-limb muscle strength difference at 9 months follow-up period was -6.8% and -8.3% for extension and flexion, respectively. This inter-limb muscle strength difference was further improved at 12 months follow-up period (-0.5% for extension, 1.3% for flexion). On initial physical examination, the altered rotational changes toward external rotation during deep knee flexion was visually observed. The altered rotational changes was improved at follow-up period. Cartilage tissue was confirmed at the transplanted site at 12 months follow-up period.

DISCUSSION: Although etiology of OCD is still unclear, OCD in this case was observed in the commonest location (posterolateral face of the medial femoral condyle)[1] which is suggested to be caused by trauma such as internal rotation of the tibia due to impact of the tibial spine against the medial condyle,[2] or repeated microtrauma with possible impairment and interruption of the local circulation, or even dissection and possible detachment of a subchondral fragment.[3] In addition to this, there are the sharing forces generated at the time of internal tibial rotation and flexion. Observed alteration in rotational alignment in this case may be compensate strategy to avoid the discomfort caused by the impact of the tibial spine against the lateral wall of the medial femoral condyle[4], or contributing factor itself of OCD. As such, close attention to the rotational changes in the tibiofemoral joint may be a key in providing better clinical outcome and effective "*Regenerative Rehabilitation*" after OAT, which should be further investigated in the future clinical practice and research.

There are some challenges to be clarify in the future clinical practice and researches.

Q1: Does compression + sharing force driven by dynamic malalignment during deep knee flexion really worse transplanted osteochondral autograft?
Q2: In this case, dynamic alignment was visually checked by experienced physical therapist. How can we objectively evaluate dynamic knee malalignment in the clinical practice that can be shared among medial doctor and physical therapists?
Q3: Does early recovery of knee range of motion always results in better functional recovery (e.g., muscle strength)?
Q4: How can we set future clinical trial design to determine whether improving dynamic alignment contribute to regenerate osteochondral tissue.

Reference

1. Mestriner LA. Osteochondritis Dissecans of the Knee: Diagnosis and Treatment. Rev Bras Ortop 2012;47:553-562.
2. Fairbank HAT. Osteo-chondritis dissecans. BJS 1933;21:67-82.
3. Flynn JM, Kocher MS, Ganley TJ. Osteochondritis dissecans of the knee. J Pediatr Orthop 2004;24:434-443.
4. Wilson JN. A diagnostic sign in osteochondritis DISSECANS OF THE KNEE. J Bone Joint Surg Am 1967;49:477-480.

Spatially Patterned Nanofibrillar Scaffolds and Rehabilitative Exercise Enhance Vascularization and Innervation to Injured Muscle

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Skeletal muscle regeneration can become permanently impaired by traumatic injuries, leading to volumetric muscle loss. Implantation of engineered biomimetic scaffolds to the site of muscle ablation may serve as an attractive off-the-shelf therapeutic approach. The objective of the study was to histologically assess the therapeutic benefit of a three-dimensional spatially patterned collagen scaffold, in conjunction with rehabilitative exercise, for treatment of volumetric muscle loss. To mimic the physiologic organization of skeletal muscle, which is generally composed of myofibers aligned in parallel, three-dimensional parallel-aligned nanofibrillar collagen scaffolds were fabricated. When implanted into the ablated murine tibialis anterior muscle, the aligned nanofibrillar scaffolds, in conjunction with voluntary caged wheel rehabilitative exercise, significantly improved the density of perfused microvessels, in comparison to treatments of the randomly oriented nanofibrillar scaffold, decellularized scaffold, or in the untreated control group. The abundance of neuromuscular junctions was 19-fold higher when treated with aligned nanofibrillar scaffolds in conjunction with exercise, in comparison to treatment of aligned scaffold without exercise. Although, the density of *de novo* myofibers was not significantly improved by aligned scaffolds, regardless of exercise activity, the cross-sectional area of regenerating myofibers was increased by >60% when treated with either aligned and randomly oriented scaffolds, in comparison to treatment of decellularized scaffold or untreated controls. These findings demonstrate that voluntary exercise improved the regenerative effect of aligned scaffolds by augmenting neurovascularization, and have important implications in the design of engineered biomimetic scaffolds for treatment of traumatic muscle injury.

Sub-additive effects of cell and physical therapy in a rodent model of stroke

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Stroke is caused by the occlusion of a cerebral artery. Blockage of cerebral blood flow can lead to death or severe, long-lasting functional impairments. Treatment options are extremely limited with rehabilitation, such as physical therapy (PT), being the only approved therapy for chronic stroke. It is extensive and cost-intensive without a guarantee to restore lost functions. Emerging approaches, such as stem-cell based therapies, are promising, potentially cheaper, and can support tissue restoration and functional recovery by integration in the peri-infarct tissue. Both approaches work putatively by promoting brain plasticity. The interaction between both interventions could hence lead to greater improvements in sensory and motor deficits. The aim of this study was to evaluate if the combination of human Neural Stem Cell (NSC) transplantation with physical therapy in a rat model of stroke will improve efficacy compared to either treatment alone. Our hypothesis suggests that aerobic exercise + stem cell therapy will demonstrate an interactive effect in the improvement of functional recovery after stroke.

A randomized control preclinical study was initiated to include adult male Sprague-Dawley rats that underwent transient middle cerebral artery occlusion (MCAo), a model of ischemic stroke. Success of MCAo was determined by T2-weighted magnetic resonance imaging (MRI). After exclusion of non-stroke and hemorrhagic animals, rats with stroke were randomly assigned to the following conditions: MCAo only, MCAo+NSCs, MCAO+PT, MCAO+NSCs+PT. Sham-operated animals served as healthy control to maintain blinding of experimenters. Groups subjected to NSCs or NSCs + PT received a perilesional NSC graft (450,000 cells) at 2 weeks post-stroke. Each rat ran at 80% of its maximum capacity (determined by using the Bruce protocol) for 30 minutes, 5 days/week. Behavioral tests were performed by blinded researchers assessing bilateral asymmetry testing, foot-fault testing and maximum capacity testing on a treadmill; following all groups for a span of 10 weeks. fMRI, DTI and CBV MRI scans were acquired to assess recovery of brain tissue and functional neural connections between groups at 10-weeks post treatment.

The location of the fMRI signal was in the SMC for all groups that received electrical stimulation to the forepaws. fMRI revealed that the combined therapy group had a significantly higher number of active voxels ($p<0.05$) in the ipsilateral hemisphere than the other groups. Exercise alone significantly increased FA in the MC, SMC, and striatum ($p<0.05$). Conversely, NSCs alone increased FA in the MC, SMC, and thalamus ($p<0.05$). The combined group had no FA increase in any of the ROIs, suggesting the combination of NSCs and exercise may not greatly affect microstructure organization. The streamline data show that transplantation of cells increased the streamline count in the MC, while exercise increased streamlines in the thalamus and SMC.

In conclusion, Physical Therapy + Cell Therapy produce sub-additive effects leading to a mild improvement in functional recovery as opposed to either intervention alone. Physical Therapy helps to improve synaptogenesis and neural connections that were previously lost after stroke damage. Stem cell implantation does this as well. However, the combined therapy does not yield improvements in neural connections as expected.

The assessment devices for sit-to-stand task in stroke patient: A review

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Title:
The assessment devices for sit-to-stand task in stroke patient.

Abstract
Objective:
The aim of this study was to review about assessment device for STS task of stroke patients and to confirm the characteristics and current state of them for further development of evaluation system.
Method:
Electronic database, MEDLINE, were searched from the beginning to September 2018 using following the key words: “stroke”, “sit-to-stand” and “assessment” (related words). There was no restriction about article types and stroke detailed content. The initial literature search identified 29 titles and abstracts for full review and 13 were retained because they met our criteria.
Result:
The 13 literature regarding sit-to-stand activity were reviewed. In total, 29 instrumentals that assessment sit-to-stand movement were identified in selected studies. The 29 instrumentals were consisted of 6 major categories: (1) three dimensional motion analysis device (2) accelerometer (3) electromyography (4) force plate (5) force (pressure) sensor (6) other. All reviewed research was carried out using not one device of them but some in combination. The force plate was tool used the most frequently to assess STS motion of stroke people.
Discussion:
In this study, we reviewed 13 articles to confirm the characteristics and current state of assessment device for STS task preformed stroke patients. As, a result, all instrumental used in reviewed research were classified into 6 categories. Moreover, multiple devices were used in combination in studies and the force plate was used most frequently. It can be thought that because stroke subjects often show asymmetry strategies while STS performance and the symmetric posture can be important element for them, force plate which has ability to observe them is necessary for assessing STS task of hemiparetic patients. Because, the high-quality and convenience assessment tool is required in clinical setting, in the future, devices which enable to quantify the motion visually with a few devices such as three-dimensional biomechanical model should be required.
Conclusion:
We revealed that force plate was the device used the most frequently and multiple devices in combination were used when assessing the STS task of hemiparetic patients. In the future, it is necessary to qualify motion and muscle activity with a few in number devices to enable high-quality evaluation at clinical site.

The correlation of kinematic changes with histomorphometric data in the rat sciatic nerve crush injury model

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Introduction

Elucidating whether there is a correlation between biomechanical functions and histomorphometric data in the rat sciatic nerve crush injury model would contribute to an accurate evaluation of the regeneration state without sacrificing animals. Kinematic analysis is considered a reliable and sensitive approach for functional evaluation, most commonly assessed as ankle angle at various phases of a gait cycle. Studies utilizing the toe angle, pelvic tilt angle or the ratio of stance for functional evaluation are scarce, and their changes following surgery remain unknown. This study assessed correlations of the toe angle, pelvic tilt angle and ratio of stance phase with histomorphometric data, aiming to determine which parameters most accurately reflect changes in histomorphometric data over time.

Methods

Six Lewis rats were designated as the control group. Of 30 rats that received surgery, six animals were randomly selected on the first, second, third, fourth, and sixth week after surgery for measurements of toe angles in the “toe-off” phase, differences between bilateral pelvic tilt angles and the ratio of stance phase accounting for a gait cycle. Histomorphometric analyses were also performed, to determine the number of myelinated nerve fibers, diameters of myelinated nerve fibers, axon diameters, and myelin sheath thicknesses. Furthermore, we investigated changes in toe angle, pelvic tilt angle, ratio of stance phase, and histomorphometric data over time, as well as correlations between three kinematic parameters above-mentioned with histomorphometric data.

Results

Both the toe angle and ratio of stance phase correlated with histomorphometric data over time and coefficients were high; the pelvic tilt angle did not correlate with histomorphometric and individual differences were considerably evident.

Conclusion

The toe angle and ratio of stance phase strongly reflect histomorphometric data over time. Nevertheless, the pelvic tilt angle could not reflect histomorphometric data, but it meant that sciatic nerve crush injury is a strong factor resulting in abnormal pelvic tilt angles.

Title: The Effect of *in vitro* Electromechanical Stimulations of iPSC-derived Myotubes on Skeletal Muscle Maturation

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Background:

Destruction of skeletal muscle and extensive degeneration leads to loss of ambulation, cardiomyopathy and death by second decade of life in Duchenne muscular dystrophy (DMD) (1). Despite the standard clinical care of corticosteroids and rehabilitation there is no effective treatment to restore normal physiologic function of skeletal muscle. Patient-derived induced pluripotent stem cell (hiPSC) is a powerful solution to study the pathophysiology of DMD, to perform de novo drug discovery and to provide material for cell therapy applications. Therefore, the purpose of this research is, 1) to develop an optimized biomimetic mechanical and electrical regimen that promotes maximal maturation of normal hiPSC-derived skeletal muscle myotubes *in vitro,* 2) to use DMD-derived iPSCs to create *in vitro* skeletal muscle myofibers that manifest DMD pathophysiology

Methods:

In a regenerative rehabilitation approach, the experiment consists of two main steps:

1) Differentiation of DMD urine-derived iPSCs to skeletal muscle myotubes (2) (Figure 1)
2) Maturation of iPSC-derived skeletal muscle myotubes by providing mechanical and electrical stimulations (Figure 2)

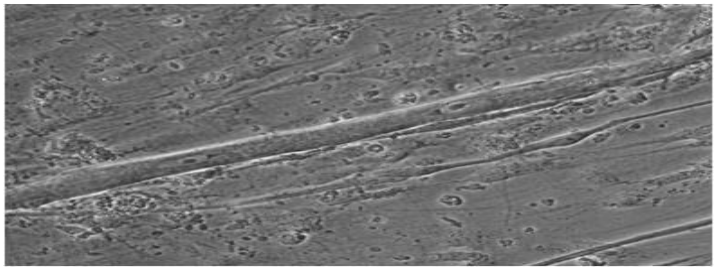

Figure 1: Normal hiPSC cell line after 30 days of myogenic differentiation

Figure 2: Strex STE-140-10 stretch and synchronous electrical stimulation system

Anticipated results:

Intermittent cyclic stretch and optimized electrical stimulations will:

- Upregulate the expression of myotube maturation genes: *MYOG, MYH1, MYH3, MYH7, MYH8, TNNT1, TNNT3, TTN, TPM1,* and *RYR1*
- Enhance sarcomere organization, T-tubule formation, and alignment of the internal F-actin cytoskeleton
- Increase cross-sectional area (hypertrophy)
- Increase fusion of myotubes, peripherally localized nuclei, and the expression of adult isoforms of contractile proteins
- Increase load-bearing capacity of muscle fibers
- Decrease resting membrane potential and cause faster calcium release and reuptake

Implications:

The complementary technologies from this research will create the first human skeletal muscle model of DMD with the phenotypic maturation sufficient to enable improved drug discovery.

References:

1) Kieny P, Chollet S, Delalande P, Le Fort M, Magot A, Pereon Y, et al. Evolution of life expectancy of patients with Duchenne muscular dystrophy. Annals of physical and rehabilitation medicine. 2013;56(6):443-54.

2) Chal J, Oginuma M, Al Tanoury Z, Gobert B, Sumara O, Hick A, et al. Differentiation of pluripotent stem cells to muscle fiber to model Duchenne muscular dystrophy. Nature biotechnology. 2015;33(9):962-9.

Title: Therapeutic Efficacy of Encapsulated Human Mesenchymal Stem Cells on Osteoarthritis

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Introduction: Osteoarthritis (OA) is a chronic disease of the joints that leads to degeneration of articular cartilage surfaces. Mesenchymal stem cells (MSC) present a promising treatment to target the disease, relying on their regenerative capacity along with their immunomodulatory and anti-inflammatory properties. However, many questions remain as to the mechanism of action of these cells following intra-articular delivery: paracrine action versus cellular engraftment [1,2]. Cellular encapsulation presents a promising means to study the paracrine factors of MSCs independent of cellular engraftment, as alginate microencapsulation provides a mechanical barrier which prohibits the direct engraftment of cells into the native host tissue. The objective of this study was to quantitatively assess the efficacy of encapsulated human MSCs (hMSC) as a disease modifying therapeutic for OA. We hypothesized that encapsulated hMSCs would have a therapeutic effect, via paracrine mediated action, on OA progression.

Materials and Methods: A medial meniscal transection (MMT) OA induction rat model was used to assess the efficacy of encapsulated hMSC intervention in OA. Intra-articular injections of either saline, empty capsules, non-encapsulated hMSCs or encapsulated hMSCs were given 1-day post-op and animals were euthanized at 3-weeks post-op, a time point where moderate OA phenotypes are exhibited in the MMT model [3]. Micro-structural changes in the articular cartilage and osteophyte formations were quantitatively assessed using equilibrium partitioning of an ionic contrast agent based micro-computed tomography (EPIC-μCT). The medial 1/3 of the medial tibial plateau was evaluated for articular cartilage parameters, as this region demonstrates high damage incidence in the MMT-induced OA model [3]. To model hMSC responsiveness, Luminex analysis was used to quantify secreted factors (41 cytokines/chemokines) of hMSCs *in vitro* stimulated with IL-1β, a key cytokine in OA pathogenesis [4].

Results: At 3-weeks post OA induction, animals in the no treatment control (MMT/Saline) group exhibited an increase in articular cartilage thickness and cartilage surface roughness, both of which are indices of early OA phenotypes [3]. MMT-animals treated with encapsulated hMSCs showed significant reduction of articular cartilage thickness and surface roughness parameters, suggesting a chondroprotective therapeutic effect on OA. Interestingly though, mineralized osteophyte volume was increased in the encapsulated hMSC group in comparison to all other MMT conditions. *In vitro* hMSC secretome studies have demonstrated the ability of IL-1β, to upregulate hMSC secretion of anti-inflammatory cytokines (Interleukin (IL)-4, IL-10) and cytokines that induce tissue regeneration in OA (Transforming Growth Factor (TGF)-β).

Conclusions: This is the first study to demonstrate that the paracrine signaling properties of hMSCs, independent of direct cellular engraftment, may exert a chondroprotective role in OA. However, these protective effects were countered by enhancements of osteophyte volumes. These augmented tissue volumes are especially relevant in clinical applications. Additionally, further work analyzing the effects of encapsulation on the hMSC secretome in both unstimulated and stimulated conditions is critical as significant differences were detected in both cartilage and osteophyte EPIC-μCT quantitative analyses between the encapsulated and non-encapsulated hMSC conditions. Future studies will aim to assess the effects of altered knee loading on encapsulated hMSC efficacy through the implementation of rehabilitation measures (running and swimming regimens).

References: [1] Horie M, et al. Osteoarthr Cartil. 2012; 20: 1197–207., [2] Sato M, et al. Arthritis Res Ther. 2012; 14: R31. [3] Thote T, et al. Osteoarthr Cartil. 2013; 21: 1132–1141. [4] Wojdasiewicz, P. et al. Mediators of Inflammation 2014. 1-19.

Transcutaneous Spinal Stimulation with Intensive Exercise Improving Functional mobility in Chronic Incomplete Cervical Spinal Cord Injury

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Introduction/Background:

Epidural stimulation results in remarkable improvements of over-ground walking during stimulation and sustained functional improvement of lower extremity function for spinal cord injury (SCI). Over the last few years, a novel strategy of non-invasive (transcutaneous) spinal stimulation on cervical and lumbosacral area demonstrates similar neurophysiological and functional improvement of upper extremity for motor incomplete SCI and lower extremity function for motor complete SCI. We hypothesized that locomotion function can be improved intensive physical therapy (PT) with transcutaneous spinal stimulation promotes more functional improvement than only intensive locomotion training in individuals with incomplete cervical SCI.

Case Presentation and Method:

A 64-year-old male with incomplete C3 spinal cord injury 4 years prior participated in the study. He is currently diagnosed as C4 AIS D central cord syndrome. He participated in the cervical transcutaneous spinal stimulation study for upper extremity function and exhibited long-lasting motor and sensory improvement of the hand functions in 2017. In March 2018, he started the current clinical trial for locomotion. The intervention involved (A) intensive PT, and (B) intensive PT with transcutaneous spinal stimulation in an A-B-A-B protocol. The intensive PT included over-ground and body weight supported treadmill walking with regular PT exercises. Transcutaneous spinal stimulation utilized 30Hz biphasic pulse consisting of 10KHz Carrier frequency with 10-60mA in cervical and lumber spine area. The walking speed and distance were the primary outcome measures. Electromyography was collected during walking.

Results:

The walking speed and distance improved throughout the course of the intervention. The physical assist level for walking remarkably improved from maximum assist with platform walker to close supervision with platform quad-cane. The functional changes demonstrated a trend that spinal stimulation with intensive PT improved functional mobility greater than performing only the intensive locomotion training. Electromyography showed more activation in distal muscles during the phase of spinal stimulation with intensive exercise.

Discussion and Significance:

We observed remarkable improvement of lower extremity function and the modulated electrophysiological state of the leg distal muscles with spinal stimulation with intensive exercise. Our results suggest that transcutaneous spinal stimulation with intensive PT could be more effective than performing only intensive locomotion training.

Bibliography:

Angeli et al. Recovery of Over-Ground Walking after Chronic Motor Complete Spinal Cord Injury. New England Journal of Medicine. 2018;379(13):1244-1250.

Rejc et al. Motor recovery after activity-based training with spinal cord epidural stimulation in a chronic motor complete paraplegic. Scientific Report. 2017;7(1): 13476.

Gerasimenko et al. Noninvasive reactivation of motor descending control after paralysis. Journal of neurotrauma. 2015;32(24):1968-1980.

Gad et al. Weight bearing over-ground stepping in an exoskeleton with non-invasive spinal cord neuromodulation after motor complete paraplegia. Frontiers in neuroscience. 2017;11:333.

Inanici et al. Transcutaneous Electrical Spinal Stimulation Promotes Long-term Recovery of Upper Extremity Function in Chronic Tetraplegia. IEEE Transactions on Neural Systems and Rehabilitation Engineering. 2018;26(6):1272-1278.

Ultrasound Therapy Suppresses Inflammatory Gene Expression in an Injured Peripheral Nerve after Sciatic Nerve Crush Injury in Rats

Akira Ito[1], Tianshu Wang[1], Ryo Nakahara[1], Hideki Kawai[1], Akihiro Nakahata[1], Jue Zhang[1], Naoko Kubo[1], Junichi Tajino[1], Tomoki Aoyama[1], Hiroshi Kuroki[1]
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[Background] Physical therapy modalities for the regeneration of peripheral nerves have been investigated; however, no modalities have been established in a clinical setting. Ultrasound (US) is one of the promising modalities for nerve regeneration. Although several studies have addressed revealing the molecular mechanism of US therapy, little evidence exists on gene expression changes such as those in inflammatory cytokines.
[Purpose] To investigate the effect of US therapy on the gene expressions in crushed peripheral nerves in rats.
[Methods] Lewis rats (12 weeks old, male) were subjected to a sciatic nerve crush injury (axonotmesis) in the left leg. After the operation, the rats were randomly divided into two groups, the sham and US groups. A US probe was set on the skin of the injury site with US transmission gel. Stimulation was initiated 3 days after the injury. One week after the injury, total RNA was extracted from the distal of the injury site. Thereafter, quantitative real-time polymerase chain reaction was performed for a gene expression analysis. Three weeks after the injury, the regeneration of the sciatic nerve was assessed by measuring the myelinated fiber density, myelinated fiber area, axon area, and myelin area. All the procedures were approved by the Institutional Animal Care and Use Committee of Kyoto University.
[Results] The myelinated fiber density, fiber area, axon area, and myelin area 3 weeks after the injury were significantly higher and larger in the US group than in the sham group, indicating an US-enhanced sciatic nerve regeneration. The mRNA expressions of IL-6 and TNF, which are known to be inflammatory cytokines, and NT-3 and semaphorin-3A, which are inhibitors of re-myelination and axonal growth, respectively, were significantly inhibited in the US group as compared with the sham group at 1 week after the injury. BDNF, a nerve trophic factor, was also significantly suppressed in the US group as compared with the sham group, although no significant differences were observed in the other growth factors such as FGF5, GDNF, and NGF.
[Conclusion] The regeneration of the sciatic nerve after a crush injury in rats was facilitated with US therapy. Our results indicate that the positive effects of US therapy are attributable to the suppression of the inflammatory and nerve growth inhibitor gene expressions.

Zinc oxide nanoparticles: Potential applications and safety issues in tissue repair

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Topical application of zinc has been shown to improve re-epithelialization, reduce inflammation and bacterial growth. Zinc oxide (ZnO) possesses antibacterial, anti-inflammatory properties and accelerates the healing of both acute and chronic wounds. It has potential application in the treatment of various conditions like dermatitis, blisters and open skin sores. Zn ions released from ZnO can enhance keratinocyte migration towards the injured site and promote healing. Nanoparticles have attracted much attention for their distinct characteristics that are never possible with conventional macroscopic materials. Zinc oxide nanoparticles (ZnO NPs) are superior to conventional ZnO. ZnO NPs antibacterial property may be due to their tiny size and high surface-to-volume ratio. Nano-ZnO possesses well-developed surface chemistry, chemical stability which makes them easier to interact with the microorganisms. Although nanoparticles possess novel properties that make them available in a vast range of applications, the questions regarding their safety arise when it comes in contact with the biological systems. Nanoformulation aided delivery, healing properties with a potential to be used for tissue repair and the possible antibacterial mechanisms, toxicity concern of ZnO NPs, will be discussed.

An Affordable Device for Reach-to-Grasp Assistance in Patients with Upper Limb Impairment

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Introduction: Upper limb assistive robotic systems offer the ability to quantify patient motion and provide exercise support outside of traditional rehabilitation facilities. We have developed a low-cost device for assistance and measurement of planar reach-to-grasp motions. Constructed from 3D-printed and off-the shelf components, the assistive system consists of three elements: a table that controls the position of the wrist, a wheelchair-mounted forearm support, and a glove for assisting with a pinch grasp. The system was developed for use in clinical and patient-home settings. We present preliminary results of the device with control and subjects with upper-limb impairment.

Methods: The assistive device operates under two control modes: position control, in which the device controls the motion of the wrist and index finger directly, and admittance control, in which the device moves the wrist and index finger in response to measured forces exerted by the subject. The admittance mode allows for observations of volitional motion under a force-based controller. Reaching and reach-to-grasp data have been collected from ten control subjects and two subjects with upper limb impairment, under both position and admittance control modes. These experiments allow for comparisons between different target positions, action intents, and patient cohorts. The smoothness of these trajectories, the presence of position error, and the overall shape of the velocity curve can be used to assess the subject's ability to perform reaching motions and the effect of grasping intent on gross arm motion. Different admittance controllers were implemented to assess variation in gross arm reaching motion and the fine stabilization required to perform successful grasps.

Results/Discussion: Figure 1 shows the results from preliminary experiments, comparing reaching trajectories of a representative control subject, a stroke subject, and an individual with early Primary Lateral Sclerosis (PLS). Between the control and impairment subjects, we observe variation in the velocity profile and peak speed during the gross transport phase, the ability of the subject to stabilize their arm at the end of the motion, and variations in the linearity and smoothness of the reaching trajectory. Prior research has shown that the reaching motions of post-stroke subjects with upper limb impairment are slower, less accurate, and have poorer coordination than non-impaired individuals []. These initial experiments appear to agree with these findings, despite the high level of functional ability seen in both subjects with upper-limb impairment. An upper limb kinematic model has been developed to relate the motions of the wrist, to their corresponding movements at the shoulder and elbow. This model has been compared to active motion capture and has been found to be an accurate method for estimating the kinematic state of the subject from the device measurements.

Conclusions: Preliminary experiments with the device demonstrate feasibility of the device for assessment and assistance of reach-to-grasp, providing a low-cost platform for clinical deployment and study. Future work will explore the development of individualized, adaptive, hybrid control schemes on larger patient cohorts, and the control of the device with an implantable brain-computer interface.

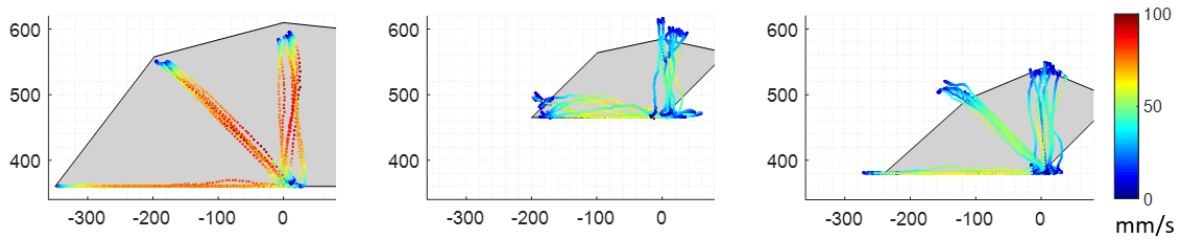

Figure 1: Sample reaching trajectories for control (left), stroke (center), and PLS (right) subjects under admittance controller. Line color corresponds to wrist velocity wrist at that point. Reachable workspace as measured by clinician is shown in gray.

Title:
Therapeutic effects of combined cell transplantation and rehabilitation in rats with brain injury

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Abstract
Background and Purpose: Cell-based therapies are attracting attention as alternative therapeutic options for brain damage following stroke or brain injury. In this study, we investigated the therapeutic effect of a combined therapy of cell transplantation and rehabilitation by evaluating the neuronal connectivity between mouse-derived cortical cells and rat host brain.
Methods: We transplanted neural cells derived from the frontal cortex of E14.5 GFP-expressing mice into the frontal lobe of three-week-old rats with brain injury, followed by treadmill training (TMT) for 14 days. Graft survival and neurite extension were examined by immunohistochemistry, and motor function of the rats was evaluated by the foot fault test.
Results: We found that the graft survival rate was higher in the TMT (+) group than the TMT (−) group (p=0.0392). In the TMT (−) group, graft-derived neurites were observed only in the striatum and internal capsule. In contrast, in the TMT (+) group, they were observed in the striatum, internal capsule, and the cerebral peduncle and spinal cord. The maximum distance from the grafts to the tip of the longest neurite was significantly longer in the TMT (+) group than in the TMT (−) group (p=0.0219). Moreover, recovery of the motor function was accelerated at one week in the TMT (+) group (p<0.05). The percentage of cells expressing C-FOS was increased in the grafts and host brain in the TMT (+) group, suggesting that the combined therapy promoted neural activity in the grafted cells and host brain.
Conclusions:
TMT promoted graft survival and neurite extension from the grafted neural cells, and the combined therapy promoted functional recovery of rats with brain injury.