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The Impact of Mechanical Strain and Immobilization on the Capacity for Skeletal Muscle-Resident CD146+ Pericytes to Secrete Extracellular Vesicles

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Purpose: Our laboratory recently reported a decline in muscle-resident pericyte quantity following hindlimb immobilization, and subsequently demonstrated the capacity for pericyte transplantation to accelerate recovery of skeletal muscle mass during the rehabilitation period. The purpose of this study was to determine the extent to which mechanical strain cues can regulate the capacity for pericytes to secrete extracellular vesicles (EVs) and determine the impact of pericyte-derived EVs on the regulation of muscle mass. **Methods:** CD146+CD31-CD45- pericytes were derived from mouse hindlimb muscle using FACS and subjected to a single bout of mechanical strain in culture (10%, 1 hour, exosome-free media). 24 hrs post-strain, CD146+ EVs were isolated from media using ultracentrifugation followed by magnetic bead sorting. Both the CD146- and CD146+ EV fractions were quantified using nanoparticle tracking analysis. EV fractions were also collected from serum of mice following acute and repeated bouts of contraction using a sciatic nerve stimulation procedure, or following unilateral hindlimb immobilization for 2 weeks. Finally, CD146+ EVs were injected intravenously and intramuscularly into mice subjected to immobilization to determine therapeutic capacity. **Results:** CD146+ EV quantity was significantly increased in media in response to mechanical strain in vitro ($P<0.05$). Serum CD146+ EV quantity was not significantly altered in response to acute or repeated bouts of contraction, yet a significant decrease was observed following hindlimb immobilization ($P<0.05$). Pericyte-derived EVs demonstrated varying capacities for muscle regrowth post-disuse based on conditions used for retrieval and administration. **Conclusion:** The results from this study suggest that CD146+ serum EVs are highly responsive to mechanical cues, and transplantation of these extracellular vesicles may possess therapeutic potential. Supported by NIH 1 R01 AR072735-01A1.

Implementation of Physical Therapy Improves the Therapeutic Efficacy of a Cellular Therapy in a Preclinical Model of Post-Traumatic Osteoarthritis

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Post-traumatic osteoarthritis (PTOA) is characterized by a chronic state of joint inflammation and cartilage degeneration and remains a significant clinical and socioeconomic challenge. Despite promising results in mitigating cartilage degradation in pre-clinical models of post-traumatic OA, no disease modifying PTOA drugs are currently FDA-approved. Intra-articular injection of human mesenchymal stem cells (hMSCs) are an attractive therapeutic option, facilitating tissue remodeling, stem/progenitor cell recruitment, and immunomodulation, and have shown promising results in improving cartilage repair in pre-clinical models. Their clinical efficacy, however, has not met their preclinical promise. We have recently shown that intra-articular injections of hMSCs can reduce cartilage thickening and surface roughness in post-traumatic OA (1). While the effect of mechanics on hMSCs are well studied *in vitro*, it remains largely unknown how exercise may alter the efficacy of cellular treatment *in vivo*. The objective of this study was to assess the combinative effects of physical and cellular therapy on tissue morphology, joint function, and pain in PTOA.

We tested the role of treadmill walking (10 m/min, 30 mins, 5 d/wk) on hMSC therapy ($5x10^5$ hMSCs injected intra-articular at 1 d post-surgery) for a pre-clinical model of PTOA (rat MMT; n=4 per group). The study concluded after 3 weeks. We assessed joint function (spatiotemporal symmetry of gait via Experimental Dynamic Gait Arena for Rodents, (2)), pain (joint hyperalgesia with a pressure application monitor, and secondary allodynia with von Frey filaments), and tissue morphology (equilibrium partitioning of a ionic contrast agent based micro-computed tomography, EPIC-µCT). We hypothesized that mild treadmill walking would enhance the therapeutic benefits of hMSCs on cartilage and osteophyte morphology, joint function, and pain beyond the effects of cell treatment alone. Preliminary data suggested that 1) mild exercise, in the form of treadmill walking, does not alter the therapeutic benefit of hMSCs on cartilage composition (still protects from proteoglycan loss) but can reduce detrimental osteophyte formation; and 3) combined hMSC and exercise therapy can reduce pain sensation and improve joint function. This pilot study showed the promise of a combinative physical and cellular therapy to attenuate morphological, functional, and pain changes associated with PTOA. We are currently completing a full study that will further elucidate the combinative effects of cellular and physical therapy on attenuating cartilage degradation, improving joint function, and alleviating pain in PTOA.

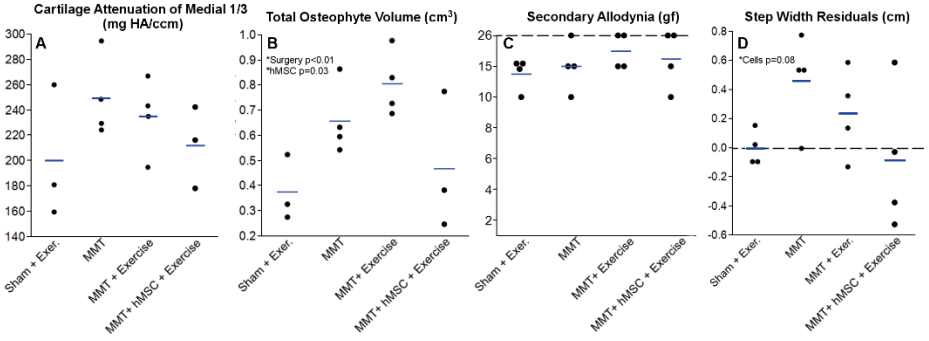

Figure. PTOA induced by MMT was associated with increased cartilage attenuation (loss of sGAG, A), osteophyte volume (B), and step width, relative to expected values for weight- and velocity-matched healthy controls (D). There were minimal differences in secondary allodynia at week 3 between the sham and MMT groups (C). Exercise alone exacberated osteophyte volume (B), but slightly reduced step width (D). Combinative therapy of hMSC injection and exercise slightly reduced cartilage attenuation (A), ostephyte volume (B) and returned step width to healthy levels (D).

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Molecular factors that contribute to muscle atrophy following ACL injury and reconstruction: A systematic review.

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Purpose/Hypothesis: ACL injury is a traumatic knee injury complicated by altered gait biomechanics and muscle function which are known to increase the risk of post-traumatic knee osteoarthritis. Despite surgical reconstruction and rehabilitation persistent impairments in quadriceps strength exist and ideal treatment approaches are not established. Alterations to the soft tissue repair process, specifically molecular mechanisms, can contribute to muscle degradation and quadriceps atrophy, but are not well understood from a clinical perspective. The purpose of this systematic review was to determine the molecular factors contributing to atrophy following ACL injury and reconstruction with regard to potential avenues for non-pharmacological therapeutic interventions (i.e. rehabilitation).

Subjects: A total of 20 studies were reviewed.

Materials and Methods: Databases MEDLINE, PubMed, and CINAHL (inception to March 2019) were utilized to identify studies that investigated cellular and transcriptional changes occurring both intraarticularly as well as within the quadriceps muscle. Study selection inclusion criteria included: ACL reconstruction, and/or osteoarthritis following both male/female participants undergoing single ligament ACL reconstruction with cytokine/growth factor sampling post-operatively. MeSH terms and the following key words were used: “ACL AND Injury”, “ACL AND Atrophy”, “ACL AND Cellular”, “ACL and Transcription”.

Results: Studies demonstrated increases IL-6 and IL-1 intra/post-operatively, while both venous and intra-articular sampling showed decreases in IGF-1 and PDGF. Studies indicate up-regulation of cytokine profiles (IL-6, TNF-α, and TGF-β) that correlate with transcriptional changes in atrogene expression and subsequent protein degradation and atrophy via the ubiquitin proteasome pathway. These studies also indicate insulin like growth factor (IGF-1) can counter effects of atrophy stimulating cytokines and increase muscle hypertrophy in the quadriceps.

Conclusion: Up-regulation of pro-inflammatory cytokines (IL-6, TNF-α, and TGF-β) correlate with transcriptional changes in atrogene expression and subsequent protein degradation and atrophy via the ubiquitin proteasome pathway. These studies also demonstrate a decrease in growth factors that correlate to an overall decrease in muscle protein synthesis and prevention of regeneration and muscle cell differentiation. These studies also suggest that regulatory growth factors can counter effects of atrophy stimulating cytokines and increase muscle hypertrophy in the quadriceps.

Clinical Relevance: These trends suggest potential cellular signaling targets that can be modulated through therapeutic intervention to reduce muscle atrophy and decrease risk for future osteoarthritis complications following ACL injury.

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Stranger Things: the upside-down of muscle, mitochondrial, and bone plasticity after volumetric muscle loss injury

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Volumetric muscle loss (VML) is characterized by a large volume of muscle tissue being removed due to surgery (e.g., sarcoma) or severe trauma (e.g., farm/industrial accident). There is currently no standard of clinical care to address the long-term functional limitations of VML patients because the pathology and plasticity of the remaining muscle and bone are unknown. C57BL/6 mice underwent unilateral VML injury to the primary ankle plantarflexors and were subsequently used to investigate the underlying pathology and plasticity of the remaining tissue. A ~20% reduction in muscle volume resulted in a ~75% reduction in muscle strength that does not recover out to 4 months post-VML. Mitochondrial function of the remaining muscle was reduced by 50% in the first week post-injury, and a 25% decrement is present out to 4 months post-VML. Employing 2-photon microscopy and utilizing the Dendra2 mitochondrial photoconvertible GFP/RFP mouse, we discovered that mitochondrial dysfunction in the remaining muscle after VML injury is accompanied by extensive mitochondrial network reorganization. Bone function, i.e., ultimate load, was 14% less in VML-injured mice compared to uninjured controls and this corresponded with decrements in bone CSA (-14%) and CSMI (-20%). Rehabilitation strategies including wheel running and neuromuscular electrical stimulation were ineffective at correcting mitochondrial and bone function and had minimal effect on muscle strength leading us to hypothesize that the plasticity of the remaining tissue is compromised after VML. To test this hypothesis, we evaluated the metabolic plasticity of the remaining tissue to an endurance exercise training stimulus. Endurance training resulted in greater muscle oxidative capacity (i.e., oxygen consumption) and mitochondrial quantity in uninjured mice whereas VML-injured mice were resilient to adaptation. We identified poor activation of the transcription factor PGC1α as a primary limitation to metabolic plasticity and showed that forced overexpression of PGC1α was sufficient to rescue metabolic plasticity in the remaining muscle tissue. Wheel running and neuromuscular electrical stimulation resulted in modest strength gains of the remaining muscle, however, the greater mechanical loads did not translate into greater bone function or quality. Muscle-bone interactions also include endocrine signaling and we have analyzed RNA-seq datasets from VML-injured rats for muscle-derived factors that may influence bone. Potential targets for future investigation include IL-6 (3.5-fold increase), IL-7 (2.4-fold increase), osteoglycin (10-fold increase), and follistatin (15-fold increase). By understanding the pathology and plasticity of the remaining tissue after VML injury we can identify technologies to combine with rehabilitation, i.e., regenerative rehabilitation, and hopefully develop standards of care for VML patients in the future.

This work was supported by the AR3T Seed Grant Program and the Department of Defense Congressionally Directed Medical Research Program to SMG & JAC.

Rehabilitative Exercise and Insulin-like Growth Factor-1 Laden Scaffolds Enhance Regeneration for Treatment of Volumetric Muscle Loss

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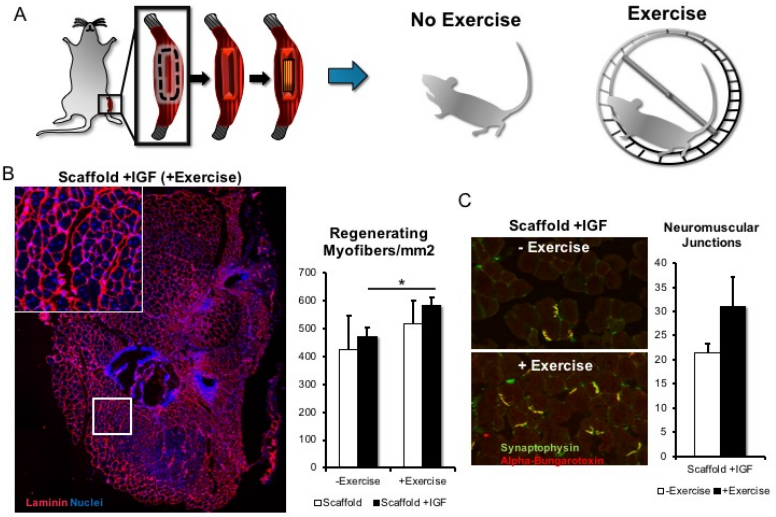
Introduction: There is an unmet clinical need for off-the-shelf therapeutics for functional tissue replacements. Muscle regeneration can be permanently impaired by traumatic injuries, despite the high regenerative capacity of skeletal muscle. Implantation of engineered biomimetic scaffolds to the site of muscle ablation may serve as an attractive therapeutic approach. Localized regeneration and long-term recovery hinge on the host foreign body response and cascade of interactions between the biomaterial and a range of immune cells, stem/progenitor cells, and the tissue microstructure environment. The objective of this study is to modulate and enhance the regenerative process via myogenic growth factors and in conjunction with rehabilitative exercise for the treatment of volumetric muscle loss.



Materials and Methods: Collagen scaffolds were fabricated by extruding high concentration rat-tail collagen-Type I (30 mg/mL) from 22G blunt tip needles into pH neutral buffer to initiate fibrillogenesis. To create a 3D scaffold bundle, 8 scaffold strips were aggregated in parallel with dimensions that were 9mm x 2mm x 3mm. Growth factor laden scaffolds were generated by incubation of dehydrated scaffolds with 250µg/ml recombinant human IGF-1 diluted in 0.1% bovine serum albumin (BSA) at 37°C and 5% CO_2 overnight. Control scaffolds were incubated in 0.1% BSA overnight. For in vitro characterization of IGF release, scaffold supernatant was collected over 7 days and IGF concentration was quantified by ELISA. For in vivo studies, scaffolds were transplanted into a mouse model of volumetric muscle loss (VML) that was created by surgical excision of 20% of the anterior tibialis (TA) muscle. Constructs were sutured at the distal and proximal ends of the defect followed by suture closure of the muscle and skin flaps. Following transplantation, animals were allowed to recover in traditional housing cages for 7 days, after which, animals were either transferred to individual cages containing cage wheels or remained in their original housing for 14 days [**Fig A**]. On day 21, the tail veins were injected with isolectin, a fluorescently labeled endothelial binding protein and the TA muscle was extracted and processed for histological analysis.

Results and Discussion: To enhance the regenerative potential of injured skeletal muscle, IGF-1 laden nano-patterned scaffolds were fabricated. Individual scaffolds released a cumulative total of 1250 ng ± 150 ng of IGF-1 in vitro over the course of 21 days. When implanted into the ablated murine tibialis anterior muscle, the growth factor laden scaffolds in conjunction with voluntary caged wheel exercise could significantly improve the density of isolectin+/CD31+ perfused microvessels by greater than 3-fold in comparison to treatment of constructs without IGF-1. Enhanced myogenesis was also observed in the muscle treated with the IGF-1 laden scaffolds combined with exercise compared to the same IGF-1 laden scaffolds transplanted into mice that did not receive exercise [**Fig B**]. Furthermore, the abundance of neuromuscular junctions was greater when treated with IGF-1 laden scaffolds in conjunction with exercise, in comparison to the same treatment without exercise [**Fig C**]. Studies are currently underway to corroborate these findings with gait analysis.

Conclusions: These findings demonstrate that voluntary exercise improved the regenerative effect of growth factor-laden scaffolds by augmenting neurovascular regeneration and myogenesis, and has important translational implications in the therapeutic design of off-the-shelf therapeutics for the treatment of traumatic muscle injury.

Eccentric Muscle Loading Improves Tendon Biomechanical Properties in a Murine Model of Achilles Tendinopathy

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INTRODUCTION: Eccentric loading of the Achilles muscle-tendon unit (muscle lengthens while it contracts) is an effective physical therapy for treatment of symptomatic human tendinopathy [1,2]. However, the mechanisms by which the presumed healing occurs are poorly understood. Recently, our research group developed a novel pre-clinical mouse model of eccentric hind limb muscle loading to investigate Achilles musculotendinous adaptation. Our initial application of this model to uninjured tendons demonstrated that mice could well tolerate the loading protocols, which did not alter tendon biomechanical properties [3]. The objectives of the current study were (1) to assess the potential of this eccentric loading protocol to improve biomechanical properties following the induction of Achilles tendinopathy, and (2) to investigate if the functional quality of the healing response was dependent on the timing of the loading treatment.

METHODS: Under IACUC approval, 12-week old C57Bl/6 male mice were injected unilaterally with two doses of 100ng of rHuTGF-β1(Peptrotech Inc.) into the Achilles tendon body [4,5] to induce tendinopathy. Eccentric loading (ECC) was initiated either two days (Early) or two weeks (Delayed) following rHuTGF-β1 injection. Under isoflurane anesthesia, one hind foot of each mouse was secured to the pedal of a 305C-5N Dual-Mode Muscle Lever system (Aurora Scientific). Electrodes were positioned beneath the skin to electrically stimulate the tibial nerve, inducing plantar flexion. Under computer control, the foot was rotated through the ankle joint range of motion while the plantar flexors simultaneously contracted to simulate body weight loading [3]. Mice were treated twice weekly for two weeks using three sets of ten repetitions. Separate, age-matched injured groups ("Early Inj", "Delayed Inj") received no eccentric loading. Twenty-four hours following the last muscle loading treatment, mice were euthanized and Achilles tendons were harvested for biomechanical evaluation (preconditioning and a load to failure test at 0.05mm/sec [4]). 2-way ANOVA and Tukey's multiple comparison tests were carried out using JMP Pro 14 (SAS).

RESULTS: For Achilles tendon cross-sectional area (CSA), treatment (p=0.0041), timing (p=0.0004), and treatment-timing interaction (p=0.0011) all led to significant differences with a decrease in CSA seen following early ECC treatment (Figure 1). A significant increase in maximum stress (p=0.0418) and modulus (p=0.06) was seen following early ECC treatment.

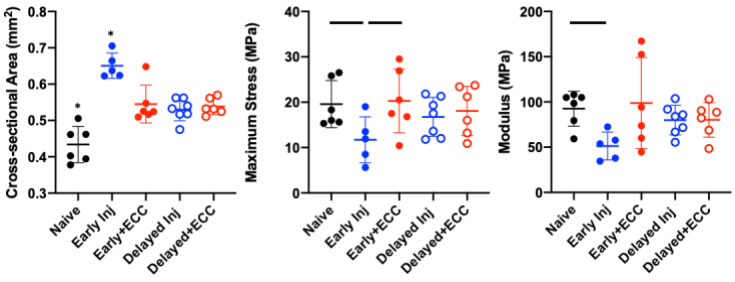

Figure 1: Geometric and material properties of Achilles tendons. Asterisks above groups denote statistically significant differences when compared to all other groups. Horizontal lines denote statistically significant differences between two

DISCUSSION: The present study used a novel, pre-clinical murine model of eccentric loading patterned after heel drop exercises widely used to effectively treat human tendinopathies. Unlike treadmill running protocols [4,6], our methodology enables a well-controlled application of muscle loading "doses" by simulating the ankle joint ROM and muscle loading patterns accompanying these clinical treatments. Our prior study [3] demonstrated that this specific muscle loading protocol does not alter the biomechanical properties of uninjured tendons. The current results indicate that early eccentric loading promotes decreased cross-sectional area and increased material properties relative to the untreated tendon injury (Figure 2). In summary, the current study (1) confirms clinical findings that eccentric muscle loading promotes tendon healing, and (2) demonstrates the importance of timing of treatment in this injury model. Ongoing studies will characterize muscle force-frequency profiles, tendon gene expression, and histologic assessment of the muscle-tendon junction to provide further insight into potential mechanisms underlying the efficacy of our novel loading approach. Future work will examine different loading parameters (treatment duration, frequency of stimulation, repetitions, etc.) to identify an optimal eccentric loading protocol to achieve tissue-level repair.

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The response of a mouse model of adult-onset muscular dystrophy to 12 weeks of non-injurious exercise, reveals a low threshold for myogenic activation

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BACKGROUND: Mutations in the DYSF gene in humans, leads to the absence or severely reduced levels, of the protein dysferlin. Dysferlin deficiency in skeletal muscle is linked to progressive muscle weakness and wasting syndromes known as dysferlin-linked muscular dystrophies or dysferlinopathies. A major challenge in the physical rehabilitative management of dysferlinopathies is preventing the complications of a sedentary lifestyle, while still protecting muscles from contraction-induced muscle damage and accelerated wasting. We hypothesized that concentrically-biased training is safe for dysferlin-deficient muscle and alters gene expression linked to muscle protection in a murine model of dysferlinopathy. METHODS: We studied the response of dysferlin-deficient mice (N = 6) and control mice (N = 6) to 12 weeks of non-injurious, concentrically-biased, forced exercise, performed with a robotic dynamometer. Each bout of exercise involved 4 sets of concentric contractions of the tibialis anterior (TA) muscle of the hindlimb (160-90 degrees of ankle dorsiflexion). Two bouts of exercise separated by 3 days, were performed each week. After 12 weeks of exercise, the mice were euthanized and their exercised (left) and unexercised (right) TA muscles were harvested and subjected to histological (H&E staining) and gene expression (array-based quantitative RT-PCR) studies. RESULTS: The exercised TA muscle of dysferlin-deficient mice had 0.77 ± 0.67% damaged fibers compared to 0.20 ± 0.11% in control mice. However, the exercised TA muscle of dysferlin-deficient mice had 23.8 ± 17.3% centrally-nucleated fibers (CNFs, marker of myogenic activity) compared to 2.9 ± 1.3% in control mice. Expression of the satellite cell quiescence gene Pax3 was downregulated ~11 fold in exercised versus unexercised dysferlin-deficient muscle. Gene expression changes relevant to apoptosis were ambiguous, since the pro-apoptotic gene caspase-3 and the anti-apoptotic gene ribosomal protein S6 kinase polypeptide 1 were both downregulated (~2 and ~5 fold, respectively) in exercised versus unexercised dysferlin-deficient muscle. CONCLUSION: Our data suggest that, despite being non-injurious in nature, concentrically-biased exercise might still trigger myogenic activity in dysferlin-deficient muscle. The translational relevance of this work is that, in order to avoid depleting the limited regenerative potential of dysferlin-deficient muscle, maintenance exercise must be carefully adjusted and monitored, to not only prevent injury but also minimize unwanted myogenic activation.

Funding to JAR from the Jain Foundation Inc., Wayne State University Startup and FRAP Awards, and NIH R03HD091648 from NICHD.

Circulating extracellular vesicles as biomarkers for the evaluation of rehabilitation outcome

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One of the main hurdle in the clinical approach to rehabilitation and treatment of neurological diseases is the lack of easily accessible and sensitive biomarkers for the evaluation of the rehabilitation outcome and the prediction of the disease progression rate. Extracellular vesicles (EVs) are nanoscaled vesicles released by almost all body cells. Those released from brain cells are known to cross the blood-brain barrier, bringing into the peripheral blood complex cargo of molecules that mediate the intercellular communication among organs and provide a snapshot of the processes occurring in the central nervous system during physiological and pathological events. EVs were shown to be involved in the regenerative and repair processes occurring after ischemic stroke[1] and in the onset and progression of many neurological disorders. For these reasons, EVs are studied as promising biomarkers of neurological disorders, with the potential to be used to monitor both regenerative processes and disease progression. Nonetheless, the initial enthusiastic approach to EVs as biomarkers has been hindered in its transfer to clinics because of technological obstacles related to their nanoscale dimensions and to their limited amount. In the strive for a reliable, sensitive and reproducible method to detect and characterize EVs, we propose a biophotonics-based biosensor that takes advantage of Surface Plasmon Resonance imaging (SPRi) technique.

We show herein the application of a recently optimized SPRi-based biosensor[2] for the detection and characterization of EVs isolated by size exclusion chromatography from the blood of stroke patients, before and after rehabilitation. The SPRi-antibody array was designed to separate EVs of different cellular origins (neurons, astrocytes, microglia, oligodendrocytes, endothelial cells) and apoptotic bodies. After the successful detection of EV subpopulations, the presence and the relative amount of specific surface molecules related to pathological or recovery processes were evaluated.

Our results demonstrated the ability of the SPRi biosensor to reveal differences not only in the relative amount of specific cell-derived EV subpopulations in blood, but also in their cargo during the disease progression and/or resolution. In particular, variations in the amount of specific receptors related to neuroinflammation and neuroregeneration were observed in the circulating EVs from stroke patients before and after rehabilitation. These data, together with the concentrations of inflammatory cytokines and BDNF provided evidence about the variations in the inflammatory state induced by the stroke event and attenuated following the rehabilitation process.

Our results provide support for using the proposed SPRi-based biosensor for the detection and characterization of circulating EVs. Besides, SPRi can be easily transferred to clinics and, thus, it represents a valuable candidate technique to evaluate the potential of EVs as peripheral biomarkers for the prediction of the recovery after stroke.

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Acknowledgments

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A Novel Role for Processing Bodies in Age-Related Impairment of Muscle Stem Cell Self-Renewal

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Introduction: Aging results in impaired physical function and decreased regenerative capacity due to metabolic and biochemical changes within the skeletal muscle. Muscle stem cells (MuSCs) represent the primary reserve cell population responsible for dictating the skeletal muscle regenerative cascade. As a result of aging, MuSCs display a decreased capacity to become activated after injury and self-renew[1]. This concept of self-renewal relies on the transfer of distinct mRNA decapping and degradation proteins via sub-cellular structures. We hypothesize that a group of cytoplasmic, non-membranous structures—known as processing bodies (p-bodies)—are responsible for the storage and transfer of mRNAs that dictate MuSC self-renewal[2]. To test this hypothesis, we investigated markers of self-renewal and p-body formation in young and old MuSCs *in vitro*.

Materials and Methods: Young (3-4 months) and old (22 months) male C57BL/6 mice were injured using a 10 μL injection of cardiotoxin (1 μg/mL) to bilateral tibialis anterior muscles. The next day, MuSCs were isolated from all limb muscles using fluorescence activated cell sorting and labeled surface markers Sca1 (-) and α-7 (+), as previously described[1]. Isolated cells were cultured in growth media for 48 hours, fixed in 2% Paraformaldehyde, and prepared for immunofluorescence staining. DCP1A and H2A.Z proteins were fluorescently tagged with antibodies against p-bodies and MuSC asymmetry, respectively. Subsequent z-stack renderings were captured using a Zeiss semi-confocal microscope, and signal intensity was analyzed by ImageJ. Since it has been suggested that p-body formation is linked to changes in the cytoplasmic environment, such as intracellular pH, young and aged MuSCs were seeded on 35 mm MatTek dishes in a second set of experiments. Using an Invitrogen pHrodo green AM fluorescent probe, pH variance before and after a stressor cocktail (Valinomycin and Nigericin) was analyzed over time, by live cell imaging.

Results and Discussion: Young MuSCs expressed a greater number of p-bodies than old MuSCs (Figure 1A). In addition, there was a significant correlation between the average intensity per cell of DCP1A and H2A.Z signal in young MuSCs. However, this relationship was disrupted with aging (Figure 1B). Live cell imaging data revealed that old MuSCs initially measured more acidic than young counterparts, which is consistent with their decreased formation of p-bodies (data not shown). Interestingly, whereas young MuSCs maintained a stable, more basic pH—even in the presence of stress—the pH intensity of old MuSCs increased in basicity by 30%.

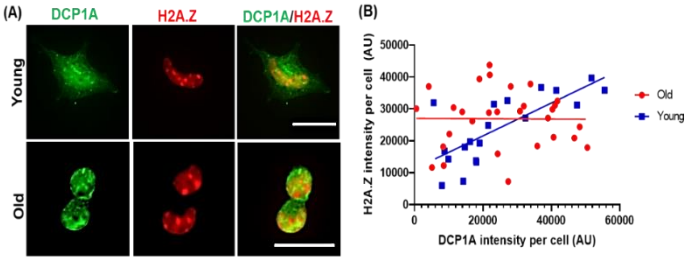

Figure 1. Aging compromises the correlation between H2A.Z and DCP1A (A) Representative confocal images, taken at 63X magnification, from immunofluorescence staining of p-bodies (DCP1A) and H2A.Z in young and old MuSCs. Scale: 15 µm. (B) Correlation of average DCP1A and H2A.Z intensities in young and aged MuSCs.

Conclusion: These experimental findings suggest a decreased capacity of old MuSCs to form discrete p-bodies structures *in vitro*. In addition, the data suggests that p-bodies may regulate self-renewal capacity of MuSCs, as demonstrated by the lack of correlation between DCP1A and H2A.Z intensities in aged cells. Furthermore, we posit that the more acidic environment of old MuSCs may inhibit p-body formation, and thus ability to self-renew. Taken together, these findings suggest that future studies that investigate the p-body cargo facilitating this self-renewal process are warranted.

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Developing new delivery methods for localized immunosuppression

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Peripheral nerve regeneration after segmental defects occurs naturally, but is often sub-optimal. The most effective current clinical option is a sensory autograft of a freshly removed nerve, with degradable biomaterial conduits and decellularized grafts of lesser efficacy. Allografting of freshly isolated live nerves is as or more effective than autografts, but is not widely practiced due to the serious risks of systemic immune suppression. Our group has been developing methods to localize the immune response surrounding only the graft in an effort to reduce risks and enable use of this highly effective strategy to regenerate peripheral nerves. Data shows that localized immune suppression achieved with drug and cell based strategies allow for full regeneration equivalent or superior to mixed nerve autografts in critical sized defects in the rat model. This strategy has also proven effective for regenerating critical sized defects that encompass bifurcations and complex nerve structures. Allografts are uniquely positioned to address this critical aspect of peripheral nerve injury.

An Inducible AGE Model for Unraveling the Effects of Aging in Musculoskeletal Tissues

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INTRODUCTION: In musculoskeletal menisci, tendons, and ligaments there is a clear link between increasing age and injuries, primarily attributed to advanced glycation end-products (AGEs).[1-2] AGEs accumulate in collagen with age, producing non-enzymatic crosslinks that alter tissue mechanics leading to more injuries. Despite ample correlative evidence linking collagen glycation to aging, little is known how AGEs impact matrix mechanics and cell-matrix interactions, limiting therapeutic options.[1-2] The objective of this study is to develop a system to trigger AGE accumulation throughout culture, while maintaining cell viability, so to investigate the effect of AGEs on tissue mechanics and cell-matrix interactions.

METHODS: Isolated bovine meniscal fibrochondrocytes were mixed with 20 mg/ml collagen at $5x10^6$ cells/ml.[4] 6 mm biopsy punches were cultured with ribose (Rb) and/or riboflavin (Rf) to induce AGE glycation. To investigate riboflavin induced glycation, constructs were soaked in 0.25 mM or 0.75 mM riboflavin for 1 hour, exposed to blue light for 40 sec, and cultured in standard growth media (DMEM) for 15 days.[5] To investigate ribose induced glycation, constructs were cultured in 0, 30, 100 or 200 mM ribose for 15 days.[1,3] To investigate combined ribose/riboflavin induced glycation, constructs were cultured in 200 mM ribose for 15 days and then exposed to riboflavin. DNA, collagen, total AGE, and specific AGE crosslink pentosidine, were determined via PicoGreen, hydroxyproline (hypro), AGE (360/460 nm), and pentosidine (328/378 nm) assays, respectively. Results were compared to aged mice tail fascicles and human meniscus[2] and cartilage.[6] Confocal was used to evaluate collagen fibril formation. All data are expressed as mean ± SD, with 2-way ANOVA and Tukey t-tests for statistical analysis ($p<0.05$ significant).

RESULTS: Riboflavin treatment and 30mM ribose did not significantly affect DNA, while 100 and 200 mM ribose significantly decreased DNA by day 9 (Fig1A). A single dose of riboflavin significantly increased total AGEs/hypro by 15 days of culture and matched total AGE and pentosidine levels produced by 3 days of 30 mM ribose (Fig 1B&C). Ribose and combined ribose/riboflavin treatment increased total AGE and pentosidine accumulation with time, in a dose dependent manner (Fig 1B&C). Aged mouse tendon, human meniscus,[2] and human cartilage [6] AGE and pentosidine levels were all reached or surpassed by a single dose of riboflavin or prolonged culture with ribose. Confocal reflectance revealed an increase in fibril formation with glycation, suggesting AGEs are actively crosslinking collagen (data not shown).

DISCUSSION: In this study we found Ribose, riboflavin, and blue light are capable of producing a wide range of AGE crosslinks which match and/or exceed human AGE levels for various tissues, ages, and diseases.[2,6] Previous work induced AGEs using 5-10x higher ribose concentrations, which reduces cell viability, making it impossible to investigate cell-matrix interactions.[1] Here, we induced native levels of AGEs with lower concentration of ribose while maintaining cell viability. Interestingly, a single 40 second dose of riboflavin and blue light produced similar levels of AGE crosslinks as 3 days of ribose treatment. This riboflavin treatment option is an exciting means to trigger AGE crosslinks on demand *in vivo* or *in vitro* without impacting cell metabolism and viability. Ongoing work is combining this inducible system with hierarchical collagen fiber formation to investigate the effect of AGEs on tissue mechanics and cell-matrix interactions at multiple levels of organization.

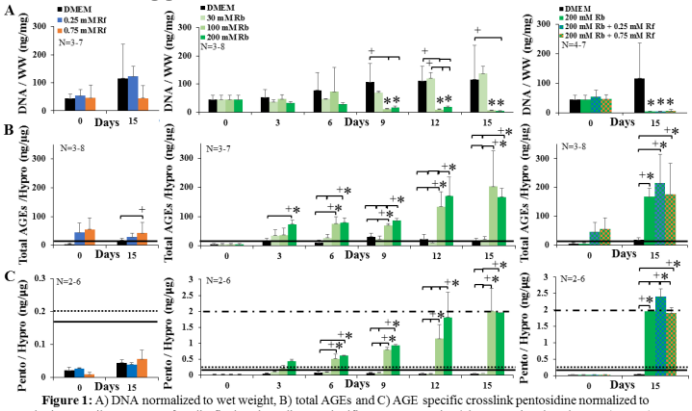

Figure 1: A) DNA normalized to wet weight, B) total AGEs and C) AGE specific crosslink pentosidine normalized to hydroxyproline content. Rf = Riboflavin, Rb = Ribose,. Significance compared to *day 0 or + bracketed group ($p<0.05$). Native 21-23 month old rat tail [2] ····· 60-70 year old human meniscus[2] - - - 80-90 year old cartilage[6]

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Regulation of mitochondrial Sirt3 by α-Klotho in muscle progenitor cells

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While youthful levels of the circulating hormone, α-Klotho, are critical for maintaining muscle stem cell mitochondrial ultrastructure and function in muscle, very little is known about the mechanism via which α-Klotho regulates mitochondrial activity within the skeletal muscle. Given that mitochondrial Sirtuin 3 (Sirt3) plays a critical role in mitochondrial homeostasis and aging, we tested whether α-Klotho may be regulating Sirt3. RNAseq analysis of injured tibialis anterior (TA) muscles revealed that Sirt3 gene was downregulated with age and that the bulk of Sirt3 interacting genes were differentially expressed with age as well. However, the expression profile was reversed to youthful levels after a systemic supplementation of α-Klotho. Likewise, aged muscle progenitors exhibited a reduced Sirt3 protein and activity level that was reversed with α-Klotho administration. Our *in vivo* studies also revealed an impaired muscle function in young Sirt3-/- mice when compared to young wild-type controls. Finally, the beneficial effect of α-Klotho administration to muscle progenitor cells was abrogated with an inhibition of Sirt3 in the recipient cells. Taken together, these studies define α-Klotho's wide-ranging mitochondrial influence and implicate α-Klotho as a potential regulator of Sirt3.

Role of the Anti-Aging Factor Alpha-Klotho in Aging-related Knee Osteoarthritis: A Pilot Study

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ABSTRACT:
INTRODUCTION: Aging is the prominent predictors of knee osteoarthritis (OA), and age-related OA is a one of the 5 major phenotypes of knee OA. However, based on systematic review, the exact working mechanisms of aging on knee OA is poorly understood. The anti-aging protein, Klotho, has been shown to regulate autophagy in a variety of cell types and potentially mediate age-related knee OA. This study is a pilot study aims to clarify the role of Klotho in the pathogenesis of age-related knee OA.

METHODS: Young (3-4 months old) and aged (21-22 months old) knee joints of male C57BL/6 mice were harvested for histopathological and immunofluorescence analyses. Cartilage degeneration and Klotho expression were compared between young and aged mice. To clarify the role of Klotho in aging-related OA, aged mice were treated with adeno-associated virus (AAV) for Klotho 19 days before the harvest.

RESULTS: As expected, aged mice demonstrated more severe cartilage degeneration compared to young mice. Klotho-positive chondrocytes were confirmed in all layers of articular cartilage in young mice, but not in aged mice. Klotho-signal intensity per cell in aged mice was lower than that in young mice. However, AAV Klotho administration prevented cartilage degeneration in aged mice.

DISCUSSION: Age-related cartilage degeneration was associated with Klotho decline, but Klotho overexpression counteracted the cartilage degeneration. These studies may provide mechanistic insight into the role of age-related declines in Klotho on OA pathogenesis.

Rehabilitative Exercise-driven Cartilage Regeneration: A Cross-Cutting Systematic Review from Ex Vivo, Animal, and Clinical Trials

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ABSTRACT:
INTRODUCTION: Therapeutic exercise is first-line treatment in knee osteoarthritis (OA) and its efficacy is similar to or surpasses the efficacy of acetaminophen, NSAIDs, and opioids in terms of pain relief. However, less is known about the benefit and risk from therapeutic exercise on articular cartilage in patients with knee OA. This "cross-cutting" systematic review aimed to clarify the effect of physical exercise on osteoarthritic cartilage in human, preclinical (in vivo), and cartilage explant (ex vivo) studies for better understanding of the potential risks and benefits of exercise intervention.

METHODS: A literature search was conducted until November 2018. We included studies investigating the dose-dependent effect of exercise or mechanical loading on OA articular cartilage. In clinical trials, studies with single intervention arms were also included. Results were narratively synthesized because of between-trial methodological heterogeneity.

RESULTS: In total, 14 studies (4 ex vivo, 4 pre-clinical, and 6 clinical trials) were included. From cartilage explant model and animal studies, exercise loading protected/aggravated histologically-detected cartilage degeneration in a dose (magnitude and duration) dependent manner, where altering signaling cascade of inflammatory and growth factors might explain the beneficial effects of exercise. These knowledge, however, did not adequately translate into human clinical trials in early or at risk of OA; that is, moderate exercise was not strictly defined with poor replicability and beneficial effect of moderate exercise on magnetic resonance imaging-detected cartilage components was controversial.

DISCUSSION: Exercise loading may affect OA cartilage health through dose-dependent manner in pre-clinical OA model and in ex vivo model with pseudo OA condition, although clinical evidence supporting this relationship is lacking in patients with early or at risk of OA. The dose-dependent response of exercise intervention on OA cartilage may be driven from altering signaling cascade of inflammatory and growth factors, which warrant future studies with the consideration of tissue-tissue interaction. Although moderate exercise intervention may benefit for cartilage composition in patients with increased risk of knee OA, the definition of "moderate" and dose threshold, which would responsible for benefit-risk balance of the exercise intervention, are currently difficult to be determined in the clinical practice.

Advanced Glycation End-Products Accumulation as a Biomarker of Clinical Severity in Knee Osteoarthritis: Data from the Osteoarthritis Initiative

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INTRODUCTION: Although knee osteoarthritis (OA) is considered a disease of mechanical etiology, mounting evidence suggests that physiologic and psychologic stress are associated with clinical severity. However, research is lacking on appropriate markers of reduced homeostatic reserve. Advanced glycation end-products (AGEs) are protein-sugar compounds that are primary factors in the systemic processes of accelerated aging. AGE accumulation is pro-inflammatory, pro-oxidative, and results in collagen dysfunction in cartilage. The purpose of this study is to examine whether AGE accumulation is associated with higher pain, higher psychological distress, and worse function in knee OA.

METHODS: Data were extracted from the Osteoarthritis Initiative for all available participants at visit 6 who received a skin autofluorescence (SAF) assessment (Table 1). SAF is a noninvasive, spectroscopy measure of the accumulation of AGEs in the dermal collagen. The output was scaled 0-100 arbitrary units and was adjusted for age, sex, and skin color. SAF was not normally distributed and organized into tertiles. Several measures were extracted for analysis: Kellgren-Lawrence score (radiographic OA severity), Charlson Comorbidity Index (CCI), Center for Epidemiologic Studies Depression Scale (CES-D), Coping Strategies Questionnaire-Catastrophizing Subscale (CSQ-CAT), Western Ontario and McMaster Universities OA Index (WOMAC), and 20 m pace (gait speed). Measures were compared across tertiles using an analysis of covariance – adjusting for age and sex – and post hoc least significant difference tests, as well as chi-square tests for categorical variables. Analyses were performed using SPSS V25 (IBM).

RESULTS: Increasing tertiles of SAF were associated with higher levels of pain, comorbidity, psychological distress (e.g., depression, pain catastrophizing), and knee symptoms (e.g., WOMAC). Gait speed was lowest in the highest tertile, but group differences did not reach significance.

DISCUSSION: The results of this study provide preliminary evidence of a link between aging pathways and indices of pain severity and function. Elevated AGE accumulation was associated with more chronic health problems and elevated psychological stress. These groups also had the highest pain and symptom severity. Noninvasive quantification of AGEs may facilitate OA prevention through earlier identification of high-risk individuals and could lead to novel interventional targets to improve participation and function.

Table 1. Demographics	**Tertile 1**	**Tertile 2**	**Tertile 3**
SAF range (AU)	17.8 – 25.6	25.7 – 30.2	30.3 – 69.1
Subjects (N)	120	115	117
% women	21 M 99 F [2,3]	41 M 74 F [1]	56 M 61 F [1]
Age (years)	62 [60, 63] [2,3]	65 [63, 67] [1,3]	68 [66, 70] [1,2]
BMI (kg/m^2)	30 [29, 31]	29 [28, 30]	29 [28, 30]
Kellgren-Lawrence score	1.5 [1.3, 1.7]	1.7 [1.4, 2.0]	1.7 [1.5, 1.8]

Values are mean [95% CI]. Superscripts denote significant ($p < 0.05$) differences between tertiles.

Table 2. Group differences	**Tertile 1**	**Tertile 2**	**Tertile 3**
CCI	0.4 [0.2, 0.5] [3]	0.5 [0.3, 0.7] [3]	0.9 [0.6, 1.1] [1,2]
CES-D	6.6 [5.4, 7.9] [2,3]	8.0 [6.5, 9.5] [1]	9.1 [7.5, 10.6] [1]
CSQ-CAT	0.5 [0.3, 0.7] [3]	0.5 [0.3, 0.7] [3]	0.9 [0.6, 1.2] [1,2]
WOMAC total score	11.1 [8.6, 13.6] [2,3]	14.4 [11.6, 17.3] [1]	16.8 [13.7, 19.9] [1]
WOMAC pain subscore	2.5 [1.9, 3.1] [2,3]	3.3 [2.6, 3.9] [1]	3.6 [2.9, 4.3] [1]
20m pace (m/s)	1.31 [1.27, 1.35]	1.29 [1.25, 1.33]	1.24 [1.20, 1.28]

Values are mean [95% CI]. Superscripts denote significant ($p < 0.05$) differences between tertiles.

A heterochronic parabiosis study implicating the paracrine function of α-Klotho on skeletal muscle regeneration

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ABSTRACT

Heterochronic parabiosis studies, surgically joining the circulation of young and old animals, have provided a platform for researchers to demonstrate the beneficial effect of 'rejuvenating factors' in a youthful circulation on the regenerative capacity of aged animals. A recent study has shown that systemic supplementation of a longevity protein, α-Klotho, enhances functional skeletal muscle regeneration suggesting a paracrine function of the protein. Here, we used a heterochronic parabiosis model to demonstrate that the circulating form of α-Klotho is a paracrine mediator for an enhanced skeletal muscle regenerative cascade. Histological analysis of cryoinjured tibialis anterior muscles revealed that parabiosing old mice with young α-Klotho$^{+/-}$ mice blunted the beneficial effect of a youthful circulation on skeletal muscle regeneration that is observed when old mice are joined with young α-Klotho$^{+/+}$ mice. This was also associated with a decrease in both circulating and muscular α-Klotho levels. *In vitro* experiments using muscle progenitor cells isolated from the parabiotes supported the *in vivo* results, finding decreased myogenicity and Klotho expression in the cells of old mice paired with young α-Klotho$^{+/-}$ mice. Taken together, these experiments demonstrate a paracrine function for circulating α-Klotho in promoting skeletal muscle regeneration.

Both functional and histological recovery are not completed within six weeks after sciatic nerve crush injury: a comprehensive 3D kinematic analysis on rats.

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[Introduction] Our previous study suggested that the ankle and toe angle in the “toe-off” phase, as reliable parameters for functional assessment, reflected histological changes over time, in the rat sciatic nerve crush injury model. However, both angles reached to normal levels while histological recovery was below the normal standard at the sixth week after surgery; the result might be attributed to the single and insufficient functional assessment method. Thus, we investigated multiple parameters for comprehensive functional assessment by using 3D kinematic analysis, in order to verify whether function is not fully recovered with six weeks after sciatic nerve crush injury as well.
[Methods] Six rats were designated as the control group , and 18 rats received surgery, six of them were randomly assigned as groups, at the first (1w), third (3w), and sixth (6w) week after surgery, for measurements of the ratio of stance to swing phase, toe and ankle angle at different phases of the step cycle, and pelvic parameters involving tilt, rotation, shift, and the trajectory of the virtual center of gravity (CoG). Histomorphometric data on sciatic nerve, including the number and diameter of myelinated nerve fibers, axon diameter and myelin sheath thickness, are investigated as well.
[Results] Values of 1w, 3w and 6w were significantly lower than that of the control group on histomorphometric data and the ratio of stance to swing phase. The unary parameters on toe, ankle, pelvic tilt, pelvic rotation and pelvic shift showed that the values at 6w were no more significantly different from the control group. However, the simultaneous change curves on toe and ankle angles of the step cycle and trajectory of CoG represented the values at 6w were significantly different, as well as 1w and 3w, from the control group.
[Conclusion] These results revealed that the functional recovery is not completed, as well as histological recovery within six weeks after sciatic nerve crush injury on rats. The 3D kinematic analysis method also showed the enormous superiority and usefulness to bring comprehensive and reliable outcomes in the field of functional assessment on rodents.

Development of Knee Adhesion Models in Rats

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【**Background**】Regenerative medicine technology has been widely used in motor disorders. The tissues adhesion caused by surgical invasion affects motor function such as range of motion. This is an important preventive and therapeutic target in physical therapy after surgical treatment. However, there is still not the adhesion model in musculoskeletal system, and the process of the early postoperative adhesion has not been clarified yet.

【**Purpose**】The aim of this study was to develop adhesion models of the knee joint in rats.

【**Methods**】18 male Wistar rats were divided into three groups. Rats from the first group were fixed with its knee joint bending deeply (Fixed group). The second group was fixed as well after the inner joint capsule was incised (Incision+fixed group). The third group, after incision of the inner joint capsule, the patella was dislocated from the femoral groove. The patellofemoral joint was exposed for 5 minutes. After that the joint capsule was sewn and fixed as well (Exposure+fixed group). All groups were fixed for 2 weeks. After that, we measured the range of motion of the knee joint with skin and muscles (knee ROM) and without them (knee structure ROM). In terms of histological analysis, we measured the length of the rear joint capsule and the adhesion of the anterior knee. The relationships between each ROM and the length of the adhesion or the rear joint capsule were verified from the measurement results.

【**Results**】Both knee ROM and knee structure ROM were significantly limited in the Exposure+fixed group than that of the other groups. Regarding histological data, the length of the adhesion was significantly longer in the Exposure+fixed group. Furthermore, the knee structure ROM correlated strongly with the length of the anterior knee adhesion although the correlation between the knee ROM and the length of the anterior knee adhesion was not observed. Interestingly, there were no or a weak correlations between the length of the rear joint capsule and the knee ROM or the knee structure ROM.

【**Conclusion**】In this study, we were able to develop adhesion models of the knee joint in rats. It was shown that adhesion of the anterior knee joint was formed within two weeks. This model might be useful for verifying process of adhesion or developing new rehabilitation programs on the field of regenerative rehabilitation with surgical invasion in the musculoskeletal system.

Using single-cell network analysis approach to develop an integrative biological age metric

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ABSTRACT

Aging causes a gradual decline in the complex biomolecular machinery. This decline rate varies among individuals but ultimately gives rise to age-related chronic diseases. Skeletal muscle health has been associated with all-cause mortality several times. ***Thus, we aim to develop an integrative biological age metric (IBAM) through quantitative exploration of protein-protein interaction (PPI) network with mouse skeletal muscle as a model system.***

Single-cell RNA-seq data was acquired across three age groups. Ten cell subpopulations were classified based on established gene markers. We generated weighted PPI network for each cell with nodes as genes with non-zero expression, and edges as physical interaction reported in STRING database. Next, we will identify subpopulations that drive the aging phenotype by analyzing differential gene expression in the context of hallmarks of aging.

If successful, biological age can be used to predict the onset of age-related diseases and for assessing the efficacy of anti-aging therapies.

Donor Age Effects on the Proliferative and Osteogenic Differentiation Potential of Equine Bone Marrow Derived Mesenchymal Stem Cells in Culture

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Introduction: Orthopedic injuries are a major cause of lameness and morbidity in horses. Bone marrow derived mesenchymal stem cells (BM-MSCs) have shown potential in cell-based therapies to facilitate the repair of orthopedic injuries and are being used increasingly in veterinary clinics. Presently, the application of MSCs in clinical practice on equine patients is most commonly designed as autologous transplants, using cells harvested from the patient shortly after the time of injury. This necessitates a delay of treatment to enable the expansion of cell numbers in culture. Of concern, however, are human and rodent studies that have shown a sharp decline in MSC quantity and quality with increasing donor age. This may be problematic for the important equine demographic of older orthopedic patients due to current recommendations that often call for 10-100 million MSCs in treatment protocols.

The aim of this study, therefore, was to investigate the relationship between donor age and MSC parameters in horses. Specific objectives were to compare the cellular proliferation and osteogenic potential of equine BM-MSCs as a function of donor age.

Materials and Methods: BM-MSCs were harvested immediately post-mortem from the sternum of horses in 5 different age groups, with 4 horses in each age group (N=20). The age groups were newborn (0 days), yearling (1-2 years), adult (5-8 years), middle-aged (12-18 years), and geriatric (≥ 22 years) horses. Cells were expanded in culture to passage 1 and then stored in liquid nitrogen. For the experiments, BM-MSCs were thawed and expanded to passage 4. The cellular proliferation capacity of the BM-MSCs was tested using an EdU incorporation assay as described previously[1]. The osteogenic differentiation potential of the same cells was compared quantitatively by measuring alkaline phosphatase activity and calcium deposition with an Alizarin Red S assay using standard protocols[2,3]. Data were analyzed by one-way analysis of variance with Tukey's *post hoc* corrections for multiple comparisons. Significance was set at $p<0.05$.

Results: Overall, the cellular proliferation of equine BM-MSCs declined with increasing donor age ($p=0.015$), exhibiting specific pairwise statistical significance in comparisons of newborn to geriatric horses ($p=0.023$), yearling to geriatric horses ($p=0.035$), and adult to geriatric horses ($p=0.025$). The osteogenic data showed that the potential of autologous equine BM-MSCs to make calcium deposits also decreased with increasing donor age ($p=0.002$), specifically between newborn and yearlings ($p=0.021$), newborn and seniors ($p=0.002$), and newborn and geriatric horses ($p=0.004$). No significant differences were found in the alkaline phosphatase activity across age groups.

Discussion: The data showed that both the cellular proliferation and osteogenic differentiation potential of equine BM-MSCs to make calcium deposits decline with increasing donor age. Interestingly, significant differences in the proliferation were only observed in pairwise comparisons involving geriatric horses. Levels of BM-MSC proliferation remained stable from newborn through middle aged horses 12-18 years of age. On the other hand, a decline in the calcium deposits was observed already in pairwise comparisons between newborn and yearlings, highlighting the importance of donor age considerations for autologous treatment of orthopedic injuries.

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Blockade of Neuromuscular Glutamate Receptors Impairs Peripheral Nerve Reinnervation Following Crush Injury

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OBJECTIVES: Motor axons in peripheral nerves are capable of regeneration following injury. However, complete recovery of motor function is rare, particularly when reinnervation is delayed. Nerve injuries are a frequent cause of permanent disability following combat wounds with over 80% of Veterans demonstrating persistent weakness after nerve injury (Rivera, 2014). We have found that NMDA-type glutamate receptors, expressed at the neuromuscular junction for only a brief perinatal window, play a crucial role in the successful innervation of muscle during development (Personius, 2016). Here, we question whether NMDA receptors play a similar role in re-establishing innervation following peripheral nerve injury in mature muscle.

METHODS: The sciatic nerves of adult mice were unilaterally crushed. We implanted a slow-release polymer infused with either glutamate receptor blockers (CNQX & AP5) or saline near the soleus muscle on the side of crush. Toe spread and surface EMG was assessed from day 0 to day 21 post-crush. Soleus muscle contractile properties and the ability of the tibial nerve to drive soleus contraction was evaluated on day 21. We determined the extent of multiple innervation (a measure of neuromuscular maturity) by immunostaining the neuromuscular junction of the soleus and extensor digitorum longus (EDL) muscles on day 15 and 21. In separate experiments, EDL and soleus muscles from mice 7 to 62 days post-crush underwent calcium imaging to assess neuromuscular responses to bath-applied NMDA.

RESULTS: The toe spread test (a measure of functional toe extension) is known to normalize by 21 days after sciatic nerve crush. Glutamate blockade prevented the return of toe extension (two-way ANOVA). Whole-leg surface EMG returned to only ~20% of initial values by 21 days in both blocked and saline groups. This result was expected since glutamate blockers were released locally near the soleus muscle. Soleus wet weight and calculated CSA was reduced in blocked compared to saline mice (one-way t-test). No differences in soleus contractile properties, however, were observed (two-way ANOVA). Glutamate blockers reduced the ability of the tibial nerve to drive soleus contraction over a five-minute period (two-way ANOVA). Glutamate blockade resulted in immature neuromuscular histology. The percent of multiply innervated neuromuscular junctions was increased in both soleus and EDL muscles at 15 and 21 day post-crush in the blocked vs. saline mice (one-way ANOVA). Bath-applied NMDA produced significant localized neuromuscular calcium release at 14 days post-crush, but not at 2-7, 21-28, 45, or 62 days (two-way t-tests).

CONCLUSIONS: These results suggest that glutamate receptors are re-expressed at the neuromuscular junction following peripheral nerve injury. Furthermore, neuromuscular glutamate receptors influence the reinnervation process and recovery of motor function. Finally, as occurs during development, glutamate signaling at the neuromuscular junction occurs primarily during a brief time-window. When reinnervation is experimentally delayed in mice to mimic the prolonged process seen in humans, reestablishment of the neuromuscular junction is abnormal, and functional recovery is poor (Sakuma, 2016). Future work will investigate whether re-expression of neuromuscular glutamate receptors improves recovery following delayed reinnervation.

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Title:

Investigating the Combinatorial Effects of Glycomaterial Implants and Neuromodulatory Electrical Stimulation on Functional Rehabilitation of Neural Networks following Severe Traumatic Brain Injuries

Abstract:

Severe traumatic Brain Injuries (sTBI) often result in devastating neurological consequences and long-term disability. There are currently no treatments to facilitate the regeneration and functional recovery of damaged neural tissue after sTBI. In a recently published study, 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 in vitro. Results from these studies suggest that electrical 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) [1]. We also demonstrated that the implantation of BDNF and FGF2 sequestering brain-mimetic glycomaterial scaffolds in an sTBI defect promoted neural stem cell proliferation, neuroprotection, and brain tissue preservation over a period of 4 weeks post-TBI [2]. Based on these findings, we hypothesized that the implantation of growth factor sequestering glycomaterial scaffolds acutely after a sTBI, coupled with the administration of activity dependent electrical stimulation would enhance the repair and re-functionalization of damaged brain tissue in a rodent model of sTBI.

In this study, we investigated the tissue-level and behavioral function recovery over a period of 20 weeks after sTBI and glycomaterial scaffold implantation. Tissue-level assessments suggest significantly enhanced neurogenesis, plasticity, and revascularization leading to a sustained chronic recovery of cerebral blood flow as well as motor function in scaffold implanted animals when compared to untreated controls. In ongoing studies, we have determined that low current, low-frequency stimulation for one week following a sTBI 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 results reveal opportunities for the combinatorial application of rationally designed glycomaterial scaffolds and electrical stimulation techniques to facilitate the directed repair and re-functionalization of damaged brain tissue post-sTBI.

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Exposure of Muscle Stem Cells to A Stiff Microenvironment Drives An "Aged" Mitochondrial Phenotype

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Age-related declines in skeletal muscle regenerative capacity and functional recovery after injury leads to increased morbidity and decreased quality of life. Muscle stem cells (MuSCs) are essential for regeneration of muscle. However, over time, MuSCs display an impaired activation, proliferation, and myogenic lineage specification. Although elegant *in vitro* studies have demonstrated that extrinsic mechanical properties have the potential to impact stem cell fate (Engler, et. al., Cell, 2006), **we know little about how native tissue stiffening associated with increased age affects stem cell function**. Our previous study demonstrated that aging causes alterations in extracellular matrix (ECM) mechanical properties in skeletal muscle and that these changes drive fibrogenic conversion of MuSCs, ultimately leading to fibrosis (Stearns-Reider, et. al., Aging Cell, 2017). Here, we tested the hypothesis that aberrant ECM stiffness drives decreased muscle regeneration and function. Furthermore, given the potential role of mitochondria in dictating stem cell fate, we investigated on mitochondrial phenotype and function. We also focused on the effect of substrate stiffness on regulation of the metabolism-associated genes.

First, to quantify how aging affects muscle stiffness, we performed biaxial mechanical testing on young and aged muscle, followed by finite element analysis. Biaxial data of young and aged muscle were utilized to calibrate the material property constants. In-plane maximum Green strain predictions revealed that collagen fibrils experience lower strain levels (i.e. are less compliant) in aged ECM when compared to young counterparts. Our analysis further revealed that the Young's Modulus (E) of aged muscle to be approximately four-fold higher than young muscle.

Next, we evaluated whether reduction of aged muscle stiffness *in vivo* improves muscle regeneration after injury. To modulate the muscle stiffness, we treated aged mice daily for six weeks with beta-aminopropionitrile (BAPN), an inhibitor of the collagen cross-linking enzyme, lysyl oxidase (LOX). Indeed, BAPN-treated aged muscle exhibited significantly enhanced regeneration and force producing capacity two weeks after an acute injury, as determined by histological analysis and *in situ* contractile testing. Furthermore, muscle quality was also assessed by half relaxation time measurements. While aged muscle typically displays increased half relaxation time, BAPN-treated aged group showed half relaxation time similar to that of young, indicating that the muscle quality was restored.

Finally, to better understand the mechanism by which stiffness may affect MuSC mitochondrial function and, thus, fate, we engineered PDMS substrates that mimics the stiffness of young and aged muscle. We then seeded the constructs with young MuSCs. Consistent with the reduced mitochondrial network size and metabolism-associated gene expression in aged MuSCs (Sahu et al., Nat. Commun., 2018), we find that young MuSCs cultured on the stiff (aged) PDMS display decreased mitochondrial network size. Moreover, seeding MuSCs onto a stiff substrate decreased the expression of the alpha-Klotho and Sirtuin 3, both of which have been shown to regulate mitochondrial function.

Taken together, these findings suggest that age-related increases in muscle stiffness may drive mitochondrial dysfunction, and that these declines may be attributed to a disruption in metabolism-associated gene expression. Our studies underscore the importance of skeletal muscle ECM stiffness on MuSC function and provide potential mechanism of myogenic-to-fibrogenic conversion of MuSC due to increased age. These findings indicate that modulation of ECM biomechanical properties could be a potential target for regenerative medicine to enhance muscle regeneration and function in elderly population.

Enhanced the myogenesis of muscle stem cells by substrate nanotopography

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In most living tissues, the extracellular matrix (ECM) exhibits different types of nanostructures, which have been shown to influence cell behaviors and tissue functions. For example, the parallel aligned structure of collagen fibers in skeletal muscle tissue is essential for muscle stem cell (MuSC) differentiation and muscle regeneration. Inspired by the nanostructures existed in the ECM, nanotopographies with different shapes and dimensions are therefore engineered and have become powerful tools in tissue engineering and regenerative medicine for controlling the cell behaviors. Although the cellular responses to engineered nanotopography have been investigated in many cell types, the current understanding of the effects of nanotopography on myogenesis of MuSCs is still limited.

To investigate the influence of nanotopography on muscle regeneration, in this study, the nanogratings were fabricated on polystyrene substrates to mimic the organization of the collagen fibers in skeletal muscle tissue. The MuSCs isolated from mice were cultured on nanogratings and flat surfaces and their morphology, proliferation and myogenic differentiation were evaluated. Our results showed that the MuSCs cultured on the nanogratings were highly aligned along the grating direction, while the cells spread randomly on flat surfaces. Compared to the flat controls, the cells on nanogratings exhibited significantly lower percentage of PAX7 positive cells and higher level of myoD positive cells, indicating that the nanogratings significantly enhanced the myogenic differentiation of MuSCs. Furthermore, the myotubes formed by the MuSCs on nanogratings had significantly higher fusion index and maturation index than the myotubes formed by the cells on flat surfaces. Mechanism study further indicated that the nanograting-regulated myogenesis of MuSCs was related to the release and content of exosomes. Our study provides insight into the rational design of biomimetic substrates for evaluating the ECM influences on skeletal muscle regeneration and creating implants to treat muscle diseases and injuries.

AAV delivery of α-Klotho to counteract age-related declines in skeletal muscle function

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Circulating levels of the longevity protein α-Klotho, have been positively associated with improved skeletal muscle regeneration and decreased loss of muscle mass in aged mice and humans. Therefore, the development of Klotho-based therapeutic interventions to improve the regeneration and maintenance of aged skeletal muscle are warranted. In this study, we utilized advancements in Adeno-Associated Virus (AAV) mediated gene delivery to upregulate Klotho in the circulation through native translation pathways. We show in aged mice that systemic upregulation of Klotho enhances skeletal muscle functional performance in conditions of homeostasis and recovery following acute injury. Furthermore, histological analyses highlight increased myofiber regeneration post injury and increased myofiber size in both homeostasis and post injury conditions. These findings suggest Klotho gene delivery is a viable option for both preserving skeletal muscle mass and improving recovery of skeletal muscle injuries in an elderly population.

Changes in physical exams for supraspinatus pathology in persons with spinal cord injury following intratendinous micro-fragmented adipose injections

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Background: Rotator cuff disease is a significant cause of shoulder pain, weakness and disability. Persons with spinal cord injury (SCI) are at increased risk of rotator cuff disease due to their dependence on shoulder function for activities and mobility with manual wheelchair use. Conservative treatment modalities include oral anti-inflammatory medications, physical therapy, and corticosteroid injections, although the long-term pain relief from these injections remains controversial. Surgical repairs, which are recommended for partial or full thickness rotator cuff tears, is not ideal for people with SCI due to the prolonged immobility after surgery. Autologous adipose-derived stem cells (ASC) have been shown to decrease disability, reduce pain in shoulder pathologies and improve defects in rotator cuff tears. However, these regenerative modalities have not been demonstrated on shoulder pain in peoples with spinal cord injury.

Methods: Manual wheelchair users with chronic SCI who had refractory single-sided shoulder pain and failed conservative treatment were selected for the study. The shoulder physical exam (PE) consisted of 11 elements: tenderness to palpation of biceps tendon, supraspinatus tendon, or acromioclavicular joint, pain with resisted internal or external rotation, Empty Can test, Painful Arc test and Neer's test. For each exam maneuver, the patient was graded with 0 (no pain), 1 (equivocal for pain), or 2 (pain), and summed to designate a physical exam score ranging from 0 to 22. Rotator cuff disease was defined as anterior shoulder pain, which manifested as pain on palpation or with any provocative maneuvers, and was confirmed via ultrasound. ASC was obtained using a Lipogems® processing system from each patient in sterile fashion. Each patient received ultrasound-guided injection of ASC into the affected supraspinatus tendon. Physical exams and scoring were performed on bilateral shoulders, one treated and one untreated, at baseline, 1, 2, 3 and 6 months after ASC injection.

Results: Eleven individuals were recruited for the study. One withdrew to explore alternative treatments for shoulder pain. Another was excluded from final analysis after receiving a platelet-rich plasma injection, for a final sample size of nine (age = 56.0years±7.8; injury duration = 21.1years±9.4; 1 Female; 1 Hispanic White, 7 Non-Hispanic White, 1 Black; 2 Tetraplegia, 7 Paraplegia). Average physical examination scores at baseline were 6.6±2.7 and 1.3±2.0 for treated and untreated shoulders, respectively. A significant interaction effect was noted ($p<.001$), which indicated that changes in PE scores over time differed between treated and untreated shoulders. Post hoc analyses revealed significant differences in PE scores between treated and untreated shoulders at baseline ($p<.001$) but no other time point. Significant decreases in PE scores between baseline and all other time points were noted in the treated side (mean decrease of 5.8, $p<.001$), but not the untreated side (mean decrease of 0.9, $p>.05$).

Conclusion
We found that supraspinatus ASC injections provided significant improvements in physical exam one month after intervention, and these effects continued for at least 6 months. The recovery of physical exam correlate with improvement in shoulder function and pain. Our findings strongly suggest that ASC is a promising therapy in supraspinatus rotator cuff disease in peoples with SCI.

The promise of autologous peripheral blood-derived cellular therapy as an adjunct to routine clinical practice in the treatment of COPD symptoms as measured on the Clinical COPD Questionnaire

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Background: COPD carries a major burden of illness and is a leading contributor to morbidity/mortality in the United States and worldwide. There are multiple validated survey-based tools to track disease related symptoms in COPD. An efficient validated survey is the Clinical COPD Questionnaire (CCQ). The changes in a CCQ score that represent a clinically significant improvement or deterioration are referred to as the Minimal Clinically Important Differences (MCID). There are regenerative medicine treatments available that are attempting to provide added clinical benefit for COPD sufferers. This abstract will evaluate a proprietary autologous peripheral blood-derived cellular therapy (Autologous Cellular Therapy or ACT for short) over a 12-month period using the CCQ results to determine if the data are suggestive of added benefit beyond routine clinical practice (RCP) alone.

Methods: A retrospective review was performed a data set of 315 COPD patients who were from the U.S., who received ACT, and who completed the CCQ at a pre-treatment baseline and 3 months, 6 months and 12 months post-treatment. To find a control population for comparison in the progression of CCQ scores and MCID over a 12-month period, a literature search was performed and revealed a 2019 BMJ Open article from Alma, et al. that was able to follow 200 Dutch patients through a year of routine clinical practice and obtain their CCQ at baseline and at 3, 6 and 12 months that was ideal for comparisons.

Results: Initial comparisons of the data show a mean change from baseline CCQ scores at 3 months of -1.16 (ACT) vs 0.00 (RCP), at 6 months of -1.09 (ACT) vs 0.00 (RCP) and at 12 months of -0.91 (ACT) vs -0.02 (RCP). All of the scores for ACT well exceeded the MCID of 0.4. Average age of the RCP was 66.69±7.91 vs 71.05±7.63 for ACT. The RCP group contained more male patients (58.5%) as did the ACT group (60.6%).

Conclusion: The compared data is suggestive of added benefit to autologous peripheral blood-derived cellular therapy as an adjunctive to routine clinical practice management of COPD symptoms over a 12-month period. However, there remains the need for further analysis to elucidate and control for differences in baseline demographics as well as mitigate sources of error. Further studies are needed that would be less likely to have differences in cultural habits and/or standards of care and a placebo-controlled study is necessary to establish the reliability of the score differences between RCP and ACT.

Development of Native-like Zonal Ligament-to-Bone Attachments for ACL Repair

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Introduction: Ligament-to-bone attachments, or entheses, are structurally complex tissues which translate load from elastic ligaments to stiff bone due to intricate zonal organization with gradients in collagen organization and mineralization. Currently, these gradients, necessary for long-term mechanical function, are not recreated after repair or in engineered replacements, leading to high failure rates.[1] Previously, we developed a novel culture system that guides ligament fibroblasts to develop aligned native sized hierarchical 30μm collagen fibers.[2] These construct hold great promise as ligament replacements, however driving organized enthesis development would aid their function *in vivo*. The objective of this study was to investigate compressive mechanical boundary conditions in addition to beta tricalcium phosphate (βTCP), known to be osteoconductive and aid in mineralization[3], on development of multizonal ligament entheses.

Methods: βTCP was synthesized as previously described[4], dried, calcined at 900˚C for 2 hrs, and ball milled into a fine powder. XRD and FTIR analyses were used to characterize the βTCP. Isolated bovine anterior cruciate ligament (ACL) fibroblasts were mixed with rat tail type I collagen to obtain 20 mg/ml collagen gels at $5x10^6$ cells/ml as previously described.[2,5] Constructs were cultured clamped up to 6 weeks with or without a topical layer of βTCP under the clamp. Constructs were sectioned into mid (M), transition (T), and clamped (C) regions (Fig 1A) for analysis of zonal organization, mechanics, and composition. SEM and Confocal were performed to analyze zonal collagen organization and mineralization in engineered and native ACL entheses. Tensile tests were performed at 0.75% strain/sec to determine mechanical properties. DNA, glycosaminoglycan, and collagen content were determined via PicoGreen, DMMB dye, and hydroxyproline assays, respectively. All data are expressed as mean ± SD ($p<0.05$ considered significant).

Results: XRD and FTIR of βTCP displayed sharp characteristic peaks and absorption bands matching known βTCP (data not shown).[4] SEM and confocal imaging revealed clamped constructs with and without βTCP developed distinct collagen fibril and fiber organization similar to immature native tissue over 6 weeks of culture (Fig 1B & 2). βTCP constructs had enhanced development compared to control, with highly aligned parallel collagen fibers in the mid-section, perpendicularly aligned fibers in the transition, and incorporation of βTCP particles into a porous collagen network in the clamped zone. SEM demonstrated βTCP constructs had enhanced fibrils organization, rounded fibrochondrocyte-like cells in the transition, and sheet-like mineralization under the clamp (Fig 1B). By 6 weeks Young's moduli of both control and βTCP show a 16x increase over 0 weeks with moduli of ~1.6 MPa (Data not shown). Composition remained relatively constant throughout culture (Data not shown).

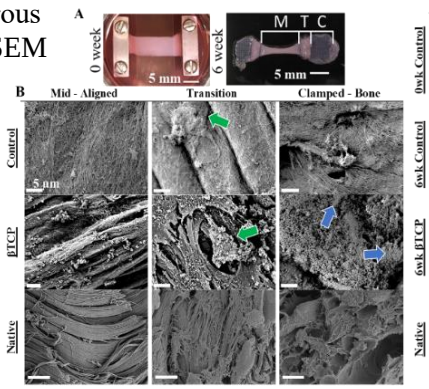

Figure 1 – A) Culture device at 0 weeks; depiction of analysis zones at 6 weeks, B) SEM of 6-week engineered samples and native tissue. Scale bar is 5 μm. Green arrow = fibrochondrocyte-like cell. Blue arrow = plate-like βTCP formation.

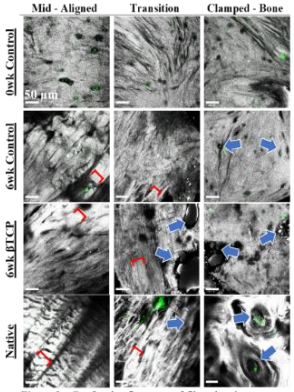

Figure 2 - Confocal reflectance of fiber development. Grey = collagen, green = cells. Arrows denote pores. Brackets denote fibers.

Discussion: Culturing with compressive boundary conditions and βTCP resulted in formation of three unique zones of collagen fibrils and fibers matching native tissue diameter and orientation, plate-like βTCP particles interconnected with collagen fibrils in the clamped zone mimicking bone formation[8], and mechanical properties matching reported bovine immature moduli for ACL fibrocartilaginous entheses.[7] SEM and Confocal imaging indicated the compressive-tensile interface of the culture system drove zonal hierarchical collagen organization. This organization was enhanced by βTCP application, suggesting βTCP is advantageous to zonal organization.

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Ink-Bath Rheological Relationship for FRESH Method Bioprinting

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The Freeform Reversible Embedding of Suspended Hydrogels (FRESH) method is a 3D printing technique that involves printing a hydrogel precursor ink into a gelled support bath. The role of the FRESH method is to print structures that cannot support themselves during the printing process, generally due to a low viscosity. The process requires a balance between the rheological properties of both the ink and bath. If the bath is too viscous, the ink is not extruded effectively from the printing needle. If the bath is not viscous enough, the ink will not be supported and the printed construct will lose its shape. In this study, we qualitatively determine the printability of different ink-bath pairings using varying concentrations of a model ink (alginic acid in phosphate-buffered saline) and a poloxamer bath (Synperonic® F-108). Measuring the rheological properties of the materials such as viscosity, elastic modulus, and shear-thinning characteristics, we determine a relationship between ink and bath for future FRESH method printing.

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Arsenic Impairs Skeletal Muscle Regenerative Cascade by Altering the Extracellular Matrix

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The extracellular matrix (ECM) provides a scaffold to the skeletal muscle for effective regeneration. Arsenic, when ingested through drinking water, impairs skeletal muscle regeneration by altering innate characteristics of the ECM. The ECM may be modified by changing myofibroblasts, which are primary producers of the ECM, perhaps by inducing mitochondrial dysfunction. We hypothesize that exposure to arsenic alters collagen types deposited by myofibroblasts as well as the cross-linking of collagen. In this study, we characterized arsenic-induced changes in the ECM to elucidate the mechanisms by which arsenic may be influencing ECM formation.

Myofibroblasts were isolated from the skeletal muscle of young, male mice and cultured in growth media. Treatment cells were exposed to 20nM sodium arsenite (As(III)) for 24 hours. Treated or control cells were plated on chamber slides and allowed to deposit ECM over 6 days. Cells were fixed, and immunofluorescence staining was performed to characterize ECM composition by targeting collagen types III, IV, and VI. Z-stacks of confocal images were captured to visualize collagen. To implicate mitochondrial change as a mediator of arsenic-induced ECM alterations, another group of arsenic-exposed cells received SS-31, a peptide shown to reverse mitochondrial dysfunction. For the *in vivo* model, mice were exposed to 0 or 100ppb As(III) in drinking water for 5 weeks. Following exposure, tibialis anterior muscles were injured with cardiotoxin. 10 days post-injury, mice were euthanized, and muscles were fixed and cryo-sectioned. Sections were stained for collagen types III, IV, VI, nuclei, laminin, and lysyl oxidase (LOX), which catalyzes crosslinking of collagen fibers. Collagen was quantified in terms of intensity per nucleus using ImageJ. Nuclear area was quantified with DAPI, and LOX was quantified by measuring LOX colocalization with DAPI. Laminin, representing the basal lamina, was utilized to determine myofiber area.

In vitro, exposure to arsenic resulted in a significant decrease in collagen types III and VI but an increase in collagen VI (Fig. 1). However, myofibroblasts treated with SS-31 following exposure to arsenic had significantly elevated levels of collagen III. *In vitro* results reflected *in vivo* findings in that collagen III and VI levels were lower for arsenic-exposed mice compared to controls. There were also fewer intracellular LOX-positive nuclei at the site of injury in the muscle of arsenic-treated mice than in control muscle. These arsenic-induced alterations were concomitant with a decline in muscle fiber cross-sectional area. Findings from both *in vivo* and *in vitro* studies suggest that arsenic promotes ECM remodeling and alters LOX expression. These matrix changes in response to arsenic may contribute to an impaired regenerative capacity of skeletal muscle. Indeed, arsenic exposure results in smaller myofiber area. The loss of nuclear LOX is consistent with larger, more open nuclei within myofibroblasts. Importantly, SS-31 treatment restored collagen III levels, implicating mitochondria as the target of arsenic-promoted dysfunctional ECM and muscle regeneration impairment.

These findings provide insight into underlying mechanisms by which the ECM may regulate skeletal muscle regenerative capacity: that arsenic leads to a decrease in collagens III and VI but an increase in collagen IV. Furthermore, these results demonstrate that such a conversion induced by arsenic may have the potential to be reversed with mitochondrial protectant. Future studies should seek to better understand how crosstalk between mitochondria and the ECM may be important to skeletal muscle health and maintenance.

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Why muscle-contraction training can enhance the efficacy of cell transplantation treatment for DMD; Focusing on macrophage dynamics.

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Duchenne muscular dystrophy (DMD) is one of the most severe muscle disorders. There is little in the way of treatment for the disease and no cure. In our research group, we are trying to establish a new cell transplantation therapy for Duchenne muscular disease (DMD) as a radical treatment. In addition, our group has shown that training (isometric muscle contractions) before the cell transplantation enhances the efficiency of the cell transplantation therapy. However, why the training has this effect is unknown.

It is already well known that macrophages play an important role in skeletal muscle regeneration. Macrophages have two subtypes, M1 and M2, which are known to function differently at different stages of skeletal muscle regeneration, and proper subtype transition is important for normal skeletal muscle regeneration. M1 macrophages promote inflammation, while M2 macrophages promote regeneration. Several studies have suggested the M1/M2 macrophage ratio contributes to the disease progression and amelioration (Villalta SA. et al., Hum Mol Genet. 2009). Intriguingly, one report found low-intensity training ameliorates the phenotype of mdx mice (DMD model mice), possibly by modulating the population ratio of M1 and M2 macrophages (Hyzewicz J. et al., Am J Pathol. 2017).

Therefore, we investigated the molecular mechanism through which the training improves the efficiency of the cell transplantation by focusing on macrophage polarization. We evaluated the changes of the total number and the population ratio of macrophages in DMD model mice (DMD-null/NSG) 24 hours after isometric muscle contractions.

Study on evaluation methods of skeletal muscle functions (muscle contraction force and endurance) after cell therapy for Duchenne Muscular Dystrophy (DMD)

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Duchenne muscular dystrophy (DMD) is characterized by the progressive degeneration and fragility of skeletal muscles. It is caused by the mutation of *DMD* gene on X chromosome, responsible for coding the dystrophin protein which is located around myofibers.

We are currently developing cell therapy as a future curative treatment for DMD. In that aim, we have succeeded in transplanting immortalized human myoblast cells Hu5/KD3 (Shiomi et al., Gene Ther. 2011) in an immunodeficient dystrophin knock-out mouse model (DMD-null/NSG). These cell transplantations showed the potential to restore dystrophin protein in the DMD mouse model's muscles. However, the functional muscle recovery still seems to be insufficient.

We are now aiming to accurately evaluate the skeletal muscle functions (muscle contractility, endurance, etc.) of the DMD mouse model, and to evaluate the therapeutic effects of cell transplantations more precisely. We have been focusing on the decline of muscle contraction force after repetitive loads of electrically-stimulated isometric contractions in various proportions of the maximal contraction force and on the time needed before its recovery. Moreover, we have also been considering other types of functional tests related to muscle endurance, such as treadmill running test or rota-rod running test, to get close to the clinical approach.

Our study will provide an accurate evaluation approach to DMD patients when the cell therapy will go to clinical trials.

Endothelial cell specific knockout of *Meis1* seems to improve the cardiac function of infarcted heart in the mouse model

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Background: A successful clinical application of cardiac regeneration would save almost 3.7 million lives per year worldwide those are caused by myocardial infarction (MI)-induced heart failure (HF) [1]. Although the implementation of the several new age therapeutic treatments of ischemic heart disease has been saving a lot of life, the life-style of the patients deteriorates significantly post treatment. Meanwhile, stem cell based therapy has caught a lot of attention serving as a promising strategy to reinstate the damaged heart which can overcome the fundamental challenge of non-proliferative nature of adult cardiomyocytes. Yet, this faces another obstruction where the newly generated cardiomyocytes located on the ischemic and fribrotic scarred tissue do not get augmented supply of blood for their survival and proliferation hindering rapid myocardial regeneration. Combinatorial approaches, such as gene-based therapy together with stem cell, facilitate the regeneration of damaged heart post-MI [2]. Recently, gene-based revascularization mediated by endothelial cells (EC) proliferation in the infarcted tissues has come out as an efficient strategy to improve the outcomes of stem cell based therapy. The recent discovery of *Myeloid ecotropic viral integration site 1* (*Meis1*) in regulating cardiomyocyte (CM) cell cycle [3] is one of them, which has opened a new window for researchers to unravel its role in the regeneration of the cardiovascular system. In the previous study, we found that EC-specific knockout (KO) of *Meis1* significantly increased blood flow of ischemic hindlimb and prevented mouse limb from necrosis and loss. We hypothesize that EC-specific knockout of *Meis1* ameliorates cardiac function via promoting revascularization in the ischemic heart.

Methods: Meis1 EC-specific KO mice [Tie2-cre$^{-/+}$;Meis1$^{-/-}$] were generated by breeding Tie2-cre$^{-/+}$ mice with wild-type (WT) Meis1 floxed (Meis1$^{f/f}$) mice. At the age of 8-10 weeks, KO and WT of both genders were used to induce MI by temporarily ligating the left anterior descending artery (LAD) followed by permanent reperfusion. Mice were housed up to 28 days and echocardiography were performed at baseline, 14th and 28th post-surgery to evaluate cardiac function. Infarcted hearts were extracted on 28th day for histological analysis.

Results: Comparison of echocardiography data from baseline, day14 and day28 showed a significant ($P<0.001$) recovery post MI in KO (n=7) than in WT (n=14), where the mean Ejection Fraction (EF) was reduced from baseline 72.2% to 39.75% on day 28 (mean reduction being 44.9%) whereas KO mean EF was only reduced by 31.39% from baseline 69.35% to 47.58%. Further, Trichrome Mason's staining of the harvested heart on day 28 demonstrated that the infarction size of KO (n=7) heart is around 16.57% less than that of WT (n=7). This observation, therefore, gives hope to the combinatorial approach of gene and stem cell based treatment for heart regeneration. The ongoing experiments include detailed analysis of ventricular chamber parameters, gene and protein expression of angiogenic specific markers, and endothelial cell characterization isolated from both KO and WT mouse heart.

Conclusion: *Meis1* negatively affects cardiac function and remodeling in post-MI mice probably through regulating EC proliferatio, thus promoting angiogenesis and or arteriogenesis. Further investigation of the mechanisms will result in better understanding of *Meis1*'s role in modulating revascularization and the findings may lead to the discovery of new therapy to treat heart patients.

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CHARACTERIZATION OF RODENT GAIT COMPENSATION STRATEGIES AFTER GASTROCNEMIUS VOLUMETRIC MUSCLE LOSS INJURY

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The rat hindlimb is a highly utilized model system for studying a variety of muscular and neurological pathologies. In order to quantify the effects of these pathologies on movement function we have previously developed a model and methodology to measure the kinematics and kinetics of rodent gait. We initially investigated the tibialis anterior (TA) and employed our motion capture approach to evaluate a 20% (by mass) volumetric muscle loss (VML) injury. This work resulted in clear statistical differences between the injured and healthy animals at all post-surgical timepoints. This was significant considering the low gait impact of the TA, as it is predominantly responsible for ankle dorsiflexion and clearing the toe during swing. We have leveraged that previous work into the investigation of VML injuries to a major gait contributor in the lateral gastrocnemius (LG). As a two joint muscle and the primary muscle for transferring energy in the lower limb during the gait cycle, healthy and injured LG muscles have a significant impact on movement ability.

The lateral gastrocnemius (LG) has only been lightly studied as a VML injury model. Thus, it is important to establish the baseline joint kinematics and kinetics over time in LG VML injured rats prior to attempting to assess efficacy of novel therapeutics for VML repair. For this study, 12-week old male Lewis rats (n=8) were tested on an instrumented rat-specific over ground walkway. After baseline data was collected, each animal was given a 20% (by mass) VML injury to the right LG. The walkway had dimensions of 8 feet long by 4 inches wide with 16 inch walls and contained two ATI 6-axis force plates. Before testing sessions, each rat was shaved to allow to accurate placement of 4mm reflective markers on the bony landmarks of the left anterior superior iliac crest (LASI), right anterior superior iliac crest (RASI), spine, tail, hip, lateral knee, ankle, and fifth metatarsal. Vicon Nexus (V2.8.1) motion capture software was utilized to record each rat moving down the walkway. All kinematic data was normalized to 100% of a complete gait cycle (heel strike to heel strike) with a minimum of three cycles averaged together for each rat. The 3-D locations of the markers on the bony landmarks collected via motion capture and the ground reaction force data collected by the force plates were exported for post-hoc kinematic and kinetic analysis using an OpenSim rat hindlimb model modified for our trials. Simulations were performed on individually scaled versions of the model to determine the kinematics and kinetics of the gait cycles. All data was filtered at 30Hz and all post-simulation analyses were performed in Matlab.

For both baseline (pre-surgery) and 12-week post-surgery spatiotemporal parameters, averages were calculated for step length as a percentage of body length (73.1±8.9 vs 72.0±5.6%, ns), cadence (142±29 vs 113±18 steps/min, $p<0.05$), walking speed (34.4±10.3 vs 29.0±5.6 cm/s, $p<0.05$), and percentage of cycle time in stance (57.9±7.0 vs 64.4±4.8%, $p<0.05$). Baseline kinematic joint trajectories compared well with previously reported data on the sagittal kinematics of normal gait in rats. Similarly, the baseline kinetic data compared well to previously reported data on healthy rats. Significant differences were detected when comparing baseline and 12-week measurements for both kinematics and kinetics. Now that we have established reliable methods of data collection and data analysis, as well as developed baseline and injured datasets for comparison, we plan to utilize what we have learned to evaluate the effects of novel regenerative therapeutics on LG VML injuries on kinematics and kinetics in a rat model.

Thanks to the AR^{3}T Pilot Funding Program and the Department of Defense (**W81XWH-18-2-0036 DoD/USAMRAA, G.J. Christ, PI**) for the funding that supported the development and execution of this study.

Finite Element Modeling of the Rat Tibialis Anterior to Predict Functional Force Deficit from Volumetric Muscle Loss

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Background: Skeletal muscle has a natural ability to repair damage to itself, surpassing the abilities of most other tissues in the body. However, in cases of severe trauma, the regenerative machinery of skeletal muscle can be overwhelmed, resulting in a permanent decrease of force-generating muscle volume. These volumetric muscle loss (VML) injuries are of great interest to the regenerative medicine community, as improvements in muscle function after injury are correlated with an increase in patient quality of life. The variety of pathologies that can produce a VML injury ensures that each instance of VML is unique in the location, shape, and volume of muscle affected. Muscle excision in rat tibialis anterior muscle is a commonly used VML model for evaluating potential regenerative therapies. Ankle dorsiflexion torque measurements from these rats are often used as a metric for muscle recovery to judge the success of potential therapies. A computational model that could evaluate the effect of VML injuries on force output could allow for an extra degree of control over experimental studies, saving valuable time and resources. In this study, we aimed to produce a computational finite element model for predicting force loss based on the position and size of a tibialis anterior VML injury.

Methods: A scalable 3D finite element model of a rat tibialis anterior muscle and tendon was designed using MRI images of a rat hindlimb coupled with image segmentation, CAD, and mesh generation software. Optimization of our finite element simulations were used to generate fiber directions and material paremeters. Results from quasi-static finite element simulations of muscle activation provided estimations of forces produced by the muscle. Validation of the finite element model geometry was done by comparing the parameters (such as pennation angle and total muscle volume) to previously reported data available in literature. Additional validation of our finite element results was accomplished by comparing our finite element model results with experimental results for whole and VML rat tibialis anterior muscle (normalized for muscle volume). A variety of VML defects were modeled along the length of the tibialis anterior muscle by removing volume from model geometry mesh at the site of injury. Finally, post-processing was performed to understand how force is transmitted along the length of the muscle through parallel and series fibers when a VML injury is added. This allowed us to explore characteristic behavior to could be used as an effective predictor of force deficit that is independent of volume loss.

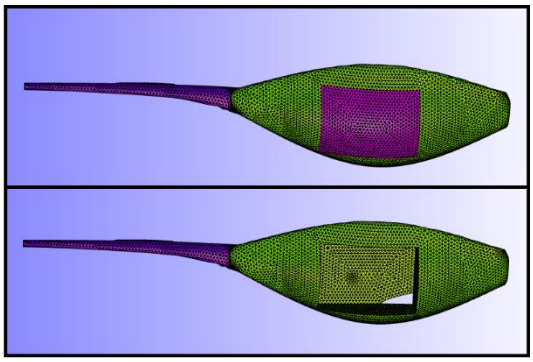

Figure 1: Building the model geometry of a VML defect

Results and Discussion: Our finite element model's pennation angle (17.6 ± 19.2^{o}) and fiber length (1.43 ± 8.2 cm) were found comparable to muscle architecture studies of similarly sized rat tibialis anterior muscles in literature. Finite element simulations of whole muscles (when scaled to equal volumes) produced similar force results to previous experimental data of rat tibialis anterior activation in healthy muscles. Additionally, the results from replicating the geometry and location of experimental VML injuries in the rat tibialis anterior in our finite element model show a similar amount of force loss, indicating that our model is predictive of both healthy and VML muscle. The amount of along-fiber (parallel fiber transmission) was found to be an indicator of total force ($R^2 = 0.551$). The largest contributor to force deficit, independent of volume loss, was found to be cross-sectional area of the defect perpendicular to muscle fibers ($R^2 = 0.6336$). This hints that the location and shape of a VML injury plays a greater role than the total volume disrupted in muscle force outcomes.

Computational model of muscle injury validated with in situ experiments: Implications for development of personalized regenerative therapeutics for volumetric muscle loss (VML) injuries

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Background

Volumetric muscle loss (VML) is a common clinical sequelae of battlefield injuries to wounded warriors. VML injuries are defined by permanent loss of muscle form and function, and treatment of these injuries is challenging because both the type of injuries and wound locations varies widely [1]. Regenerative medicine is a promising method for the treatment of VML injuries; however, within the literature, there is variability in the *in vivo* models used to study these injuries as well as the methods used to assess functional repair [2-3,4]. *In silico* analysis is a tool that can capture the variability of VML injuries' sizes and locations as well as simulate different experimental conditions.

The goal of this study was to couple a finite element (FE) computational model with *in vivo* studies of muscle contractile responses. The latissimus dorsi (LD) of rodents was selected because it is a large flat muscle on the back that allows for a range of VML injury sizes and locations to be tested. Additionally, its heterogeneous muscle architecture consists of a series of both parallel and pennate fibers creating complex relationships between injury magnitude, location, and functional loss. In short, the LD provides a more biologically-relevant preclinical VML injury, that scales well to the some of the muscles of the face, hand and shoulders of wounded wariors with craniofacial or extremity trauma. Functional assessment of the LD can be tested using an *in situ* setup, which maintains the physiological conditions of the LD and captures the force of both parallel and pennate fibers. We developed an FE model of the rat latissimus dorsi (LD) and used it as a both a predictive tool to focus our *in vivo* studies, as well as to provide new insights into the complex biomechanics of the force deficits that result from distinct VML injuries.

Methods

FE model development. The three-dimensional FE model of an intact rat LD was created based on measurements from dissected LDs of rats and was simplified to have a shape of one rectangle plus one triangle [5]. The LD was modeled as a transversely isotropic, hyperelastic and quasi-incompressible material [6], and the fiber direction was assigned from the origin along the spine to the insertion at the humerus using computational fluid dynamics [7]. Boundary conditions were assigned to replicate *in situ* experimental testing conditions, and maximum muscle activation was set for all simulations. The native LD model was calibrated to fit native experimental data using a sensitivity analysis of the peak isometric stress parameter. Then an injury 11x15 mm was created at various locations, including at the very top, very bottom, mid, and far side, within the muscle model and isometric contractions were simulated.

Experimental studies of LD muscle contraction *in situ*. Using the *in silico* framework as a screening tool, only two injuries were tested experimentally saving valuable time and resources. First an injury in the mid-section (original injury) was tested *in situ* to validate the predictions of the computational model with a mid-range force value [4]. Then an injury near the top of the muscle (high injury) was tested using the *in situ* setup to further confirm the range of model predictions and determine if that injury location would provide the largest decrease from native muscle force production. In the *in situ* method, the LD muscle was minimally dissected and maintained in its native environment with blood supply and nerve innervation. A nerve cuff was placed around the motor nerve and the muscle's distal tendon was attached to the lever arm

of a force transducer. The nerve was then stimulated and the peak isometric forces were measured at a range of stimulation frequencies.

Results

Experimental validation of model. The isometric simulations of five different injuries demonstrated that the location of an injury has a dramatic impact on the LD's total force of contraction. In the model, the intact muscle force production is 6.436 N, and an injured muscle can generate 3.247 – 4.784 N of force, depending on the injury location. The 36% reduction in force of the original injury compared to native LD was validated experimentally. An injury near the top of the muscle generated the largest decrease from native at 50%. The *in situ* experimental testing of the high injury confirmed the model's force prediction. Using the FE model as a predictive tool, we were able to determine the preferred injury location to maximize the decrease between native and injured force production. For the development of novel therapies, an increased difference would help to increase the margin of difference between treatments and better aid development.

Computational biomechanical analysis. The FE model can also be used to better understand the biomechanics of force production and the difference between the original and high injuries. A breakdown of the force production from each component of the LD showed that the original injury triangle generated 3.204 N compared to only 2.269 N from the high injury triangle portion. The increased number of intact fibers present in the original injury likely contributed to the increased force production of the original injury location. Analysis of along-fiber stretch shows that the high injury LD generated a larger along-fiber stretch within the region of intact fibers of the triangle compared to the original injury. This indicates that the fibers of the high injury LD operate on the descending limb of the force-length curve and are unable to generate maximum force. This is an analysis we are not able to experimentally and demonstrates the power of coupling experimental studies with computational tools.

Conclusions

These studies demonstrate that a computational and experimentally coupled framework can be used to optimize the experimentally desired force deficits for any given set of studies over a range of values (3.2 – 6.4 N). As importantly, a corrolate of this would be that for a given individual we could predict the force deficits based on the size and location of that injury. In the end, this approach has the potential not only to improve the efficiency of preclinical evaluation of regenerative therapeutics, but as importantly, it can aid in developing more effective personalized regenerative therapeutics and rehabilitation regimens that take into account symptom-based and patient-specific nature of VML injuries to wounded warriors.

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Learning Objectives

1. In silico models can be used to evaluate potential therapeutics prior to experimental work.
3. Personalized tissue engineered therapeutics can be developed using a multidisciplinary approach.
3. Injury location can have a dramatic effect on the force production of the muscle.

Unique Outdoor Running Activities Captured Using Wearable Sensors in Adult Competitive Runners

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Context: Laboratory gait analyses are frequently used to quantify running mechanics, however treadmill-based analyses cannot effectively mimic outdoor training and racing demands. Wearable sensors offer a means to transcend laboratory settings using lightweight technology capable of measuring biomechanics in natural environments.

Methods: Heel-mounted footpod sensors (RunScribe™, Half Moon Bay, CA) measured individual runners' biomechanics during unique outdoor running demands. Participants completed a week's worth of typical running wearing the sensors and maintained self-reported running logs. Individual runners (4 females, 1 male; 32.8±6.6 years) completed hill sprints (Runner1) and track interval workouts (Runner2), and competed in a 21-kilometer road race (Runner3). Two runners competed in the same 5-K trail race (Runners4 and 5). Step-by-step datasheets from each activity were extracted. Walking events were identified from the flight ratio variable data falling to zero and were eliminated from analysis. The 21-K race was broken down into four sub-sections (5-, 10-, 16-, and 21-K, steps: 19,944), and the 5-K race was broken into thirds (miles 1, 2, and 3; Runner4 steps: 4,476; Runner5 steps: 4,470) using stride length data as a distance guide. Step rate (SR) and step length (SL) were analyzed for all steps in each activity using means with 95% confidence intervals (CIs), and violin plots with overlaid box plots (Figure).

Results: Biomechanical patterns were identified during the individual training and racing situations. SR and SL increased during the uphill sprints of the hill intervals (steps: 576) compared to downhill (steps: 164) across repetitions (SR_{uphill}: 191.7±2.9 steps/min., $SR_{downhill}$: 161.8±0.8 steps/min.; SL_{uphill}: 1.30±0.04 m, $SL_{downhill}$: 0.97±0.05 m), and increased with shorter track distances (SR_{1600}: 182.1±2.2 steps/min., SR_{800}: 186.2±3.2 steps/min., SR_{400}: 191.1±3.6 steps/min., SR_{200}: 200.9±4.1 steps/min.). SL decreased during the second repeat of all track intervals (steps: 4,770). Average SL decreased over the course of the 21-kilometer race (SL_{5K}: 1.24±0.04 m, SL_{10K}: 1.24±0.03 m, SL_{16K}: 1.21±0.03 m, SL_{21K}: 1.15± 0.04 m). During the first and final thirds of the 5-kilometer trail race, average SR was increased (Runner4 – SR_{Mile1}: 173.8±7.9 steps/min., SR_{Mile2}: 172.2±6.5 steps/min., SR_{Mile3}: 174.4±9.0 steps/min.; Runner5 – SR_{Mile1}: 175.9±9.4 steps/min., SR_{Mile2}: 170.9±7.6 steps/min., SR_{Mile3}: 177.0±10.8 steps/min.), and the same trends were found for SL (Runner4 – SL_{Mile1}: 1.15±0.18 m, SL_{Mile2}: 1.05±0.13 m, SL_{Mile3}: 1.13±0.18 m; Runner5 – SL_{Mile1}: 1.17±0.19 m, SL_{Mile2}: 1.13±0.11 m, SL_{Mile3}:1.14±0.17 m).

Conclusions: The wearable sensors identified spatiotemporal patterns in running activities that would not have been feasible using laboratory technology. SL and SR progressively increased across track intervals and at the beginning and end of a trail race, while SL decreased over the course of a 21-K race. These data lend insight into individuals' adaptations to external demands encountered in the field.

TITLE: Soft Tissue Manipulation as a Non-Invasive Intervention in Regenerative Rehabilitation: Visual Monitoring Improves Consistency of Force Application

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Purpose/Hypothesis: Soft tissue mobilization/manipulation (STM) is a non-invasive intervention used to reduce pain, improve scar mobility and promote soft tissue healing, repair and regeneration. Instrument-assisted soft tissue manipulation (IASTM) uses rigid devices. The amount of force delivered during STM manual therapy matters, leading to different biological and clinical outcomes. However, it is unknown whether therapists apply consistent force during STM. The purpose of this study was to investigate the consistency of force application during a common STM technique, with and without visual feedback.

Materials and Methods: Two experienced physical therapists, each with > 25yrs of similar background in IASTM participated. A mechatronic hand-held IASTM force-sensing device measured and graphically displayed the amount of force applied. A linear, cross fiber massage-like stroke pattern was used on a hard padded surface. In sessions without visual monitoring, examiners administered what they subjectively perceived as “high” force. In sessions with visual feedback, examiners applied 15 N, a high clinical force as determined by preliminary testing, while allowed to look as desired at the graphic display. Each examiner conducted 30 trials, 15 sec/trial, with 30 sec rest between trials, during each session. Statistics were calculated using SPSS.24.

Results: Without visual monitoring, a significant mean difference of 25% (5N ± 1.4N; p=0.000) between experienced examiners’ average peak forces was found. With visual monitoring, this difference was minimized to an insignificant level of 3% (0.4 ± .1N). Interclass correlation coefficient of IASTM inter-rater reliability with visual feedback improved (ICC 0.27) from without (ICC 0.0). Differences in examiners’ method of force application and frequency of visual monitoring were observed.

Conclusions: With visual monitoring, the average peak force was more consistent between examiners, with less than a 1N difference found during a STM technique. This is within a clinically acceptable range of ± 5% agreement. Comparatively, more than a 5N difference existed when examiners relied on subjective perception alone, falling only within 25% agreement. This initial study used a smooth inanimate surface with a linear stroke to reduce variability stemming from a living subject and strokes applied in multiple directions. Future studies conducted with novice therapists, using additional STM stroke parameters and patterns, and on humans are needed.

Clinical Relevance: This research suggests visual feedback improves the consistency of STM force application between experienced therapists. This is important since variable STM force application likely leads to inconsistent clinical outcomes. Further research on the use of visual feedback is required to optimize STM outcomes as a conservative treatment option in regenerative rehabilitation.

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A pilot study of autologous, micro-fragmented adipose tissue for the treatment of chronic, refractory shoulder pain in wheelchair users with spinal cord injury

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Background
Wheelchair users with spinal cord injury (SCI) have a high risk of shoulder pain and injury. After conservative treatments fail, surgery is generally the next option. However, surgery is not always effective and restricts independence by requiring hospitalization, power wheelchair use, and dependence upon others. Autologous, biologic treatments are one potential alternative, yet no clinical trials have been conducted to evaluate efficacy in this population; thus, more research is needed to determine their efficacy. The objectives of this pilot study were threefold: to evaluate the 1) safety and 2) potential treatment effect of an autologous, micro-fragmented adipose tissue (MFAT) injection for shoulder pain treatment, and 3) the feasibility of conducting a larger clinical trial. A future trial would be considered feasible if the dropout rate in this pilot study was less than 20% and missing data rate was less than 5%.

Methods
Ten manual wheelchair users with SCI who had chronic shoulder pain due to rotator cuff disease (confirmed via history, physical and ultrasound examinations) and had failed at least 6 months of conservative therapy. With ultrasound-guidance, damaged shoulder structures were treated with a single injection of autologous MFAT. All participants received MFAT injections into their supraspinatus tendons. Other targeted shoulder structures included the biceps tendon and/or sheath, acromioclavicular joint, infraspinatus tendon, and glenohumeral joint. Participants continued their usual activities of daily living, as tolerated, after 24 hours post-injection. Participants began standardized shoulder stretching and strengthening programs 24 hours and 4 weeks post-injection, respectively. Numerical pain rating scales (NRS), Brief Pain Inventory Interference Items (BPI), and the Wheelchair User's Shoulder Pain Index (WUSPI) were collected 1, 2, 3, and 6 months post-injection. Adverse events were monitored.

Results
No adverse events were reported. One participant (10%) dropped out of the study to seek alternative treatments for their shoulder pain. Two data collection points were missed out of the potential 45 (4.4%). Linear mixed-models revealed significant decreases in WUSPI (mean decrease = 49.1±48.1, $p<.001$), BPI (mean decrease = 4.0±2.2, $p<.001$), and NRS (mean decrease = 4.0±2.2, $p<.001$) scores 6 months post-treatment.

Conclusion
Autologous MFAT injections appear to be safe, and may be efficacious for treating chronic shoulder pain in wheelchair users with SCI. Low dropout rates and limited missing data were noted. A larger clinical trial is feasible and necessary to evaluate efficacy. Although dropout and missing data rates were low, these may change with the introduction of an active control. Additional resources may be necessary to maintain low dropout and missing data rates in a larger trial.

3 Cavalier Project: Predicting Functional Deficits after Volumetric Muscle Loss in the Lower Extremity

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University of Virginia Departments of Orthopaedic Surgery, Biomedical Engineering, and Kinesiology

Abstract:

During the recent conflicts in Iraq and Afghanistan the percentage of surviving wounded continued to increase from previous wars due to improved body armor and rapid medical care and transport. However many of the wounded warriors sustained extremity injuries and due to the high energy nature of the wounds there is often a significant component of Volumetric Muscle Loss (VML) which to date has limited treatment options, primarily bracing and physical therapy. While we can identify grossly that VML has occurred and likewise can identify some effect on function, no study has yet been done to see if we can quantify the specific muscle lost and what effect this may have on the remaining muscle in the extremity and how we might target efforts to compensate or restore the lost function. In the civilian world, VML can occur with open fractures, large lacerations, industrial accidents (including blasts and explosions) , and crush injuries. This is planned as a pilot study of 15 patients who have sustained lower extremity VML as the result of an injury and who have a functional deficit. The study involves identifying the specific VML through MRI, ultrasound, zebrascope (laser doppler to study sarcomere function) biodex strength testing and gait lab evaluation. These will be performed at study initiation and 6 months later to evaluate functional improvement.

Desired Outcomes: If we can identify the specific injuries unique to each patient as well as the functional deficit related to these injuries then we can develop a personalized treatment plan to address the specific deficit which should lead to faster and ultimately better recovery from injury. Obtaining pilot data on 15 patients would show the viability of this approach and allow pursuit of further funding primarily through the DOD. The military has been very interested in treatment strategies for VML as a high percentage of wounded soldiers who are medically retired have musculoskeletal injuries and improved treatment would increase the ability of some of the wounded to return to active duty and lessen the permanent impairment of all the injured. As new treatments for VML become available, having a validated approach to evaluation can also help determine the effectiveness of these treatments and perhaps identify focus areas for future research.

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Return to Play Testing in Individuals with ACL-Reconstructed Knees: Does Timing of the Assessment Matter?

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Context: Traditional return to play assessments following anterior cruciate ligament reconstruction (ACLR) identify large muscular deficits at 6 months post-surgery. This is concerning with majority of patients being cleared for sports on time alone. It is unknown if individuals post-ACLR show improved outcomes if assessed later than 6 months post ACLR. The purpose of this study was to examine patient function in individuals stratified by months post-ACLR.
Methods: A total of 293 individuals following ACLR (23.2±10.1 years, 142 Female, 6.4±.9 mo post-ACLR) participated in the study. Participants were stratified based on the timing of their evaluation in months since ACLR: 5-6 mo: n=122, 6-7 mo: n=102, 7-8 mo: n=43, 8-9 mo: n=26. Subjective knee function was assessed through the International Knee Documentation Committee (IKDC) Subjective Form. Mass-normalized maximal voluntary isometric contraction (MVIC) and limb symmetry indexes (LSI) were collected on knee extensor and flexor muscle groups. Non-parametric statistics were run due to violation of the assumption of normality. Measures of subjective and muscular function were compared through Kruskal-Wallis with *post-hoc* partial eta squared values for effect sizes.
Results: There were significant difference between the 5-6 mo. vs 6-7 mo groups (η^2=.04) and the 5-6 mo vs 8-9 mo groups (η^2=.04) for subjective function (*P*=.04). There were significant differences between the 5-6 mo vs 8-9 mo groups (η^2=.07) and the 6-7 mo vs 8-9 mo groups (η^2=.04) for MVIC Extension (*P*=.14). No differences were seen between groups for MVIC for knee extension (*P*=.14) or flexion (*P*=.97) or knee flexor LSI (*P*=.60) (Table 1).
Conclusions: There are significant differences which demonstrate progressively increasing subjective function and knee extension symmetry when tested at later timepoints from surgery. However, the observed values are low suggesting even at 9-months post ACLR patients are demonstrating deficits that may be improving.

Table 1: Between Group Differences: Median (IQR)

Mo. Post-ACLR	5-6	6-7	7-8	8-9	P-Value	Effect Size (η^2)
IKDC	79.7[a,b] (70.1, 88.5)	83.9[a] (74.5,92.0)	79.3 (73.6, 88.8)	89.1[b] (75.8,92.3)	.019[a] .026[b]	.04 [a] .04 [b]
MVIC Extension (Nm/kg)	1.46 (1.16,1.87)	1.60 (1.26, 2.03)	1.59 (1.23,2.07)	1.65 (1.39, 2.05)	-	-
MVIC Flexion (Nm/kg)	.737 (.51, 1.01)	.76 (.59,.98)	.77 (.57, .89)	.66 (.511, 1.11)	-	-
MVIC Extension LSI (%)	60.0[b] (49.8,76.2)	67.9[c] (52.2,79.1)	67.7 (59.2,80.0)	76.7[b,c] (64.0, 90.5)	.002[b] .021[c]	.07 [b] .04 [c]
MVIC Flexion LSI (%)	89.5 (71.0,105.0)	84.2 (68.8,98.1)	88.5 (66.7, 99.2)	84.3 (69.4, 95.8)	-	-

[a] Significant difference between 5- and 6-month groups. [b] Significant difference between 5- and 8-month groups. [c] Significant difference between 6- and 8-month groups.

Quadriceps Oxygen Consumption During Exercise in Patients with ACL-Reconstruction

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PURPOSE:

Patients with ACL reconstructed knees (ACLR) commonly experience persistent muscle weakness. Altered oxygen consumption (OC) during voluntary rehabilitation exercises of the quadriceps may be a contributing factor. The purpose was to compare quadriceps muscle OC during knee extension exercises in patients with ACLR versus healthy controls.

METHODS:

Ten patients with primary, unilateral ACLR (7M/3F, 22.9±3.5y, 170.81 ± 7.93cm, 73.7 ± 15.1kg) and 10 matched controls (7M/3F, 22.9±3.5y, 170.4 ± 10.7cm, 68.86 ± 9.51kg) participated. Each participant completed a single data collection session consisting of 5-second isometric contractions at 25, 50 & 75% of the volitional maximum followed by a 30s maximal isometric knee extension contraction. We continuously recorded measures of oxygenated hemoglobin (O2Hb) on the reconstructed thigh (versus the non-dominant thigh of healthy controls) using three wearable, wireless near-infrared spectroscopy units placed superficial to the vastus medialis, lateralis and rectus femoris muscles. Relative changes in OC were ensemble averaged and plotted for each contraction intensity with associated 90% confidence intervals. Statistically significant differences were defined as portions of the exercise trials where confidence intervals of the O2Hb graph that did not overlap. Effect sizes were calculated for statistical significance.

RESULTS:

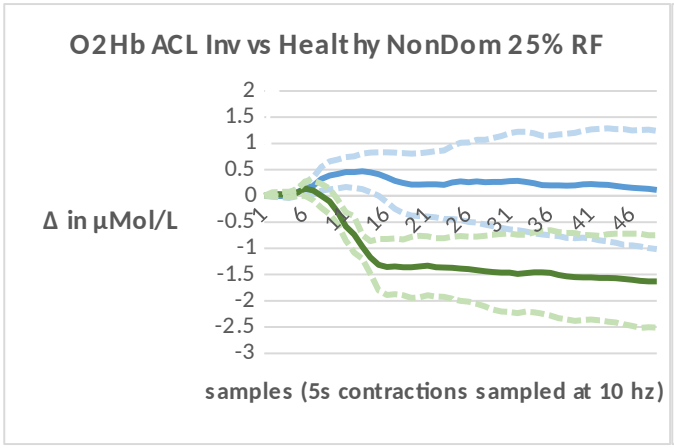
We observed significantly lower relative change in O2Hb for ACLR compared to healthy controls in the rectus femoris at 25% (2.1[1.5-2.7]), 50% (2.8[2.6-2.9], 75% 2.0[1.9-2.2] and for the vastus medialis at 75% (1.5[1.4-1.5] and 100% (2.6[2.5-2.7] (Figure 1). No other statistically significant differences were observed.

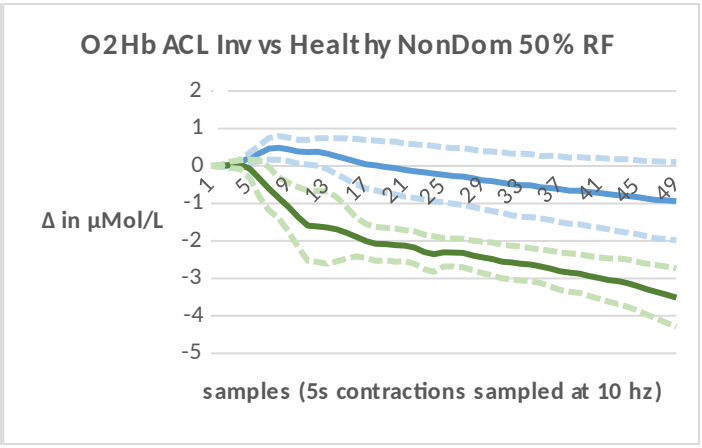
CONCLUSION:

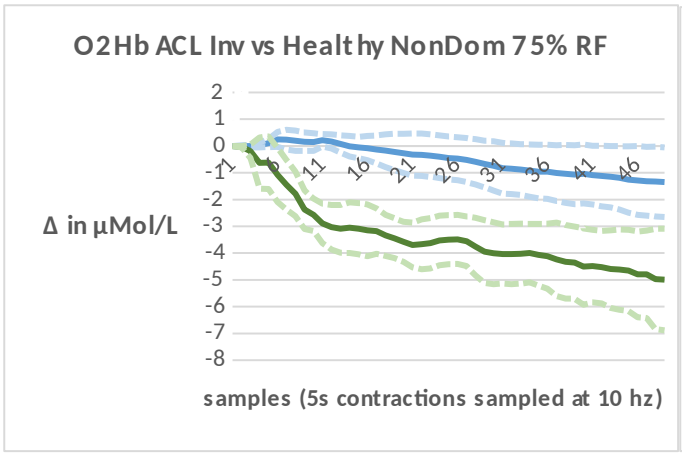
Differences exist in quadriceps muscle perfusion in patients with ACLR during the same isometric exercises versus healthy controls. Individuals who have undergone ACLR have significantly lower consumption of oxygen in their quadriceps when compared to their healthy controls or to the contralateral limb. However not all portions of the quadriceps are affected uniformly across contraction intensities.This is the first study that has investigated the perfusion changes in the quadriceps after ACLR. Patients after ACLR develop persistent quadricep weakness the cause of which are not fully discovered yet. This study provides another piece to the puzzle in understanding the potential mechanism behind the persistent weakness of the quadriceps muscles even after completion of the rigorous rehabilitation programs.

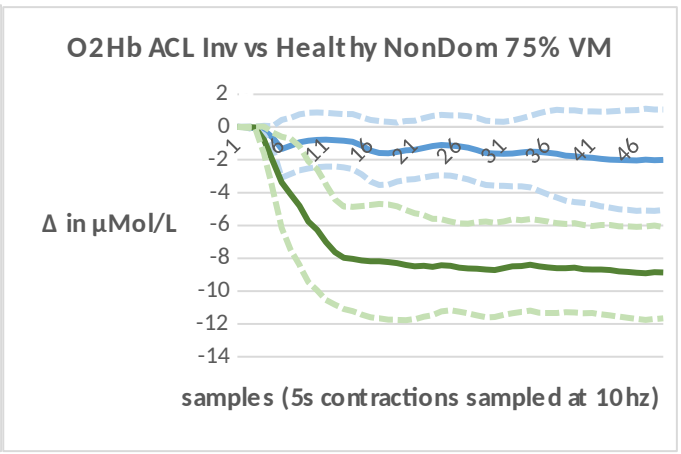
Figure 1: O2Hb in ACLR thighs compared to a healthy matched thighs at different exercise intensities for rectus femoris (RF) and vastus medialis (VM). Blue Solid lines represent ACLR, green lines represent Healthy and dotted lines represent 90% confidence intervals over the course

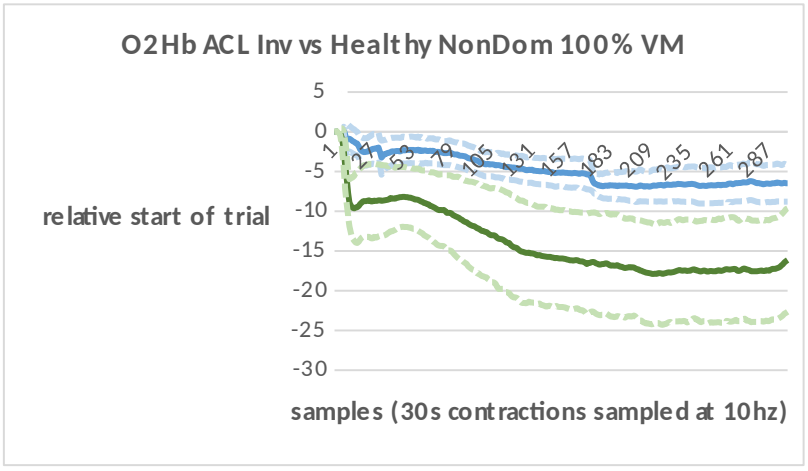
of knee extension isometric contraction trials. Data presented as changes relative to the start of the trial.











Are muscle strength asymmetries associated with decreased self-reported measures, physical function and time since surgery following ACL reconstruction?

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Objectives: Numerous factors can contribute to the achievement of symmetrical muscle function following anterior cruciate ligament (ACL) reconstruction. This study aimed to explore the relationship between thigh muscle strength limb symmetry index (LSI) with single leg hop (SLH) distance LSI, time since surgery and the Knee Osteoarthritis and Injury Score (KOOS) following ACL reconstruction (ACLR).

Methods: Thirty-three participants between ages 20-52 years who underwent ACLR 2-20 years ago with any type of graft were recruited. Concentric quadriceps, eccentric quadriceps and concentric hamstrings peak torque were measured using the isokinetic dynamometer. Participants completed the KOOS questionnaires and physical performance was measured using the single leg hop for distance. Thigh muscle strength LSI were analysed against KOOS4, SLH distance LSI and time since surgery for associations using multiple regression.

Results Significant association (p=<0.001) was found for concentric quadriceps peak torque LSI with SLH distance LSI (p=0.003) and time since surgery (p=0.008). A significant association between eccentric quadriceps peak torque LSI and SLH distance LSI (p=0.022), however, the overall regression for eccentric quadriceps LSI was not significant (p=0.096). All other models of regression and variable associations were not significant.

Conclusion Single leg hop for distance may be used as a predictor for quadriceps strength asymmetry in patients with ACLR more than two years after surgery. Further as time since ACLR increases patients' quadriceps asymmetry becomes more significant indicating quadriceps weakness over time.

Anodal Transcranial Direct Current Stimulation with Eccentric Exercise Improves Neural Excitability & Dynamic Balance in Individuals with Chronic Ankle Instability

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Background *

High recurrence rates following ankle sprains have been attributed to changes in brain function, suggesting current rehabilitation is insufficient. Maladaptive neuroplasticity-related changes in patients with chronic ankle instability (CAI) indicate decreased neural excitability potentially impairing joint stability. Anodal transcranial direct current stimulation (aTDCS) may increase excitability of intracortical neurons, facilitating long-term potentiation-like changes and enhancing motor learning. This may provide an adjuvant therapy for rehabilitation, enhancing the ability of strengthening to induce neural changes. The neuromodulatory interventions may therefore improve function in patients with CAI by correcting potentially maladaptive neuroplasticity.

Objective(s) *
We aimed to determine the effects of eccentric ankle strengthening with aTDCS or sham stimulation on cortical and reflexive excitability and dynamic balance in individuals with CAI.

Design & Methods *
We implemented a randomized controlled trial design. Dependent variables included neural excitability to peroneus longus (PL) via Hoffmann reflex and transcranial magnetic stimulation (Motor Threshold, MT; I_{50}); and postural stability indices (PSI's) from a hop-to-stabilization tested at baseline, week-2, week-4, and week-6. Twenty-two individuals with CAI (IdFAI>10) completed 10 sessions of eccentric ankle evertor strengthening over four weeks with aTDCS (21.8±2.7yrs, 5M/6F) or sham aTDCS (22.3±3.1yrs, 3M/8F) over the motor cortex. The aTDCS group received 1.5mA of cortical stimulation for 18-minutes; the sham group received 1-minute of stimulation. Differences were assessed with group-by-time factorial ANOVA (α=0.05).

Results *
A significant time-by-group interaction was seen for MT ($F=3.401$, $p=0.025$). There was a significant decrease from baseline (aTDCS: 36.92±11.53%; Sham: 36.67±12.74%) within sham (week-2: 27.86±14.69%, p=0.022) then increased (week-4: p=0.022, week-6: p=0.006); in aTDCS (week-6: 32.91±12.33%, p=0.024). Significant time-by-group interaction was seen for I_{50} ($F=5.290$, $p=0.003$). A significant decrease from baseline (aTDCS: 51.97±6.47%; Sham: 51.11±11.27%) was observed at week-2 in sham (45.47±10.62%) then increased (week-4: p=0.019, week-6: p=0.001); and week-6 in aTDCS (47.42±5.63%, p=0.025). H-reflex showed no time-by-group interaction ($F=1.165$, $p=0.331$). PSI's demonstrated a significant time-by-group interaction (F=3.087, p=0.034) indicating decreases from baseline to week-6 in the aTDCS group ($p \leq 0.026$).

Conclusions

These results indicate 4-weeks of aTDCS with eccentric exercise increased cortical excitability and improved balance when compared to sham stimulation, suggesting aTDCS may be an efficacious adjuvant to CAI rehabilitation. These changes may reverse maladaptive neuroplasticity and subsequently improve function, warranting further investigation into aTDCS in joint instability rehabilitation.