PROGRAM

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ABSTRACTS

Regional ABC Sydney

08 December 2020

Macquarie University

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Thank you for supporting Regional ABC Sydney 2020; and a big thank you to Australian New Zealand Society of Biomechanics for their support also! This has certainly been a year like no other, amongst the challenges there have been a lot of opportunities for growth in learning how to get things done i.e., meetings, research, collaborations, in ways we had not previously thought of. But despite our best use of Zoom, Teams, FaceTime, Skype, etc. there is something about face-to-face, in-person interactions that can't be replicated digitally, as such it is great that we have this chance to get together for a boutique conference experience.

We also wish to thank Vicon and iMeasureU for their support of the networking function.

On behalf of the Organising Committee we welcome you to Regional ABC Sydney 2020 hosted at Macquarie University, Sydney.

Organising Committee:

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Program Detail

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Melissa Knothe Tate	Life's Mechanobiological Adaptation Across Length Scales, from Bones to Trees, and Through the Lens of Virtual Power
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First Author	Abstract Title
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Individuals with gluteal tendon repair display similar hip biomechanics to those of a healthy cohort during a sit-to-stand task

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INTRODUCTION

Gluteal-tendon repair is reported to be effective for relieving pain and improving clinical function in patients with gluteal-tendon tears (1). However, biomechanical deficiencies during gait have recently been reported indicating functional abnormalities. The sit-to-stand (STS) task is an important activity of daily living, and is often used to assess functional capacity in clinical populations (2). Performing STS requires a minimum level of muscle strength and force development. Therefore, our aim was to investigate how biomechanical parameters during STS differ between age- and sex-matched participants with and without gluteal-tendon repair.

METHODS

27 participants with a gluteal tendon repair and 29 healthy participants performed the STS task. Data were acquired using the Vicon three-dimensional motion capture system. Outcome measures were task duration, vGRF, rate of force development, trunk, pelvis, and hip joint angles, and sagittal and frontal hip joint moments and powers. Participant characteristics, task completion time, maximums, and minimums throughout the movement cycle were compared between groups using independent t-tests. Between-group differences in continuous angle, moment and power data for the trunk, pelvis, and hip were evaluated using an independent sample t-test with statistical parametric mapping methods.

RESULTS AND DISCUSSION

The GTR patients performed the STS movement over a significantly longer period (1.41s +/- 0. 1.41s +/- 0.40) compared to controls (1.07s +/- 0.24) (mean difference -0.3s;95% CI -0.5 to -0.2; p=0.00). Furthermore, the GTR group demonstrated significantly lower rate of force development over 10 milliseconds (mean difference: 4.78 N/kg/s; 95% CI 0.7 to 8.8; P=0.016). There were no differences in maximal vertical ground reaction force or symmetry. There were no group differences for hip, pelvis or trunk angle over the movement cycle or for maximal or minimal values. Furthermore, there were no significant between-group differences were detected in mass normalised hip joint kinetics (Figure 1). However, there appeared to be substantial between subject variability indication different patient specific movements patterns.

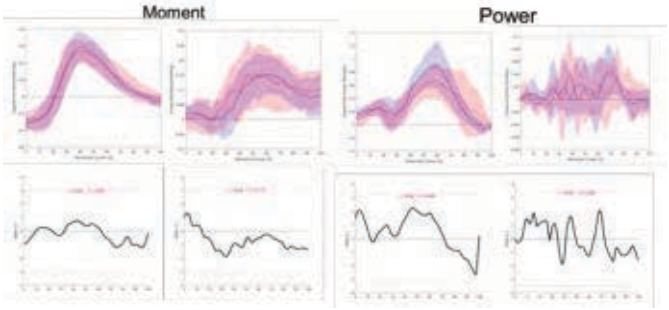

Figure 1 *Means (95% CI) for healthy (blue) and GTR (red) sagittal and frontal hip moments and powers Lower graphs represent corresponding t-values (black - solid) and t-threshold (red - dashed) for SPM analyses.*

CONCLUSIONS

The GTR group overall performed the STS task about 20% slower than healthy controls with a lower rate development. However, there were no differences between the groups for hip-flexion or adduction angles, moments and powers There was substantial between patient variability indicating that different were strategies employed in order to perform this task. While the lack of differences between groups could suggest that GTR helps restore function and corrects the proposed underlying aetiology, it is possible that the STS task was not sufficiently challenging to discriminate between groups. Future work is required to characterise different STS strategies.

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Table 1: Comparisons of STS time and vertical ground reaction force between healthy and GTR groups.

	Control	GTR	Mean Diff (95% CI)
Sit-to-Stand Time (s)	**1.1 +/- 0.2**	**1.4 +/- 0.4**	**-0.3 (-0.5 to -0.2)**
Maximal Vertical GRF (N/kg)	6.0 +/- 0.5	5.9 +/- 0.7	0.1 (-0.2 to 0.5)
Maximal Side-to-Side Vertical GRF Difference (N/kg)	1.0 +/- 0.7	1.5 +/- 1.3	-0.5 (-1.1 to 0.0)
Rate of Force Development -Δ10ms (N/kg/s)	***35.1 +/- 5.7***	***30.3 +/- 8.5***	**4.8 (0.7 to 8.8)**

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ASSOCIATIONS BETWEEN RUNNING KINEMATICS AND KINETICS AND PREVIOUS HAMSTRING STRAIN INJURY IN MALE SUB-ELITE ATHLETES: PRELIMINARY PHD FINDINGS

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INTRODUCTION

Hamstring strain injuries (HSI) typically occur during high speed running.[1] Recurrence rates are high and often subsequent injuries are more severe than the primary injury, resulting in a longer recovery time.[2] Altered hip and trunk kinematics and kinetics have been identified in athletes who have recovered from HSI and this may contribue to re-injury by placing the hamstrings under additional strain during high speed running.[3,4] However the exact nature of this relationship remains uncertain. The purpose of this study was to determine whether there is an association between sprint kinematics and kinetics and previous HSI, in male sub-elite athletes.

METHODS

Twenty male sub-elite athletes between the ages of 18-35 years old who were free from injury at the time of testing, with (n=10) or without (n=10) a history of HSI within the previous 18 months, participated in this cross-sectional study. 3D hip, lumbopelvic and thoracolumbar joint angles and net joint moments data were collected during 15m sub-maximal overground sprints using 16 motion capture cameras (Oqus 700+) and two multichannel Kistler force platforms. Visual 3D was used for data reduction and analysis. Mixed factorial analyses of variance (ANOVA) calculations identified mean differences between groups (history of HSI vs. control) for joint angles ($P<0.05$). Tukey post hoc tests were performed to further explore statistical relationships. Independent t-test were used to ascertain group differences in sprint speed and net joint moments

RESULTS AND DISCUSSION

The only statistically significant between group differences identified for hip, lumbopelvic or thoracolumbar joint angles or moments at any temporal event was a significant angle*event interaction for hip joint angle. However, *post hoc* testing identified no statistical significance but a trend towards moderate effect in the reduction in hip flexion angle in the swing phase exhibited by the history of HSI group compared to the control group. This reduced hip flexion in the swing phase may be due to an effect of speed as the history group sprinted at a moderately slower speed, and speed can alter hip kinematics.[5]

A moderate effect for higher thoracolumbar net extension joint moment during the swing phase in history compared to control group was also observed. Less thoracolumbar compliance has been associated with a more upright running position,[6] however, the lack of between-group difference in trunk strategies could not explain this higher spinal loading.

CONCLUSIONS

Swing phase moderate effect sizes found at the hip joint angle and thoracolumbar extension moment may suggest an underlying difference between groups. Continued data collection may elucidate these differences. This study is ongoing.

ACKNOWLEDGEMENTS

This study was financially supported by General Electric and National Basketball Association Orthopaedics and Sports Medicine collaboration.

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Table 1: Moderate main effects between groups.

Joint angle/moment variable	History (mean(SD))	Control (mean(SD))	Effect size (d)	*95% confidence intervals (p*-value)
Hip joint angle (°) early swing	68.2 ± 12.1	77.2 ± 9.6	0.78	-1.1 to 19.1 (0.08)
Hip joint angle (°) mid swing	60.5 ± 14.2	67.4 ± 7.2	0.60	-3.7 to 17.4 (0.19)
Thoracolumbar extension joint moment swing	0.73 ± 0.47	0.52 ± 0.17	0.59	-0.1 to 0.5 (0.19)
Speed (m/s)	2.7 ± 0.2	2.5 ± 0.2	0.77	0.0 to 0.2 (0.09)

The influence of total knee replacement design on knee flexion kinematics: A prospective randomised clinical trial

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[2] University of Canberra, Canberra
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INTRODUCTION

Modern total knee replacement prostheses are designed to restore healthy kinematics including high flexion. The survivorship of a TKR is dependent on the kinematics in six-degrees-of-freedom (6-DoF). Kneeling is a high flexion, kinematically demanding activity after TKR which is reported as highly desireable by patients (1). Implant choice is guided by implant survival, surgeon preference and kinematic performance (2). However, the debate over which implant to use is still unresolved. The purpose of this randomised clinical trial was to prospectively compare the six-degree-of-freedom kinematics of posterior-stabilised fixed-bearing (PS-FB), cruciate-retaining fixed-bearing (CR-FB) and cruciate-retaining rotating-platform (CR-RP) designs during deep kneeling

METHODS

68 patients were randomised to either a posterior stabilized (PS), cruciate retaining (CR) or rotating platform (RP) design. Sixty-four of these patients completed a minimum 1-year follow-up. Patients completed full-flexion kneeling while being imaged using single-plane fluoroscopy. Six degree of freedom kinematics were generated by registering the femoral and tibial computer-aided design (CAD) models for each of the TKR designs to the fluoroscopic images using bespoke software Orthovis. Linear mixed effects models were used to test the effect of implant design on kinematics throughout the flexion range.

RESULTS AND DISCUSSION

The CR-RP (123 ± 1.9) and PS-FB (125 ± 1.9) designs achieved higher maximal flexion than CR-FB (117 ± 1.8) (p=0.002). There were no design differences for anterior-posterior translation range. However, posterior-stabilised designs were more posterior at each flexion angle compared to both cruciate-retaining designs (Figure 1-top). The CR-RP design displayed more external-femoral rotation at each angle throughout flexion when compared to the other designs (Figure 1-bottom). In contrast, CR-FB were less externally rotated at 130° of flexion compared to PS-FB and CR-RP. The total rotation range between 90° to maximal flexion did not differ between designs.

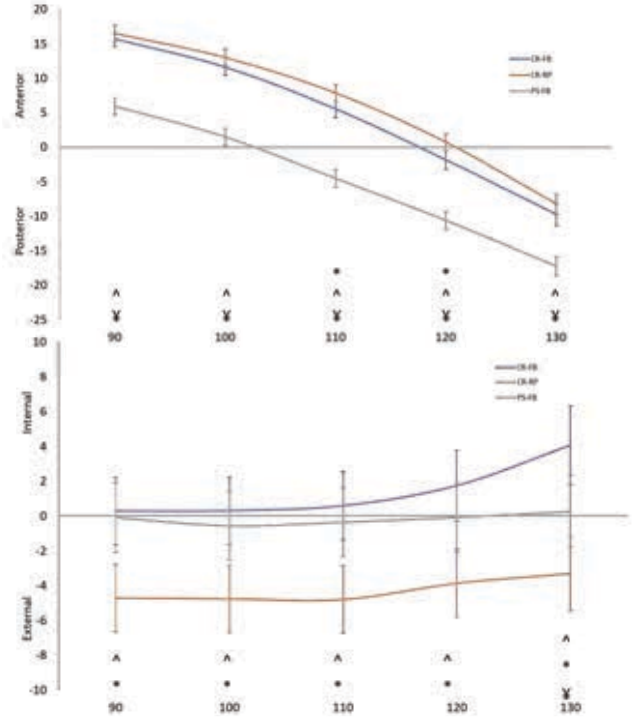

Figure 1 *Output from the mixed effect linear models for kneeling kinematics from 90° to 130°of flexion for top) anterior posterior position (mm); and bottom) internal external rotation (°)*

CONCLUSIONS

The increased maximal flexion found in the PS-FB and CR-RP designs were likely achieved in different ways. The PS-FB design uses a cam-post to hold the femur more posteriorly preventing posterior impingement. The external rotation within the CR-RP design was surprising and has not previously been reported. It is likely due to the polyethylene bearing being decoupled from flexion. The findings of this study provide insights into the function of knee replacement designs in the context during deep kneeling. They also provide clinicians with a more kinematically informed choice for implant selection and provide better management of patient's functional expectations.

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THE LONG-TERM EFFECT OF FLEXIBLE SHOES ON CHILDREN'S FOOT STRENGTH AND FUNCTIONAL PERFORMANCE

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INTRODUCTION

Barefoot childhood results in better foot strength and performance outcomes than shod childhood [1-2]. Laboratory studies have established standard school shoes restrict foot motion and alter foot kinematics [3].

No long-term studies into the effect of different children's shoe design on foot and low limb strength development exist to date. The aim of this prospective, longitudinal, randomised controlled trial was to establish whether a child's foot strength and low limb functional performance improves with flexible shoe use.

METHODS

Seventy 9-12 year old healthy children were recruited from a Sydney School and randomly assigned control or experimental shoes. Exclusion criteria were: < 3-month-old foot/ankle injury, orthotic use, general ligament laxity, > 4 hours/week of gymnastics/dance, BMI > 95th percentile.

The control group wore standard school shoes. The experimental group wore shoes previously established in pilot laboratory studies to have minimal foot motion restriction. The shoes were worn for non-sports days, during school hours (±18hrs/wk) for 9 months. Pre- and post-intervention measures were taken.

Primary outcome measures were cross-sectional areas (CSA) of Abductor Hallucis (AH) and Flexor Digitorum Brevis (FDB) muscles, and toe flexor strength (TFS) of hallux and lesser toes separately. Single leg balance (SLB), Y-balance test (YBT) and standing long jump (SLJ) were secondary outcome measures.

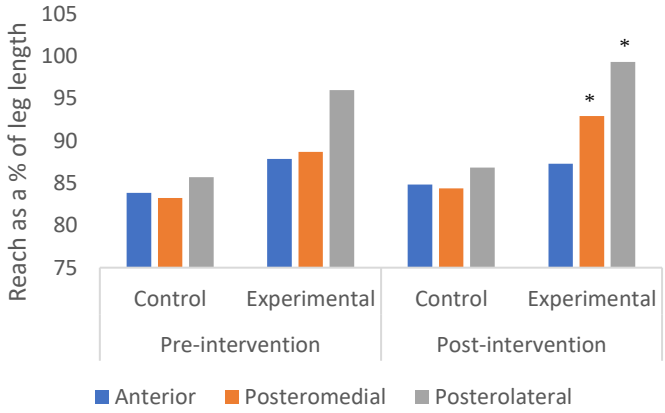
RESULTS AND DISCUSSION

Flexible shoes resulted in moderate but not significant improvement in CSA (AH η^2_p = .04, FDB η^2_p = .05) and TFS (hallux η^2_p = .05, lesser toes η^2_p = .04) differences.

The experimental group had significantly greater reach during the YBT in the postero-medial (P = .04, η^2_p = .07) and postero-lateral (P = .01, η^2_p = .10) directions (Table 1). YBT performance (anterior, postero-medial and postero-lateral) was positively correlated with hallux TFS (R = .29, .27 and .33 respectively), lesser toes TFS (R = .28, .35 and .38 respectively) and SLJ (R = .30, .39 and .57 respectively). CSA of FDB was positively correlated with SLJ (R = .34) and SLB (R = .42).

This was the first study to establish long-term effects of different shoe designs on children's foot development and low limb functional performance. There is strong potential for flexible shoes to positively influence the growth and development of children's feet, with added performance benefits to balance and lower limb power. Children would benefit from a growing awareness of the impact of shoe design amongst parents, health professionals and children's shoe manufacturers.

Table 1: Y-Balance Test



*P < 0.05

CONCLUSIONS

Wearing flexible shoes long-term improves balance in children. Toe flexor strength in children is correlated with better balance and standing long jump performance.

ACKNOWLEDGEMENTS

Prof Stephen Lord (NEURa) for assistance with balance tests.

Oxford Falls Grammar for supporting the study at the school.

The children and parents for partaking in this study.

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OPTIMISATION OF RUNNING GAIT COORDINATION TESTING FOR APPLICATION TO FIELD-BASED ATHLETE MONITORING

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INTRODUCTION

Assessing stride interval long-range correlations can be used to monitor motor control in running athletes during heaving training [1]. This can identify athletes at risk of overtraining during planned heavy training prescriptions designed to promote training adaptation (functional overreaching) [1]. However, this method has relied on wearable devices with high sampling rates (2000 Hz) that are difficult to obtain in common commercial devices. Additionally, identifying the minimal stride number needed to monitor motor control is important to inform field testing, which must be time-efficient. Therefore, the aim of this study was to investigate the effect of lowering sampling rate and stride number on the ability to detect changes in athlete motor control during heavy training.

METHODS

Data were obtained from a past study [1] assessing stride intervals before and after 2 weeks of heavy training, and after a 10-day taper. The heavy training was designed to induce functional overreaching. Times series of 300 strides were originally collected at 2000 Hz using in-shoe force-sensitive resistors (Trigno™, Delsys Inc, Natick, MA). Treadmill tests were performed at speeds equivalent to 65% and 85% maximum heart rate (HR_{max}).

The force-sensitive resistor data were re-sampled at 1600, 1200, 800, 400, and 100 Hz using custom MATLAB code (R2018a, MathWorks, Natick, MA). The original 300 stride time series were reformatted to 200 and 100 strides. This produced six time series sampling rate iterations and three time series length iterations for secondary analysis.

Long-range correlations (α) in each time series were determined using detrended fluctuation analysis [2]. Strides with long-range correlations across all times produce an α value of 1.0, while stride-to-stride fluctuations that are unpredictable are associated with α values closer to 0.5. Linear mixed models were used to determine the effect of sampling rate and series length on α values across the three assessment timepoints (baseline, heavy and taper) and two speeds.

RESULTS AND DISCUSSION

There were training*speed interactions ($p<0.03$) whereby α values at the 65% HR_{max} speed were decreased after heavy and remained low after taper training (Figure 1). This indicates that stride interval monitoring detects changes in running gait coordination during functional overreaching and these changes are best detected at lower (65% HR_{max}) compared to higher intensity (85% HR_{max}). These coordination changes may have implications for injury risk.

Sampling rates between 400-2000 Hz resulted in equivalent α values but α values were reduced at 100 Hz (Figure 1). Series lengths of 200 and 300 strides produced equivalent α values but the 100-stride series length reduced α values (Figure 1). Lower sampling rates and shorter series lengths should facilitate field-based applications of these testing methods.

CONCLUSIONS

Wearable devices monitoring running stride coordination can use low sampling rates of 400 but not 100 Hz and testing should collect a minimum of 200 strides. These technical specifications and testing requirements appear feasible for field-based athlete monitoring applications.

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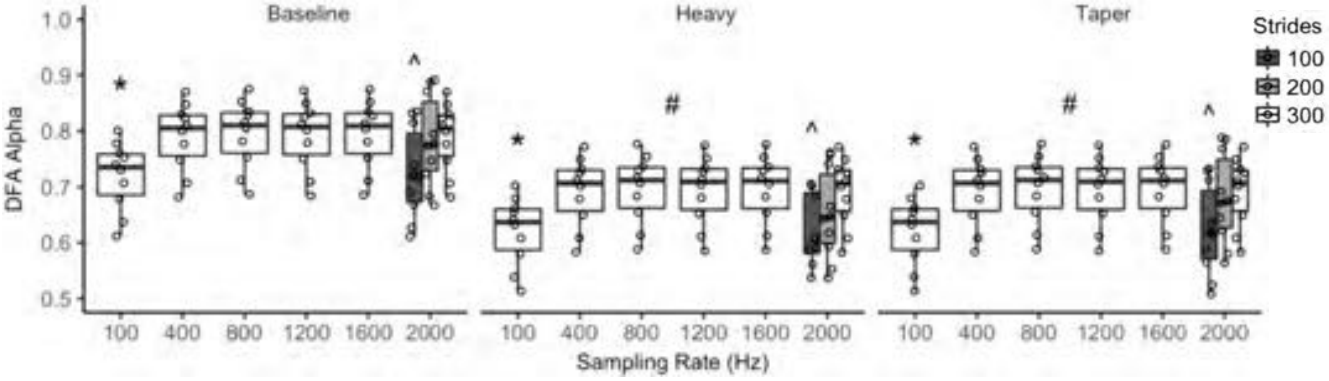

Figure 1 *Sampling rate effects on detrended fluctuation analysis (DFA) of running strides captured at 65% maximum heart rate during overreaching. Symbols indicate difference ($p<0.05$) from baseline (#), 400-2000 Hz (*) and 200-300 strides (^).*

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THE EFFECT OF GAIT RETRAINING ON VERTICAL LOADING RATES IN DISTANCE RUNNERS: A SYSTEMATIC REVIEW AND META-ANALYSIS

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INTRODUCTION

Numerous researchers and clinicians have attempted to retrain running gait for injury treatment [1,2] or prevention [3], based on theoretical links between running biomechanics and running-related injury. There is emerging evidence that runners can successfully modify their running biomechanics using various gait retraining feedback strategies [4-5]. However, a comprehensive review which summarises the effectiveness of gait retraining strategies is lacking. This review aims to synthesise evidence from randomised controlled trials (RCTs) that investigated the effect of gait retraining on running kinematics, kinetics, performance, pain, and injury in distance runners. The findings from the review will inform clinicians and researchers about the most effective gait retraining strategies that may be translated into clinical practice and future research studies. This report is limited to the findings related to impact variables.

METHODS

We searched MEDLINE, EMBASE, CINAHL, Web of Science, SPORTDiscus and PEDro from inception to April 2020 for RCTs that investigated the effects of running gait retraining compared to no intervention, usual training, placebo, or standard care, and reporting biomechanical, physiological, performance, or clinical outcomes. Gait retraining interventions were categorised into step-rate, non-rearfoot footstrike, impact, and technical subgroups. We conducted meta-analysis where appropriate, with standardised mean differences (SMD). SMDs were described as trivial (<0.20), small (0.20–0.59), moderate (0.60–1.19), large (1.20–1.99), and very large (≥2.00). The overall quality of the evidence synthesis was rated high, moderate, low or very low using the Grading of Recommendations Assessment, Development and Evaluation system.

RESULTS AND DISCUSSION

This review included 18 studies from 15 independent cohorts involving 683 participants. We found moderate-quality evidence of a small, significant decrease in average vertical loading rate (AVLR) in response to step rate-based retraining (SMD: -0.54 ±0.46), and low-quality evidence of a moderate, significant decrease in instantaneous vertical loading rate (IVLR) following impact gait retraining (SMD: -1.01 ±0.49), compared to control conditions (Figure 1). Additionally, we found very low and low-quality evidence of a non-significant decrease in AVLR in response to impact (SMD: -0.99 ±1.17) and technical-based retraining (SMD: -0.55 ±0.79), respectively. The observed reductions in AVLR and IVLR are likely to be clinically meaningful because elevated vertical loading rates are linked to lower limb stress-fractures [6-7]. These reductions occurred after step rate- and impact-based retraining methods. The effect of technical-based retraining on AVLR and IVLR was less clear, and no data were available for non-rearfoot footstrike retraining.

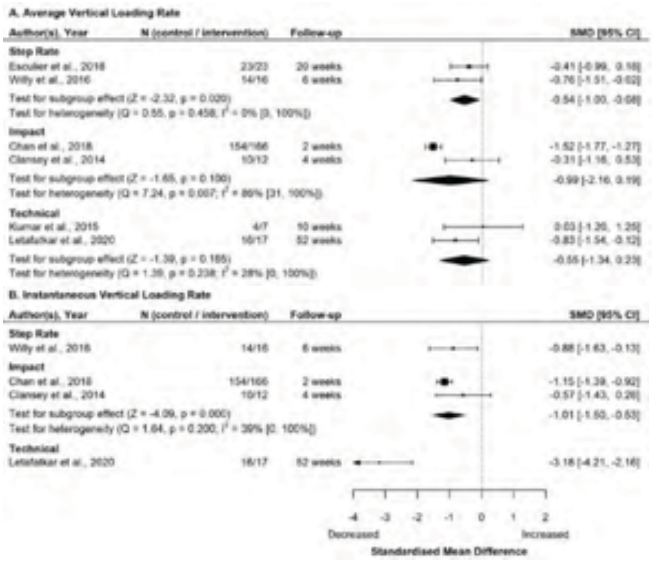

Figure 1 *Forrest plot detailing standardised mean differences (SMD) for A) Average vertical loading rate and B) Instantaneous vertical loading rate when comparing runners who received gait retraining to controls.*

CONCLUSIONS

Step rate and impact-based gait retraining reduce vertical loading rates in distance runners. Long-term research is needed to determine possible injury prevention benefits of lowering vertical loading rates in runners and whether gait retraining can improve outcomes in injured runners.

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LARGER UPPER AIRWAY DILATORY MOVEMENT DURING INSPIRATION REFLECTS LARGER NEURAL DRIVE TO THE GENIOGLOSSUS

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INTRODUCTION

Upper airway dilatory movement is critical to maintain pharyngeal airway patency during inspiration. Previous studies using tagged magnetic resonance imaging (tMRI) have shown that the posterior region of the tongue moves anteriorly during inspiration [1], and movements differ between people with and without obstructive sleep apnoea (OSA) [2, 3].

Dilatory movement measured with tMRI is thought to be the biomechanical result of neural drive to the genioglossus, the largest dilatory muscle of the upper airway, but knowledge of the relationship between neural drive and muscle mechanics is incomplete. Thus, this study aimed to examine the relationship between inspiratory tongue movement, as measured by tMRI, and genioglossus neural drive, as quantified by electromyography (EMG), and investigate how this is related to OSA pathophysiology. We hypothesised that muscle activation would peak before peak airway dilation and that greater dilatory movement would be associated with greater genioglossus EMG activity since upper airway muscle EMG has been previously related with task requirements [4].

METHODS

The study was approved by the South Eastern Sydney Local Health District Human Research Ethics Committee (HREC/13/POWH/745). Forty-two participants (10 women, aged 20–68 years) were studied. The severity of OSA was measured by the apnoea hypopnoea index (AHI), which is the number of apnoeas and hypopnoeas per hour of sleep. Eight participants had no OSA [AHI < 5 events h^{-1}], 12 had untreated mild OSA (5 < AHI ≤ 15 events h^{-1}), 11 had untreated moderate OSA (15 < AHI ≤ 30 events h^{-1}) and 11 had untreated severe OSA (AHI > 30 events h^{-1}).

tMRI and EMG measurements were obtained for the anterior and posterior regions of the horizontal and oblique neuromuscular compartments of genioglossus during nasal breathing in the supine position awake. These regions are innervated by different branches of the hypoglossal nerve and may function independently. Airway dilatory movement was quantified over 3 inspirations as the genioglossus anterior-posterior movement using harmonic phase methods. Neural drive was measured as the phasic EMG, peak EMG, and inspiratory EMG activity during inspiration and was normalised to a maximum voluntary contraction (tongue protrusion) over 54±33 [14-195] breaths. Timing of peak EMG and maximal dilatory movement were measured relative to the respiratory cycle.

RESULTS AND DISCUSSION

tMRI and EMG measurement were obtained for 115 neuromuscular compartments out of 168 (i.e 4 compartments for every 42 participants, 68%). Across all these compartments and after statistically accounting for the fact that several measurements were made within the same participants, three main results were observed.

First, peak EMG occurred prior to maximal dilatory movement (16.8 ± 5.5% vs 21.4 ± 13.4% of the respiratory cycle, general linear model, n = 115, P= 0.02).

Second, for a given neuromuscular compartment, larger dilatory movement was associated with larger inspiratory EMG activity (partial Pearson, n=112, r=-0.19, P=0.04), but not with peak and inspiratory EMG (P= 0.07, and P= 0.24).

Third, when also controlling for age and BMI, a higher AHI was associated with larger dilatory movement (n=110, partial Pearson, r=-0.24, P= 0.01), but not with increased EMG (phasic: P= 0.40, peak: P= 0.66, inspiration: P= 0.94).

CONCLUSIONS

These results show that larger dilatory movement during inspiration reflects increased drive to genioglossus. This suggests that the biomechanical function of the tongue in OSA patients remains closely linked to the drive to the muscle. However, while dilatory movement appears to be increased with increasing OSA severity, this was not the case for EMG. This suggests that airway patency is adequatley preserved during wakefulness in people with OSA despite relatively lower neural drive in people with severe OSA.

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Regional ABC Sydney
08 December 2020

IMAGING PIPELINE FOR MAGNETIC RESONANCE ELASTOGRAPHY RECONSTRUCTION OF ANISOTROPIC TISSUE

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INTRODUCTION

Magnetic resonance elastography (MRE) provides a non-invasive method for quantifying changes in the mechanical properties of soft tissues. The implementaion of MRE commonly used in clinical practice assumes that soft tissues are isotropic. This limits the abitlity of these methods to capture chages in anisotropic tissues, such as muscles.

Previous methods for anisotropic reconstruction have introduced bias into their estimates, by either relying on an assumed fibre direction [1], or selectively fitering the MRE wave data [2]. The reconstruction technique used in this study prevents this by including the local fibre orientation to estimate the stiffness at each image voxel.

This study aimed to validate the proposed reconstruction technique against *in silico and ex vivo* phantoms, and demonstrate its application with image data of the human calf muscles in different states of stretch.

METHODS

To validate the estimated shear moduli, the reconstruction was performed with simulated wave data corresponding to a range of material properties, fibre structure, and additive noise.

To test the method with real data, *ex vivo* samples of bovine muscles were imaged in a 3T MRI scanner (Philips Ingenia CX). To assess the accuracy of the estimate, eccentric rheometry was also used to estimate the muscle stiffness.

Finally, six healthy controls underwent (aged 49±13 years; weight 88±17 kg; height 170±9 cm) underwent imaging of their lower right leg. Images were collected while the participant's ankle was in a neutral position. Scans were repeated with the ankle maximally plantarflex and thendorsiflexed, to passively stretch the anterior (tibialis anterior, TA) or posterior (soleus, SOL; medial gastrocnemius, MG) muscles, respectively.

The same imaging pipeline was used for the *ex vivo* and *in vivo* data. MRE: the transducer coil excited the tissue, and the sinusoidal displacements were sampled at multiple time dynamics. Diffusion weighted imaging (DWI): 33 gradient directions collected, with the same FOV and voxel size as the MRE images. mDIXON images were acquired at a higher in plane resolution, but same slice thickness to provide an anatomical reference image for registration.

To correct for small distortions in the DWI, an affine transform was applied to align the DWI with the MRE. Muscle fibre tracts were then estimated from the aligned DWI images. The fibre tracts were used to provide a voxel wise vector map of the fibre orientation for the transversely isotropic reconstruction.

RESULTS AND DISCUSSION

The new method robustly extracted the transverse shear moduli from the *in silico* phantom. Minimal bias was introduced by noise in the fibre orientation or wave data, and the method produced quantitatively accurate estimates of all moduli.

There was greater variation in the estimated shear moduli for the *ex/in vivo* data sets, as blood vessels and connective tissues violate the assumption of local homogeneity.

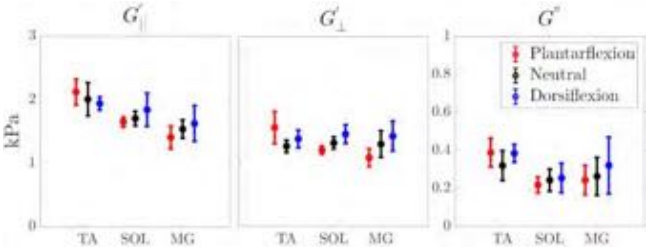

Figure 1 *Mean stiffness and 95% confidence intervals for the estimated group stiffness of the TA, SOL and MG, in different stretched states. From left to right, plots show the parallel shear storage (G'), perpendicular shear storage, and the shear loss moduli (G'').*

Fig. 1 shows the group data highlighting the change in muscle stiffness with ankle position. On average, G' was greater in the TA while plantarflexed compared to dorsiflexed, whereas the SOL and MG G' was greater in dorsiflexion, consistent with expected muscle stretch.

CONCLUSIONS

In silico testing showed the anisotropic reconstruction method can reliably reproduce the shear moduli with realistic levels of noise in the MRE and DWI data. This imaging pipeline was applied to imaging data of the human lower leg, demonstrating it could detect changes in stiffness due to stretch. This indicates that this method can detect changes in anisotropic tissue mechanics.

ACKNOWLEDGEMENTS

Supported by a Disocivery grant from the ARC (DP160100061). L.E.B. is supported by senior research fellowships from the NHMRC (APP1077934, APP1172988).

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Regional ABC Sydney
08 December 2020

New insights into the organisation and role of elastic fibres in the intervertebral disc

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INTRODUCTION

Understanding the ultrastructural organization and mechanical properties of elastic fibers in the disc is pivotal to identify their clinical relevance. Light microscopic studies using histological methods and mechanical measurements using enzymatic digestions may enhance our understanding of the contribution of elastic fibers to the structural integrity of the disc. However, these approaches are not able to reveal the structural complexity of elastic fibers at the nano-scale or to measure their intrinsic mechanical properties [1].

We have developed a new technique to eliminate all non-elastic components from the disc tissue to, for the first time, visualize the ultrastructure of elastic fibers in different regions of the disc and quantify their organisation and mechanical properties, in-situ.

METHODS

The cost-effective and efficient method that we developed was based on alkali digestion of disc tissue with the simultaneous application of sonic waves to eliminate the extracellular matrix. Tissue samples (thickness 50µm – 1 mm) were harvested from different disc regions and placed in NaOH solution (1 M) while sonicated. Collagen fibres were removed using a post-heating process (at 70^{o}C for 20 min in water) with Orcein staining confirming the efficacy of the technique.

Micro-mechanical loading was applied to both digested and undigested tissue in shear and tension directions to identify the mechanical role of elastic fibres.

RESULTS AND DISCUSSION

Our results revealed that elastic fibres are well-organised across the disc creating a continuous network with orthotropic characteristics (Figure 1). Elastic fibres form a network across the nucleus pulposus (NP) consisting of straight and thick (890 nm) parallel fibres that were interconnected by wavy fine fibres (215 nm).

Further analysis identified a dense elastic fibre network at the interface of the NP and annulus fibrosus (AF), a region known as the transition zone (TZ), consisting of major thick elastic fibres (> 1000 nm) that were interconnected with delicate (< 200 nm) elastic fibres.

Within the AF lamellar space (LAM), a loose network of elastic fibres was observed consisting of almost parallel thick fibres (300-500 nm) and fine interconnecting fibres of less than 300 nm. Visualization of the inter-lamellar matrix (ILM) under high magnification revealed a dense and complex network of elastic fibres, including thick (2000 nm) and thin (100 nm) elastic fibres. Similar to the ILM, elastic fibres within the partition boundaries (PB) formed a complex and dense network comprised of thick (1000-2000 nm) and thin (100 nm) fibres [2, 3].

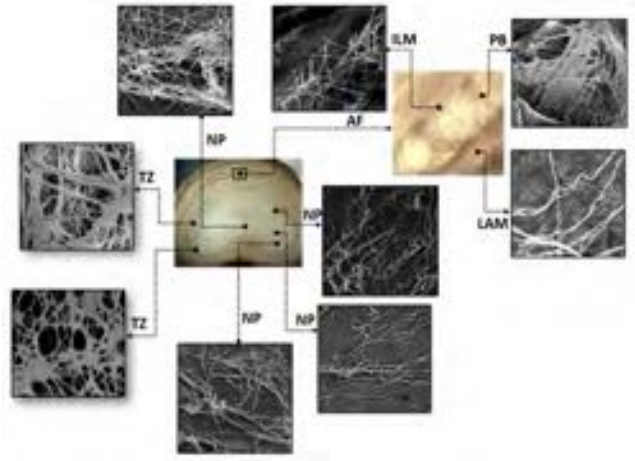

Figure 1 *The ultrastructural organization of elastic* fibres *in the disc. The AF, NP, TZ, ILM, PB, and LAM stand for annulus, nucleus, transition zone, Inter-lamellar matrix, partition boundaries, and lamella, respectively.*

We found a strain-rate dependent response for the elastic fibres in the ILM during dynamic loading, particularly for phase angle, normalized linear, and average loading stiffness. The elastic fibres in the ILM demonstrated a significantly higher capacity for energy absorption at slow compared to medium and fast strain rates as well as in shear compared to tension loading. In addition, when tested to failure, a significantly higher normalized failure force was observed in tension compared to the shear direction of loading. The well-organized elastic fibres that create a highly cross-linked and orthotropic network, significantly contribute to both nonlinear elastic and failure mechanical properties of the ILM [4].

CONCLUSIONS

A detailed examination of the elastic network led to new understanding of the disc structure and function. The disc can be viewed as a modular structure organized into compartments of collagen bundles enclosed by an elastic sheath. These fundamental structural findings contribute to the fabrication of more realistic tissue-engineered disc models.

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Regional ABC Sydney
08 December 2020

Viscoelasticity Investigation of 3D Printed Neural Cell Containing GelMA Hydrogels by Confocal Brillouin Microscopy

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INTRODUCTION

Biomechanical properties of the extracellular matrix (ECM) play a significant role in regulation of central nervous system (CNS) cell behaviours after spinal cord injury (SCI) [1]. Recently, Brillouin microscopy, a non-destructive, label- and contact-free method has been applied successfully to probe mechanical properties of cells and tissues on a microscopic scale [2]. In this work, the viscoalastic behaviour of 3D printed cellular and acellular GelMA hydrogels with varoius solid fractions was charactized using Brillouin microscopy.

METHODS

Different GeLMA hydrogel solid fractions (polymer concentration) of 5%, 10% and 15% (w/v) were prepared with mixing GeLMA powder and LAP photoinitiator, followed by mixing with cells at a ratio 10:1. GeLMA bioprinting was performed using a BIO X 3D Bioprinter and rapidly crosslinked by UV light irradiation to maintain the bioprinted desired structure. The live/dead assays was performed to analyse the viability of the NG 108-15 neuronal cell line (Mouse neuroblastoma x Rat glioma hybrid) in hydrogels over time.

The Brillouin microscopy system consisted of a single-frequency solid-state laser (Torus by Laser Quantum, 660 nm), a confocal microscope, a 3D scanning microscopy stage and a tandem Fabry-Perot interferometer (TFP1, Table Stable Ltd). The spectrometer resolution is approximately 70 MHz and the spectra extinction ratio is above 10^{10} [3].

RESULTS AND DISCUSSION

Brillouin microscopy provides a viable alternative to rheology for measurement of material elasticity and viscosity, allowing determination of the complex longitudinal modulus in the GHz frequency range [2]. The viscoelasticity of cellular and acellular GelMA hydrogels was investigated, as shown in Fig 1. The Brillouin frequency shifts based on the averaged Stokes and anti-Stokes peaks were 6.2245 ± 0.0027 GHz, 6.3868 ± 0.003 GHz and 6.5366 ± 0.0032 GHz for the hydrogel concentrations of 5, 10 and 15% (w/v), respectively. A positive correlation between the Brillouin frequency shift/linewidth and the hydrogel solid fraction was observed, as depicted in Fig 1(a). Furthermore, to investigate effect of encapsulation of cells in a hydrogel, the stiffness of cellular GelMA hydrogels and acellular hydrogels was compared. Before the Brillouin measurements, live/dead viability assay was performed and confirmed the high viability of cells in 3D printed GelMA hydrogels with 5% (w/v) concentration. Then, the Brillouin measurement results obtained from lateral line scans across the gel containing cells, confirmed the lower stiffness (~6.20 GHz) of cellular GelMA hydrogels compared with acellular hydrogels (~6.37 GHz), as shown in Fig. 1(b). It seems that the cells may be remodelling the matrix as they do *in vivo*.

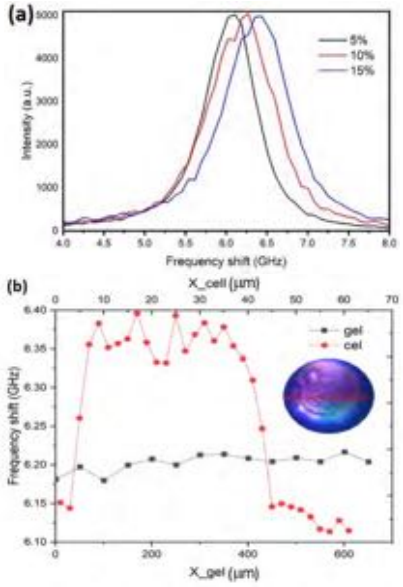

Figure 1. (a) Brillouin spectra of acellular GelMA hydrogels with 5, 10, and 15% (w/v) concentrations. (b) Lateral scanning of cellular (red line) and acellular (black line) GelMA hydrogels.

CONCLUSIONS

In conclusion, we have demonstrated that Brillouin microscopy can be used to map the mechanical properties of hydrogel constructs immersed in a liquid solution. In this study, Brillouin microscopy successfully resolved differences between 5, 10 and 15% (w/v) solid fraction gels, as well as gels containing neural cells. Ultimately, this finding may lead to the development of future therapies for neural regeneration after SCI.

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Regional ABC Sydney
08 December 2020

Mechanomics Engineering of Stem Cell Structure and Function –

Multidisciplinary Therapeutic Approaches in Regenerative Medicine

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INTRODUCTION

Mechanomics describes the regulatory role of mechanics in biological processes including stem cell lineage commitment, which itself underpins the emergence of structure-function relationships across length scales, from sub-cell to cell to tissue to organ and organism [1]. Our working hypothesis is that

- mechanical signals in the SCs' environment modulate both the building of raw materials (transcription of structural proteins that make up cell and extracellular matrix structure) and
- the distribution of these materials in space (*e.g.* geometry, architecture at different length scales) resulting in emergent tissue structure and function.

Multidisciplinary approaches are needed

- to probe mechanisms underpinning emergence of tissue structure and function from single cells, and
- to apply this knowledge for the design of engineered tissues/biomaterials, and devices.

This study uses multidisciplinary tools to elucidate underpinning mechanisms of stem cell mechanoadaptation including

- chemistry, for spatiotemporal control of drug delivery;
- mechanics, for control delivery of mechanical cues; and
- physics for imaging and quantification of murine embryonic SCs (C3H10T1/2) mechanoadaptation during adhesion, ingression, and other function in tissue genesis.

METHODS

Polymeric drug carriers are developed using a one pot miniemulsion polymerisation technique to encapsulate cytoskeleton (actin & tubulin) or ion channel-modulating drugs and to control release drugs via reduction of disulphide bonds by high intracellular glutathione concentration. The ProFlow chamber (Harvard apparatus) [2], [3] is used to deliver controlled volume and shape changing stresses, via seeding density and fluid flow. Changes in shape, volume and cytoskeleton spatial distribution are visualised via confocal live imaging and quantified using custom-built MATLAB code.

RESULTS AND DISCUSSION

Paclitaxel exposure reduces the expression of tubulin is in SCs. Pilot data show significant, dose-dependent decreases in proliferation (Fig. 2A) as well as increases in volume and surface area (Fig. 2B&C).

These changes may provide a means for SCs to adapt to the reduced structure and functional capacity of the tubulin (compression bearing) cytoskeleton. We hypothesise that the SCs compensate for loss in tubulin through increases in actin polymerisation (tension bearing cytoskeleton, Fig. 1).

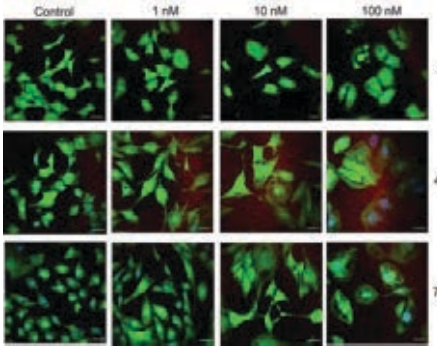

Figure 1 *Confocal microscope images depicting the spatiotemporal, dose-dependent changes in SC shape and volume upon paclitaxel (PAX) treatment over 3 days. Scale bar represents 50 µm.*

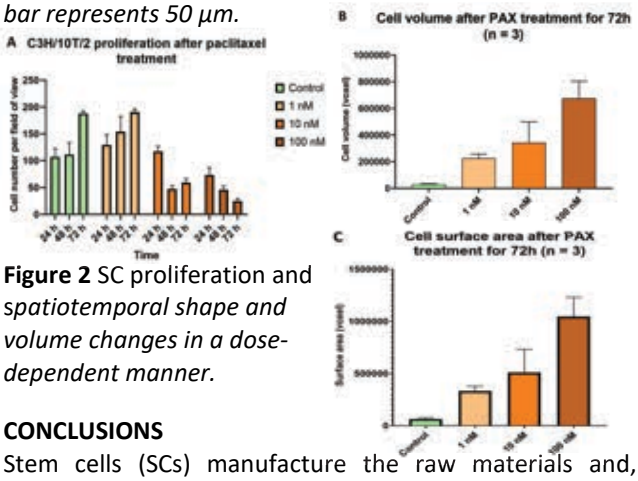

Figure 2 SC proliferation and *spatiotemporal shape and volume changes in a dose-dependent manner.*

CONCLUSIONS

Stem cells (SCs) manufacture the raw materials and, together with mechanical and biochemical cues, modulate their spatial organisation for tissue in development and healing. The multidisciplinary approaches of mechanomics engineering enable probing of SCs' mechanoadaptation, as well as linking mechanical and chemical modulation of SC structure (shape) and function (fate).

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Regional ABC Sydney
08 December 2020

Life's Mechanobiological Adaptation Across Length Scales, from Bones to Trees, and Through the Lens of Virtual Power

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Mechanics, *i.e.* motion and forces producing displacements and resulting in work (force on an object exerted over a distance, Fx) , is central to life, for motile and sessile organisms alike. Biomechanics and mechanobiology begin to address the interplay between mechanics, biology, and physiology of organisms from the animal and plant kingdoms. Yet new paradigms are needed to address the mechanics of advanced functional materials manufactured by living organisms, including biomaterials and smart or advanced materials engineered and manufactured by humans.

"Brainless" cells have the capacity not only to manufacture extracellular matrix, *e.g.* from biofilms to cartilage to gum tree bark, but also to serve as architects of structures with advanced properties. Darwin described *intelligence* in terms of the efficiency of species in "doing the things they need to survive". In that context, cells and the stimuli-responsive materials they produce, are not only by definition *smart* but also intrinsically intelligent, in that they thereby promote their own survival.

Emergent properties include sentience in plants and cognition in humans; cognition emerges from the connectome of the brain, artificial intelligence emerges from human-made computer algorithms, and smart, advanced functional properties of materials emerge from material and structures created by cells.

Mechanical forces, associated with life in Earth's gravitational field, are inextricably to the organization of living matter on Earth. Tissue structure and architectures represent a repository or rendering of the cells' collective response to stimuli throughout their lifecycle, and thus physically encode memories throughout life, underpinning sentience/intelligence in all organisms, from plants to people.

This *Keynote Lecture* provides a progress report and critical review of the field [1], presenting the virtual power principal as a means to address the transformation/recycling of energy via materials' manufacture and organisation, in space and time (*i.e.* geometry and architecture) throughout their lifespan and in different time domains relevant to life on Earth. It provides insights into new ways of integrating the physical and biological sciences to understand and engineer novel, advanced functional materials.

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Regional ABC Sydney
08 December 2020

Multi-Modal Multi-Scale Cytokine Modulated Molecular Transport to and between Knee Joint Compartments

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INTRODUCTION

Osteoarthritis (OA) is a leading, global cause of disability. OA is characterised by chronic pain, progressive degeneration and loss of function of the synovial joint. The precise pathogenesis of OA remains elusive and involves a complex interplay between the musculoskeletal compartments of articular joints, as well as the cardiovascular and the immune systems. No TGA/FDA-approved pharmaceutical interventions are currently available to attenuate and reverse OA progression [1].

Local and systemic inflammatory events are implicated in the pathogenesis of OA. However, it remains unknown how acute increases in inflammatory cytokines affect tissue barrier function at tissue interfaces within the joint and with the circulatory system. Yet, molecular transport underpins the joints' physiologic function. We hypothesise that disruptions to transport within and between joint tissue compartments plays an important role in OA pathogenesis.

METHODS

To investigate impact of inflammatory events on molecular transport, a single mixed bolus of 70kDa tagged dextran (Texas-Red) with one of two inflammatory cytokines (TNF-α or TGF-β) was administered intracardially to 11–13-month-old Dunkin-Hartley guinea pigs, a spontaneous model of OA. After allowing five minutes' circulation, animals were euthanised and whole knee joints were resected.

Left hindlimbs were cryo-embedded then imaged using near-cell resolution 3-D fluorescent blockface cryo-imaging, enabling visualisation of tracer within the entire joint. The tissue compartments were segmented to quantify tissue permeability within the joint. Contralateral hindlimbs were resin embedded (PMMA) and underwent confocal and scanning electron microscopy. Tracer intensity were quantified within knee joint compartments.

RESULTS AND DISCUSSION

Cryo-imaging revealed significant decreases in tracer within the entire joint in groups where TGF-β was administered. TNF-α and TGF-β significantly decreased tracer in bone, cartilage and marrow space, compartments where tracer was abundant in control animals. However, cytokines to not appear to alter tracer concentrations within other tissue compartments including muscle, muscle vasculature and bone vasculature, which implicates the periosteum as a functional tissue barrier between the circulatory system and the joint.

We observed separation of the tracer throughout joint tissue compartments at multiple length scales, down to pericellular spaces. Quantification of tracer revealed no significant differences in knee joint tissue compartments between TNF-α or TGF-β groups. TNF-α animals exhibited significant differences between the vasculature and a greater number of tissue compartments than TGF-β animals ($p < 0.05$).

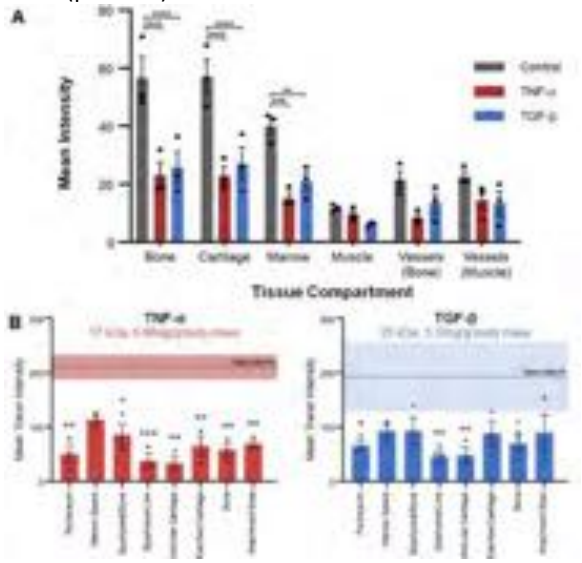

Figure 1. (A)*Tracer intensity across tissue compartments and (B) tracer intensity within tissue compartments.*

CONCLUSIONS

This study implicates the acute spike in circulating cytokines for a near instantaneous modulation of barrier function at the interfaces between the tissues of the circulatory and musculoskeletal system; this change in barrier function is observed to modulate molecular transport between and within the tissue compartments of the joint as well. The data provide further evidence for the role of systemic inflammation and crosstalk in OA pathogenesis. Cytokine-modulated transport to and within the joint warrants further study, given its implications for joint patho-/physiology and its potential for the development of novel pharmaceutical and physical therapeutic strategies to mitigate OA progression.

ACKNOWLEDGEMENTS

Supported in part through the Paul Trainor Foundation and a NHMRC Development Grant.

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Regional ABC Sydney
08 December 2020

PRIOR KNEE INJURIES AND ACL RUPTURE

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INTRODUCTION

The anterior cruciate ligament (ACL) is a major stabilising knee structure restraining tibial translation and rotation. Several risk factors for ACL rupture are known (e.g. gender and participation in certain sports) but despite programs targeting these, the rate of ACL rupture is increasing [1]. Rupture has associated social, economic and physical burdens and the traumatic event is likely to lead to early onset Osteoarthritis (OA), a debilitating musculoskeletal disease [2]. Mild injuries (e.g. joint sprains) are more prevalent amongst sports players than severe ones but the consequence of these on ACL biomechanics is under-investigated [3].

With knowledge of the role of prior mild knee injuries, intervention that could prevent rupture may be possible. This could be achieved through improved tracking and management of sports players who have sustained a mild knee injury. This study employed a mouse model of mild knee injury to better understand if previous injury/injuries may predispose the ACL to rupture.

METHODS

A randomised, blinded study was performed. Mice were assigned to receive zero (naïve mice), one, or two mild knee injuries (n = 110). Those undergoing two injuries, sustained their second at either 2- or 4-weeks after the first. A mouse knee loading apparatus was used to cause injury under anaesthesia without acutely failing structures within the joint. Mice were euthanised 4, 6, or 8 weeks after first injury, and an isolated ACL tensile test was performed to measure ACL stiffness and failure load.

Biomechanical analyses were conducted on force-displacement data using a customised MATLAB script. Statistical analyses in the form of mixed model linear regression were carried out followed by Benjamini-Hochberg adjustment for multiple pair-wise comparisons.

RESULTS AND DISCUSSION

Mild injury loading and joint stiffness measurements *during injury* did not differ significantly between injury groups. This indicates that there were no variances in the injury inductions that would need to be accounted for in subsequent ACL failure data analyses.

ACL failure load was not significantly different between any of the study arms (Figure 1). There were no trends in the mean failure load between age similar injury groups. Significant intra-group differences in ACL stiffness were found for naïve, one and two (with 4-weeks recovery) mild injury mice. However, the intra-group nature of these differences and the changes observed in the naïve group suggests that these variances are unlikely to stem from mild injury exposure.

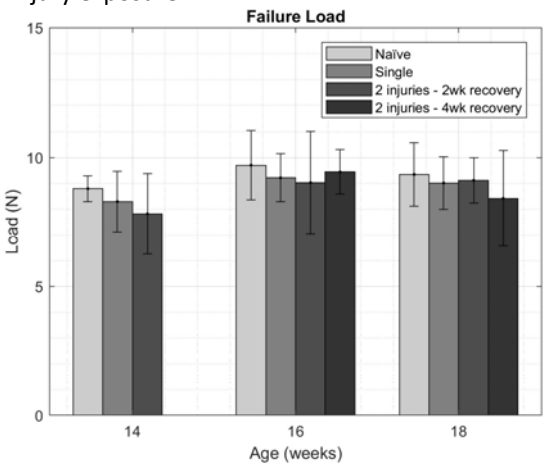

Figure 1 *Isolated ACL failure load*

The finding in this study that ACL strength is not affected by prior mild knee injuries differs from other results from our research group [4]. Slight changes made to the apparatus may have had inadvertent effects on the repeatability of the methods followed and should be explored further. The mean and standard deviation (SD) of the ACL failure load in each naïve group was 8.79 ± 0.50N, 9.70 ± 1.35N and 9.34 ± 1.22N (Figure 1), where SD indicates variability. This may tell of the natural biological variation that exists within a population and/or experiment variability in the ACL failure testing.

CONCLUSIONS

In this study, ACL biomechanics was not affected by prior mild knee injuries, nor varying the length of the recovery period between the first and second injury. Whilst significant intra-group differences were found between failure stiffness for some study arms, this is unlikely to be attributed to exposure to injury/injuries.

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Regional ABC Sydney
08 December 2020

THE EFFECTS OF DECELLULARISATION AND STERILISATION PROCESSING ON KANGAROO TENDON STRENGTH

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INTRODUCTION

Rupture of the anterior cruciate ligament (ACL) is one of the most common musculoskeletal injuries in humans. Surgical reconstruction requires the use of autograft or allograft tendons, or synthetic constructs; each with inherent limitations. Kangaroo tendon, processed into an acellular tissue, has potential as a new xenograft source for ACL reconstruction. A major challenge faced by load-bearing xenografts is retaining sufficient mechanical strength following necessary decellularisation and sterilisation processing.

The present study screened four candidate decellularisation protocols (A-D) and two doses of gamma irradiation, assessing their effect on kangaroo tendon strength.

METHODS

Kangaroo tendons were processed using one of four proprietary decellularisation protocols (A-D), followed by gamma irradiation with either low (15kGy) or standard (25kGy) dose (n=8/group). Unprocessed ("*Ex vivo*"), and non-irradiated (decellularised only) controls were also prepared. Biomechanical evaluation was performed by tensile loading to failure at 5% strain/s, with ultimate tensile strength normalised (%) to *ex vivo* controls.

Results were analysed using mixed model regression, followed by pairwise comparisons between groups, adjusting for multiple comparisons using the Benjamini Hochberg procedure with false discovery rate of 0.05.

RESULTS AND DISCUSSION

All four decellularisation protocols significantly reduced non-irradiated tendon strength compared to *ex vivo* controls ($p \leq 0.007$). Decellularisation protocol A retained the highest tendon strength (89.0% of *ex vivo*) compared to protocol B (64.7%, $p<0.001$), protocol C (76.2%, $p<0.001$) and protocol D (64.1%, $p<0.001$) in non-irradiated groups (Figure 1). Gamma irradiation further reduced tendon strength when compared to non-irradiated controls ($p<0.001$ both doses), however there was no main effect of irradiation dose ($p=0.351$ between doses).

Pairwise comparisons between, and within, decellularisation groups showed no consistent effect of decellularisation or sterilization protocol on the reduction in tendon strength (Figure 1). Significant differences observed between non-irradiated decellularisation groups were not maintained after sterilisation; with the exception of protocol C (62.5%) retaining significantly higher strength than protocol B (53.3%) in the low irradiation dose group only ($p<0.001$).

The present study assessed the feasibility of four proprietary decellularisation protocols and two doses of gamma irradiation as processing methods for a kangaroo xenograft using strength as the initial primary outcome. Despite processing-induced reductions, all combinations of decellularisation and sterilization protocols retained a higher strength when compared to published values for native human ACL [1-2].

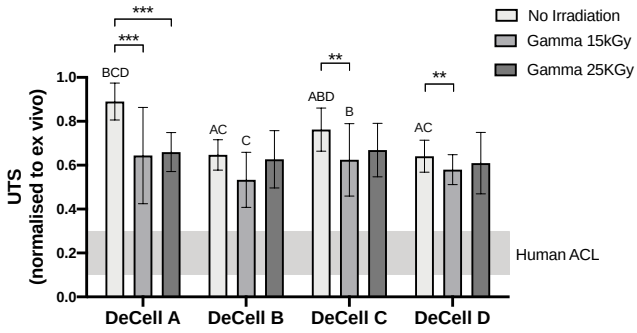

Figure 1. Effects of Decellularisation and Sterilisation Protocols on Kangaroo Xenograft Strength. *Ultimate tensile strength (UTS) of kangaroo tendons processed by decellularisation protocol (A-D) and sterilization dose (no/15kGy/25kGy) normalized to ex vivo (mean±SD). After Benjamini-Hochberg adjustment (5% FDR), statistical significance: (i) between sterilisation protocols identified by: (**) p<0.01, (***) p<0.001; and (ii) between decellularisation groups for the same sterilization dose identified by: (A) different to A, (B) different to B, (C) different to C, (D) different to D; all p<0.001.*

CONCLUSIONS

All four decellularisation protocols and both irradiation doses provide grafts with sufficient mechanical strength and remain as viable candidate methods for future development of this novel ACL xenograft. Sufficient mechanical strength only represents one facet of a successful graft, and ongoing biocompatibility studies may highlight more imperative differences between processing protocols to direct future xenograft development.

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Regional ABC Sydney
08 December 2020

EFFECTS OF FREEZER TEMPERATURE, STORAGE DURATION, AND FREEZE-THAW CYCLING ON THE BIOMECHANICAL STRENGTH OF KANGAROO TENDON

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INTRODUCTION

Anterior cruciate ligament (ACL) rupture is one of the most common knee injuries. The rate of reconstructive surgery in Australia is increasing, particularly for revisions [1]. Most commonly, a surgeon elects to harvest an autograft taken from the patient's hamstring or patella tendon [2]. However, drawbacks include increased surgical time, potential donor-site complications, and possible absence due to prior harvest. Allografts from cadavers avoid these issues, however are limited in availability [2].

A kangaroo tendon xenograft is currently in development with potential benefits including increased flexibility and availability when compared to allografts. The strength of these tendons is to be verified following freezer storage, analysing multiple timepoints up to 12 months. An additional study investigating the strength of tendons after multiple freeze-thaw cycles has been conducted.

METHODS

Kangaroo tendons were harvested and divided into 12 treatment groups, one experiment (n=205) analysing duration of storage (1,3,6,9,12 months) and another (n=270) freeze-thaw cycling (1,2,3,4,5,10 cycles). Left and right paired tendons were stored in -20C and -80C freezers respectively.

After treatment, the central portion of the tendons were placed in a custom-made ratchet micrometer [3] to determine cross-sectional area. All tendons then underwent uniaxial tensile testing to failure at a strain rate of 10%/sec. Ultimate tensile strength and elastic modulus were calculated using a customised Matlab script, followed by mixed model regression statistical analysis controlling for individual animals and tendon portions.

RESULTS AND DISCUSSION

There were no significant differences for ultimate tensile strength (UTS) or elastic modulus in either experiment when comparing the freezer temperature between tendons. This is supported in the literature [4].

In the long-term storage experiment, there were significant differences between multiple timepoints. When normalised to the 1 month treatment group, the 6, 9 and 12 month time points showed increases of over 25% for tensile strength (Figure 1). This is in contrast to a rat achilles tendon study which had significant reductions in strength [4], and a bone-patellar tendon-bone graft study with consistent strength [5], for 9-month storage samples.

The freeze-thaw cycling experiment had no significant differences between treatment groups, which is consistent with studies analysing a range of human cadaver tendons [6,7]. The results confirm that removal and return of tendons to freezer storage will have no effect on the biomechanical properties of the tendons.

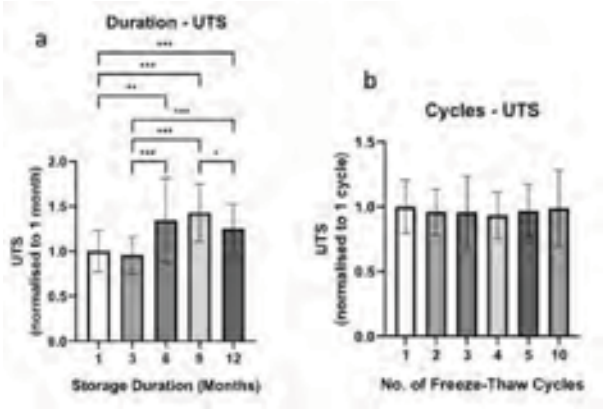

Figure 1a *Ultimate tensile strength (UTS) of kangaroo tendon stored for various durations. Results shown are normalised to the lowest storage duration.* **1b** *UTS of kangaroo tendon after multiple freeze-thaw cycles. Results shown are normalised to the lowest number of cycles.*

CONCLUSIONS

Although significant differences were found for longer storage duration, all tendons across both experiments showed strength greater than native ACL values found in literature. This suggests that the kangaroo tendon will retain strength properties after long-term freezer storage; a valuable asset in establishing the kangaroo xenograft within the ACL reconstruction market.

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Regional ABC Sydney
08 December 2020

IN VITRO DEGRADATION OF COMMONLY USED TENDON GRAFTS FOR ACL RECONSTRUCTION

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INTRODUCTION

In Australia alone approximately 17,000 anterior cruciate ligament (ACL) reconstructions are performed each year, with numbers continuing to rise [1]. Surgical repair of tendon and ligament injuries commonly involve the use of various autograft and allograft tendons with similar morphometry and mechanical properties to that of the native tendon or ligament. *In vivo* remodelling of implanted tendons has the potential to alter the tensile strength and lead to early graft failure.

In vitro enzymatic degradation assays are commonly used as an indicator of *in vivo* resorption and remodelling rates [2] . This study aimed to compare *in vitro* degradation rates of different tendons commonly used as grafts for anterior cruciate ligament reconstruction.

METHODS

Tendons were retrieved from fresh-frozen human cadavers (3 male, 3 female; 49-62 years). For each donor, samples of ~10 mm × 2 mm × 2 mm were dissected from the patellar, semitendinosus, gracilis, achilles, tibialis anterior, and tibialis posterior tendons. The initial dry weight was recorded before rehydration and exposure to 100 U of bacterial collagenase under constant agitation for 1, 2, 4 or 8 hours (n=4-6/tendon/time point). Final dry weight was measured after inactivation of collagenase activity by 0.025M EDTA.

The change in dry weight was evaluated using mixed model linear regression, accounting for paired donors, and including time, sex and initial dry weight as covariates. Benjamini-Hochberg adjustments were performed on the comparisons between tendons.

RESULTS AND DISCUSSION

All tendons demonstrated a significant reduction in tissue mass over time which is evident in Figure 1. At 1 hour the average loss in mass was greatest in the patellar tendon (degraded by more than 25%) compared to all other tendons which retained more than 75% of their initial mass. After 4 hours, the patellar tendon continued to degrade more rapidly, losing 77.2% of its mass compared to less than 46.89% for all other tendons. Despite the higher degradation rate the patellar tendon is still one of the most used tendons for ACL reconstruction [3].

By 8 hours, the average percentage loss was greater than 90.45% for patellar, tibialis anterior and tibialis posterior tendons. Gracilis, achilles and semitendinosus tendons were reduced by 81.53%, 70.31% and 70.08% respectively. The greater resistance to degradation in the latter three tendons may suggest a more favourable graft option. Overall, change in mass was significantly greater in the patellar tendon compared with all other tendons (P≤0.004). Tissue loss was also significantly higher in tibialis posterior compared with semitendinosus (P=0.0128).

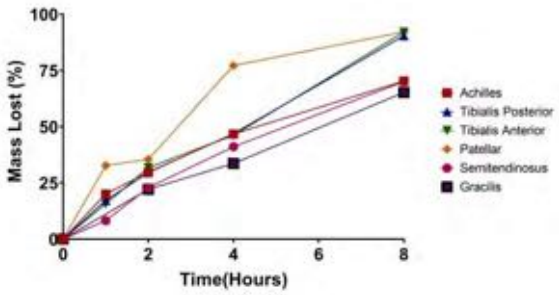

Figure 1 *Mean degradation rate of common tendons used for ACL reconstruction (Achilles, Tibialis Anterior, Tibialis Posterior, Patellar, Semitendinosus and Gracilis) when exposed to bacterial collagenase*

CONCLUSIONS

The patellar tendon degraded at an increased rate when compared to the other tissue samples. Despite this, the patellar tendon is one of the most common and successful grafts for ACL reconstruction. This suggests that *in vitro* enzymatic degradation may therefore not act as an effective indicator for *in vivo* performance.

ACKNOWLEDGEMENTS

The authors acknowledge the assistance of Dr Nick Hartnell, Dr Patrick Haubruck and Miss Samantha Hefferan in the retrieval of tendons from tissue donors.

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Thank you

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