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RESEARCH

The revitalisation of flexor tendon allografts with bone marrow stromal cells and mechanical stimulation

AN *EX VIVO* MODEL REVITALISING FLEXOR TENDON ALLOGRAFTS

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Objectives

The present study describes a novel technique for revitalising allogenic intrasynovial tendons by combining cell-based therapy and mechanical stimulation in an *ex vivo* canine model.

Methods

Specifically, canine flexor digitorum profundus tendons were used for this study and were divided into the following groups: (1) untreated, unprocessed normal tendon; (2) decellularised tendon; (3) bone marrow stromal cell (BMSC)-seeded tendon; and (4) BMSC-seeded and cyclically stretched tendon. Lateral slits were introduced on the tendon to facilitate cell seeding. Tendons from all four study groups were distracted by a servohydraulic testing machine. Tensile force and displacement data were continuously recorded at a sample rate of 20 Hz until 200 Newton of force was reached. Before testing, the cross-sectional dimensions of each tendon were measured with a digital caliper. Young's modulus was calculated from the slope of the linear region of the stress-strain curve. The BMSCs were labeled for histological and cell viability evaluation on the decellularized tendon scaffold under a confocal microscope. Gene expression levels of selected extracellular matrix tendon growth factor genes were measured. Results were reported as mean ± SD and data was analyzed with one-way ANOVAs followed by Tukey's post hoc multiple-comparison test.

Results

We observed no significant difference in cross-sectional area or in Young's modulus among the four study groups. In addition, histological sections showed that the BMSCs were aligned well and viable on the tendon slices after two-week culture in groups three and four. Expression levels of several extracellular matrix tendon growth factors, including collagen type I, collagen type III, and matrix metalloproteinase were significantly higher in group four than in group three ($p < 0.05$).

Conclusion

Lateral slits introduced into de-cellularised tendon is a promising method of delivery of BMSCs without compromising cell viability and tendon mechanical properties. In addition, mechanical stimulation of a cell-seeded tendon can promote cell proliferation and enhance expression of collagen types I and III *in vitro*.

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Keywords: BMSCs, Bone marrow stromal cells, Stretch, Tendon allografts revitalisation, Tendon slice, Tissue engineering

Article focus

- A novel technique, lateral slits, was developed to improve delivery of BMSCs into decellularised tendon without compromising cell viability and tendon mechanical properties.
- Revitalised allogenic intrasynovial tendons were further accessed by combining tissue engineering, mechanical stimulation and cellular and molecular analyses using an *ex vivo* canine model.

Key messages

- The newly designed and lateral fan-shaped slits improved the efficiency of delivery of viable BMSCs, thus promoting tendon remodeling under mechanical stimulation.

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Strengths and limitations

- Well-designed study groups to allow comprehensive comparison among unprocessed tendons, decellularised tendons, cell-seeded decellularised tendons and cell-seeded decellularised tendons under mechanical stretch.
- Novel design of lateral fan-shaped slits to allow more variable BMSCs to be delivered at the target tendon site.
- The stem cells studied were from a single source, i.e., bone marrow stromal cells (BMSCs).
- Cell number and viability were not quantitatively determined after two weeks of mechanical stimulation.
- All of the constructs were evaluated at only one strain level (3.0% strain) and one time point (two weeks).

Introduction

Flexor tendon injuries are common, especially in the young and working-age population. Failure in repairing flexor tendon injuries results in considerable disability, which can prevent patients from working and can increase healthcare costs.[1] Repair of flexor tendon injuries requires adhesion-free healing with smooth tendon surfaces and good gliding ability to restore hand function, which remains a great challenge for hand surgeons.[2] Tendon graft repair is therapeutically indicated when the direct repair fails primarily due to severe adhesions and ruptures of the repaired tendon, which occurs in an estimated 10% to 30% of cases.[3] The clinical standard of care for tendon graft repair is flexor tendon reconstruction using autologous extrasynovial tendons.[4] Palmaris longus, plantaris, and toe extensor autografts are most often used as the sources of tendon grafts in the hand.[5] However, the drawbacks of using extrasynovial tendons include rough tendon surfaces, high frictional resistance, and inferior structure compared with intrasynovial flexor tendons, all of which lead to poor clinical outcomes associated with more adhesions and diminished restoration of function.[6,7] Although autologous intrasynovial tendons are ideal, they are rarely available in the clinical setting.

Decellularised allograft tendons represent an attractive alternative to autografts in tendon or ligament reconstruction for several reasons including reduced immunogenicity, abundant availability, potential off-the-shelf access, absence of donor morbidity, expedited surgeries, and cost reduction.[3] The decellularised tendon needs to be repopulated with either intrinsic or therapeutically derived cells. However, this can be challenging since tendon is a highly dense connective tissue, which is slow to repopulate cells. The result is that allograft tendons require a long period of time to revitalise and become viable, as demonstrated experimentally and clinically.[2] An additional challenge of the hypocellular tendon allograft is delayed intrinsic healing. This slow rate of intrinsic healing reduces the integration potential, ultimately resulting in weakening at the repair site and increased likelihood of tendon rupture.[2]

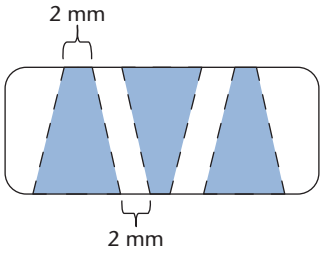

Fig. 1

Diagram of newly designed, lateral fan-shaped slits. The alternating single-sided, fan-shaped slits (shaded area) were made on the tendon at 2-mm intervals over a 2-cm length.

In a recent study, the authors introduced multiple slits into the tendon allograft surface to habour transplanted bone marrow stromal cells (BMSCs)[8] and allograft vitalisation was accelerated. *In vitro* results demonstrated that processing the allograft in this way did not alter tendon mechanical properties, and transplanted cells remained viable at the treatment site for two weeks. Due to the fact that tendons are subjected to a steady tensile load in daily life, mechanical stimulation may be a key component for transplanted cell tenogenesis and appropriate matrix alignment. The goal of the present study was to investigate novel techniques for revitalising allogenic intrasynovial tendons by combining cell-based therapy and mechanical stimulation. We hypothesised that cells can be seeded and survive in the native slit-tendon scaffold, and mechanical stimulation of the cell-seeded tendon scaffold can promote tenogenesis through increasing cell proliferation and matrix gene expression.

Materials and Methods

Flexor tendon harvest. Tendons were obtained from four mixed-breed dogs (weight, 21 kg to 26 kg) killed for other studies approved by the Mayo Clinic Institutional Animal Care and Use Committee (IACUC). These studies were unrelated to the tendons, and study treatments had no relevant effect on tendon tissue. In total, 32 flexor digitorum profundus (FDP) tendons from the second through to the fifth forepaw digits were chosen randomly and were divided distally at the bony insertion and proximally immediately distal to the common FDP, at the location where the four FDP tendons fuse together. At harvest, tendons were frozen at –80°C. Tendons were assigned to one of four groups with each group having 8 tendons: (1) untreated, unprocessed normal tendons (normal group); (2) tendons decellularised with trypsin and non-ionised detergent (Triton X-100; Alfa Aesar, Ward Hill, Massachusetts) (decellularised group); (3) tendons decellularised as in group two and perforated with short lateral slits into which BMSCs were seeded followed by culturing (BMSCs group); and (4) tendons as in group

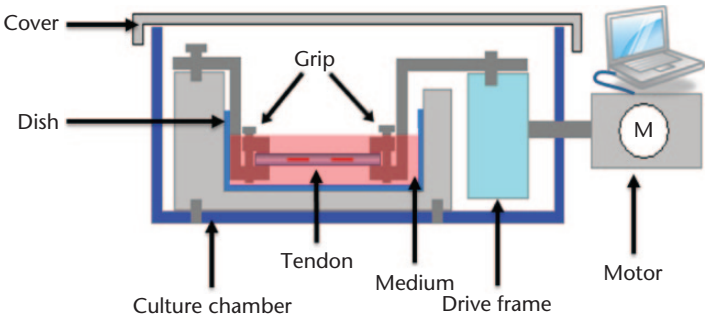

Fig. 2

A custom-made mechanical stimulation device. Schematic diagram of the stretch unit, including a cell-slice construct loaded between stainless steel grips within a dish.

three and stretched by a mechanical device in culture (BMSCs + loading group). The BMSC-seeded tendons were cultured for two weeks before seeding.

Decellularised and tendon slitting. Frozen tendons were thawed at room temperature before use. In the BMSCs + loading group, alternating single-sided, fan-shaped incisions at 2 mm intervals were made with a No. 11 scalpel (Fig. 1). The tendons were then immersed in trypsin 0.05%/0.53 mM ethylenediaminetetraacetic acid for 24 hours at 37°C, followed by 0.5% Triton X-100 (Alfa Aesar) for 24 hours at room temperature. The decellularised tendons were washed in phosphate-buffered saline for 24 hours.

BMSC harvesting and seeding. Bone marrow was harvested from the experimental mixed-breed dogs that were sacrificed under IACUC approved studies that did not have any effects on bone marrow cells using an established protocols.[9] Briefly, immediately after killing, 8.0 mL of bone marrow were aspirated from each tibia using a 15 mL syringe containing 2.0 mL of heparin solution. The heparin was removed by centrifugation at 1500 rpm for five minutes at room temperature, and the bone marrow cells from one dog were divided into three 100 mm dishes in 10 mL of standard medium, which consisted of minimal essential medium with Earle's salts (Gibco, Thermo Fisher Scientific Inc., Grand Island, New York), 10% foetal bovine serum, and 1% antibiotics (antibiotic-antimycotic; Gibco, Thermo Fisher Scientific Inc). The bone marrow cells were incubated at 37°C with 5% CO_2. Adherent cells were allowed to grow in minimal essential medium 10% antibiotic-antimycotic, with medium replaced every third day. After 70% to 80% confluence was reached, the BMSCs were subcultured. BMSCs of passage 4 or less were used in all experiments. A cell suspension of 2×10^7 cells/mL in culture medium was prepared, and 50 µL of this cell suspension was seeded directly into each slit with a micropipette. After one hour of incubation at 37°C in a 5% CO_2-humidified incubator, culture medium was added and the BMSC-seeded tendons were cultured for two weeks. The culture medium was changed every three days.

Mechanical stimulation device and dynamic culture of engineered tendon constructs under stretch. A custom-made mechanical stimulation device, as described previously for tendon mechanical stimulation,[10] was used to apply cyclic uniaxial strains to the cell-seeded constructs in an incubated environment (Fig. 2). The device included a component that applied mechanical stretching and a microcontroller that regulated the stepper motor driving the motion. The device included three separate wells, and each well was capable of containing up to four scaffolds in the culture medium. This unit allowed control over the frequency and amplitude of the strain applied to the scaffolds, and timers could introduce automatic rest periods in the cycle. The instrument was designed to fit inside a standard cell culture incubator.

Before the BMSCs were seeded into the tendon slits, the ends of each tendon were clamped into the grips of the mechanical stimulation device, maintaining an exposed span of tendon 25 mm in length. The allografts were arranged such that there were four tendons in each of the three partitions of the device. The cell-seeded composites were cultured statically for two days, and then subjected to a cyclic loading protocol with a peak displacement amplitude of 0.75 mm at a frequency of 0.2 Hz for 20 minutes of each hour. The protocol was applied for 12 concurrent hours each day followed by 12 hours of rest over a two-week period at 37°C in a 5% CO_2-humidified incubator. Statically cultivated composites were not subjected to cyclic loading but were similarly placed in culture for two weeks after two days of static culture. All conditions were the same except for mechanical loading. The engineered tendon constructs were collected after two weeks of culture for further evaluation.

Biomechanical assessment. All tendons were distracted by a servo-hydraulic testing machine (MTS 858 Mini Bionix II; MTS Systems Corp., Eden Prairie, Minnesota) after two weeks of culture to evaluate mechanical properties. A region of the tendon, from a point just proximal to where the proximal pulley would lie in the neutral position, to 2 cm distal to this point, was included in the gauge length. Prior to testing, the cross-sectional dimensions (major

Table I. Primer sequences for the analysis of gene expression.

	Forward primer	Reverse primer
GUSB	5′-CATGCTGGTCCAGAGCTACA-3′	5′-CAGGCTTCAGGAAGGAAGTG-3′
COL-I	5′-TGGTTCTCCTGGCAAAGAT-3′	5′-ATCACCGGGTTCACCTTTA-3′
COL-III	5′-ACAGCAGCAAGCTATTGAT-3′	5′-GGACAGTCTAATTCTTGTTCGT-3′
MMP3	5′-GGAGAGGCTGACATAAAGATT-3′	5′-GATGTATCGCTTGTCCATTG-3′
MMP9	5′-TGCCTGAGACTGGAGAG-3′	5′-GCAAGTCTTCCGAGTAGTT-3′
MMP13	5′-TACAACTTGTTCCTTGTCGC-3′	5′-CTGGGCCATAGAGAGACT-3′
IGF-1	5′-TTGCACTTCAGAAGCAATGG-3′	5′-CAAGCACAGTGCCAGGTAGA-3′
Tenomodulin	5′-GATCCCATGCTGGATGAG-3′	5′-TACAAGGCATGATGACACG-3′

COL1, collagen type I; COL3, collagen type III; MMP9, gelatinase; MMP13, collagenase; TNMD, tenomodulin; GUSB, β-glucuronidase; IFG-1, insulin-like growth factor 1.

and minor diameter) of each tendon were measured with a RS232 digital caliper (Aklands Grainger, Brantford, Canada). Capliper tests were blinded to tendon group. The caliper has a rated accuracy of 0.02 mm and a resolution of 0.01 mm. Measurements were obtained at three different levels (proximal end, distal end, and midpoint). The area was calculated based on the assumption that the tendon cross-section was elliptical. The cross-sectional area of the tendon was averaged over the three levels. Tissue adjacent to the gauge length region was gripped in the clamps of the rest machine along with a surrounding sheet of sandpaper to aid gripping. At the start of each test, the tendon was preconditioned with ten cycles of loading from 10 N to 50 N at a rate of 20 mm per minute. Following the tenth cycle, the tendon was distracted at a rate of 20 mm per minute. The tendons were moistened with a saline mist throughout the testing. Tensile force and displacement data were continuously recorded at a sample rate of 20 Hz until 150 N was reached. The stiffness and Young's modulus were calculated from the slope of the linear region of the force-displacement and stress-strain curve, respectively.

Cell stress-strain viability assessment. The same tendons from the four study groups described previously were also subjected to cell stress-strain viability assessment. For tracing living cells in the engineered tendon patches, all BMSCs were labelled with the Vybrant DiL cell-labelling solution (Molecular Probes, Inc., Eugene, Oregon) before seeding on the decellularised tendon scaffold. Cells were labelled according to the manufacturer's instructions and as previously described.[8] Briefly, before transplantation the BMSCs were labelled with 2.5 µL/mL Vybrant DiL cell solution for 30 minutes and protected from light at 37°C in a 5% CO_2-humidified atmosphere and followed by three washes with phosphate-buffered saline. After tissue culture, the tendon segments were embedded in the optical cutting temperature compound (Tissue-Tek; Sakura Finetek USA Inc., Torrance, California) and cut into 7 mm slices with a cryostat (Leica CM 1850; Leica Microsystems GmbH, Wetzlar, Germany). Sections were also stained with haematoxylin-eosin (H&E) stain using standard technique.[11]

To evaluate cell viability, three carbocyanine dye DiL-stained tendons in each group were incubated for 30 minutes before being viewed with a confocal microscope (LSM 510; Carl Zeiss Microscopy, Oberkochen, Germany). Eight tendons were selected randomly from each experimental group and underwent haematoxylin-eosin staining. Cell nuclei were counterstained using 4′, 6-diamidino-2-phenylindole (DAPI) following the standard protocol (Vector Laboratories, Inc., Burlingame, California). Eight different fields of view were randomly chosen from each slide under the confocal microscope. Digital images were recorded of each field of view. Decellularised tendons were used as controls.

Gene expression by qRT-PCR. qRT-PCR was performed to measure the gene expression levels of several extracellular matrix tendon growth factors, including tenomodulin (TNMD, a marker for tenocyte differentiation), collagen types I (COL1) and III (COL3), matrix metalloprotease 9 (MMP9), and MMP13.[12] Briefly, 20-mm sections from groups three and four were snap frozen in liquid nitrogen and stored at -80°C until ribonucleic acid (RNA) extraction. Total RNA was isolated from the tendons and cultured cells using TRIzol reagent (Invitrogen; Life Technologies, Corp., Grand Island, New York). cDNA was synthesised using the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Inc, Hercules, California). qRT-PCR was performed with C1000 Touch Thermal Cycler (Bio-Rad Laboratories). The expression level was normalised to the housekeeping β-glucuronidase (GUSB) gene, which was used as internal normalising control. Eight samples from groups three and four were used for assessment of gene expression with the sequences for the primers presented in Table I.

Statistical analysis. The mean (standard error, SE) expression in each gene and biomechanical parameter (Young's modulus and stiffness) were calculated for each group. The overall comparisons of gene expression, biomechanical parameter among the control group, and BMSCs-seeded tendon composition groups were analysed with one-way analysis of variance (ANOVA). The Tukey-Kramer significant difference test was used as a *post hoc* test. The significance level was set at $p < 0.05$ in all cases. All statistical analyses were performed using JMP software version 9.0.1 (SAS Institute Inc., Cary, North Carolina).

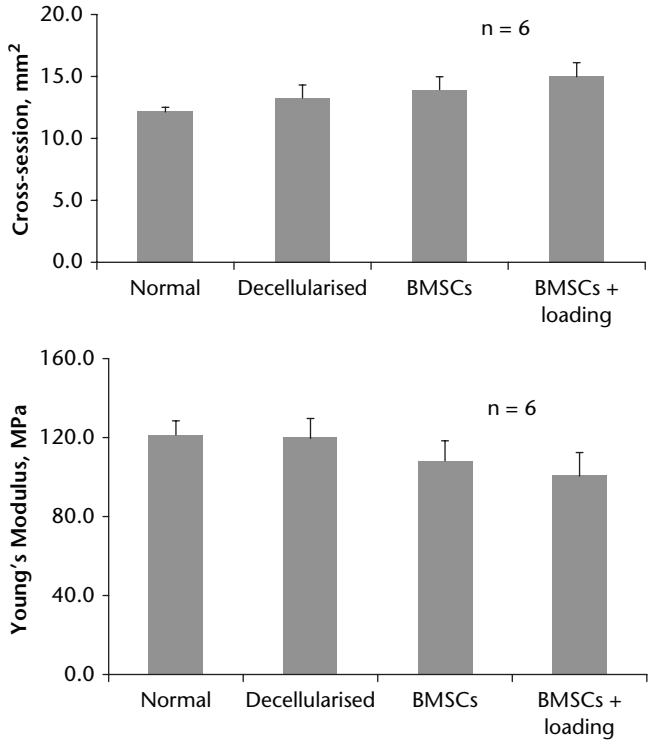

Fig. 3

Comparison of mechanical characteristics among the four study groups. The mean cross-sectional area (a) and (b) Young's modulus of the normal group, decellularised group, bone marrow stromal cells (BMSCs) group, and BMSCs with mechanical stimulation group (BMSCs + loading). Error bars represent SE. N=6, 6 samples out of 8 tendons in each group were randomly selected for measurement. BMSCs indicate bone marrow stromal cells.

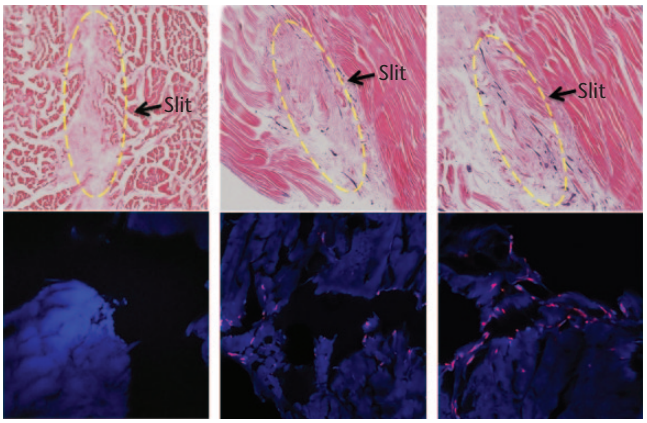

Fig. 4a Fig. 4b Fig. 4c

Histological findings of longitudinal sections of tendon scaffolds in H&E staining (top) and 6-diamidino-2-phenylindole (DAPI) (DAPI) cell tracker staining (bottom) demonstrated that no cells were seen in the decellularised tendons a). Seeded cells were present in the bone marrow stromal cell-seeded tendons with H&E staining and DAPI cell tracker staining (bottom) b). In the cell-seeded tendon with cyclic loading group, the viable cells were also observed inside the tendon with cell migration into tendon substance under both H&E (top) and DAPI cell tracking (bottom) images c).

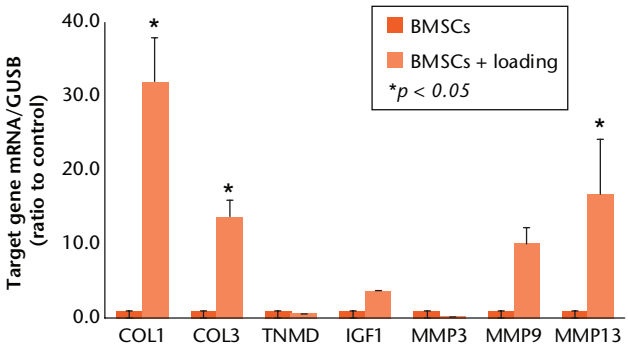

Fig. 5

Comparison of extracellular matrix tendon growth factor gene expression in BMSCs and bone marrow stromal cells (BMSCs) + loading groups. The expression level was normalised to that of the GUSB gene. Error bars indicate mean (SE); * significant difference ($p < 0.05$). BMSCs, bone marrow stromal cells; COL1, collagen type I; COL3, collagen type III; MMP9, gelatinase; MMP13, collagenase; TNMD, tenomodulin; IFG-1, insulin-like growth factor 1; GUSB, β-glucuronidase.

Results

Mechanical properties. Since all failures occurred at the clamping site, we did not report the maximum strength. Young's modulus calculated within the linear region of the stress/strain curve was used to characterise the tendon mechanical properties and compared across the four study groups. We observed no significant difference of cross-sectional area and Young's modulus among the groups (Fig. 3).

Morphological characterisations and viability of BMSCs. Representative histological findings are noted in Figure 4. Specifically, no cells were observed in the tendons from the decellularised group with haematoxylin-eosin and DAPI staining (Fig. 4a).

Histologic sections showed that the BMSCs aligned on the tendon slits after being cultured for two weeks in groups three and four (Figs 4b and 4c). DAPI-labelled BMSCs were also detected in the composite of BMSCs and tendon group with and without mechanical stimulation after two weeks' incubation (Figs 4b and 4c). Some labelled cells also spread between the collagen fibres.

Expression of extracellular matrix tendon growth factor genes. Gene expression levels of several extracellular matrix tendon growth factor genes were measured with qRT-PCR and are summarised in Figure 5. No mRNA was detected in the decellularised group. Gene expression of COL1, COL3, and MMP13 was significantly increased in the BMSC mechanical stimulation conditions as compared with the BMSCs alone ($p < 0.05$).

Discussion

Delayed healing is a common problem associated with tendon or ligament reconstruction when tendon allografts are used; the delay is primarily due to the lack of cellularity.[13] The tendon-healing process is a combination of extrinsic and intrinsic cellular activity after tendon grafting.[3] However, decellularised allograft tendons do

not have viable intrinsic cells and rely on the extrinsic cell repopulation, which results in a long period for regeneration of tendon tissues. Increased cellularity of the decellularised tendon scaffold may speed healing and reduce adhesion failure.[14]

The present study described newly designed, fan-shaped lateral slits, and the effect of cyclic uniaxial strain on the proliferation of BMSCs for flexor tendon graft engineering. This alternating single-side fan-shaped design of incisions not only allows for maximum cell seeding, but also maintains the gliding functionality of the tendons by leaving the gliding surfaces of the tendons intact. Woon et al[15] showed that cell-seeded tendon scaffolds can exhibit superior strength compared with unseeded tendon scaffolds, however, they did not evaluate the gliding potential of these treatments. Ozasa et al[8] demonstrated that multiple short slits on the tendon surface can successfully increase the ability of BMSCs to penetrate a decellularised tendon scaffold without compromising the tendon stiffness. However, the gliding resistance of the tendon with multiple slits was significantly higher than that of normal tendon because the multiple slits were created in the volar-dorsal direction, roughening the tendon's gliding surface. In our study, we modified the slit direction from volar-dorsal to lateral-lateral, which enabled the gliding surface to remain intact. Furthermore, a lateral fan-shaped slit provided a larger space for seeding the BMSCs than the previous rectangular slit. We also found no significant difference in stiffness or in Young's modulus among the four groups, suggesting that the slits did not diminish the tendon's biomechanical properties.

Mechanical stimulation of cultured tendon cells can enhance cell proliferation and matrix production.[10,16] More than 80% of the dry weight of a tendon is composed of COL1 and COL3.[17] Production of COL1 and COL3 indicates the cell tenogenic phenotype and activity.[10] Our study showed that mechanical stimulation with seeded BMSCs upregulated COL1 expression more than 30-fold and COL3 expression more than ten-fold compared with BMSC treatments. COL1 is the primary constituent of the tendon extracellular matrix and the main protein responsible for load bearing,[18,19] whereas COL3 is generally thought to be involved in the early stage of tendon healing.[19] We also found that the expression of MMP13 was increased more than ten-fold following mechanical stimulation, which could indicate that mechanical stimulation enhances the tendon remodeling process.[20] MMP13 expression is an important marker of matrix degradation and remodeling in tendon tissue, and is regulated in both loading and unloading conditions.[21] Qin et al reported [22] that expression of MMP13 in tenocytes with mechanical loading in an *in vivo* model was decreased. However, by blocking Interleukin (IL)-1 beta, the MMP13 expression was increased, which suggested MMP13 is regulated by both IL-1beta-dependent, and IL-1beta-independent pathways. In addition, MMP13 has been shown to have substrate specificity for a range of matrix components, such as MMP2 and MMP9.[22]

Riboh et al[16] studied the effects of three patterns of cyclic uniaxial strain on epitenon tenocytes, sheath fibroblasts, BMSCs, and adipose-derived stem cells. Their results showed that intermittent cyclic strain can increase cell proliferation, promote COL1 production, and maintain tenocyte morphologic characteristics *in vitro*.[16] Qin et al[22] also reported that mechanical stimulation of decellularised tendon slices seeded with BMSCs (BMSCs-DTSs) increased the expression of tendon-related genes (COL1, decorin, and tenomodulin) after seven days in culture. Our findings were consistent with these previous studies and provide additional evidence to support the benefit of mechanical stimulation of cell-based constructs before implantation. However, expression of tenomodulin, a tenocyte marker, was not increased after two weeks of mechanical stimulation, in contrast to Qin's report that tenomodulin expression increased after one week of mechanical stimulation. This finding might be due to the fact that tenomodulin expression was measured at different time points. In the current study, two weeks' mechanical stimulation not only increased collagen production but also increased MMP13 expression, indicating that tendon regeneration and degradation are progressing at the same time. This combined anabolic and metabolic response to mechanical stimulation may alter the expression of other cytokines, such as tenomodulin.

There were several limitations in this study. First, only one strain level (3.0% strain) with a single culture time point was studied. The incubation period of two weeks might not be adequate for full integration of the cell suspension composite into the host tendon. As this was a proof-of-concept study, further studies on gene expression and matrix protein structure, formation and quality of these constructs should also be performed with different strain levels in a longer period of follow-up. Second, we did not test the tendon gliding ability after cell seeding. Overgrowth of the seeded cells on the tendon surface is certainly a possibility that should be studied in future *in vivo* work. Third, the results derived from an *in vitro* model could be very different from an *in vivo* model. However, one of the goals of this study was to characterise viability and differentiation of BMSCs seeded on the lateral sliced tendon, as a prelude to a longer study in a canine tendon repair model *in vivo*. Since we have shown that the cells can survive when seeded through our newly designed lateral slit on the tendon model *in vitro*, we will now set up a study of extended duration, e.g. up to six or eight weeks, to evaluate biomechanical parameters from the tendon healing perspective *in vivo* using an animal model. Finally, we did not quantitatively measure the cell number and viability after two weeks of mechanical stimulation, nor did we study the stem cells derived from

other tissue sources such as mesenchymal stem cells, embryonic stem cells or induced pluripotent stem cells[23]. Kryger et al[19] have demonstrated that tendon sheath fibroblasts, BMSCs and adipose-derived mesenchymal stem cells were similar in growth characteristics. In addition, both Chong's and Costa's studies on tenocyte proliferation and reseeded tendon constructs showed that the tendon-derived stem cells are particularly difficult to harvest.[14,24] Future in-depth work should evaluate other sources of stem and stromal cells for optimising both culture conditions and tendon regeneration.

In conclusion, we have demonstrated that the newly designed lateral slit on the tendon model is an effective approach to seed BMSCs without compromising cell viability or altering tendon mechanical properties. Cyclic mechanical loading applied to a cell-seeded tendon can stimulate the expression of COL1 and COL3 *in vitro*, and may therefore promote tendon remodeling. This revitalised, engineered allograft may be clinically effective for intrasynovial tendon reconstruction.

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Author Contribution

- J. H. Wu: Study design, Performed experiment, Data acquisition and analysis, Data interpretation, Drafting of manuscript, Manuscript edit and approval.
- A. R. Thoreson: Study design, Performed experiment, Data acquisition and analysis, Data interpretation, Manuscript edit and approval.
- A. Gingery: Study design, Performed experiment, Data acquisition and analysis, Data interpretation, Manuscript edit and approval.
- K. N. An: Data interpretation, Manuscript edit and approval.
- S. L. Moran: Data interpretation, Manuscript edit and approval.
- P. C. Amadio: Data interpretation, Manuscript edit and approval.
- C. Zhao: Study design, Performed experiment, Data acquisition and analysis, Data interpretation, Drafting of manuscript, Manuscript edit and approval.

ICMJE Conflicts of Interest

- None declared.

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