Introduction. Tendons (rotator cuff, Achilles and patellar tendons) are among the most commonly injured soft tissues [1]. Many repairs/reconstructions have been attempted (e.g. sutures, resorbable biomaterials, autografts, and allografts) with varying success. A tissue engineered repair using mesenchymal stem cells (MSCs) is attractive [2-5] but tissue stiffness and strength need to be improved [6].

The current study was undertaken to determine how mechanical stimulation in culture of MSC-type I collagen sponge constructs affects the biomechanics and histology of rabbit patellar tendon (PT) defect repairs 12 weeks after surgery. The hypotheses to be tested were that mechanical stimulation would: 1) significantly improve repair biomechanics and histological appearance, 2) result in higher failure forces than measured in vivo forces in normal rabbit PT during an inclined hopping activity [7], and 3) result in repairs that match the tangent stiffness of normal PT above these peak in vivo forces.

Methods. Ten one-year-old female New Zealand White rabbits were assigned for biomechanical (n = 7) and histological (n = 3) evaluations at 12 weeks post surgery. MSCs were isolated from the iliac crest of each rabbit and then culture expanded for five weeks until passage two for PT surgery. All constructs (n = 8 for each animal) were created by seeding these MSCs (0.14 x 10^6 cells/construct) on a type I collagen sponge (Kensey Nash Corporation, Exton, PA). Half of the constructs (n = 4 for each animal) were then mechanically stimulated once every five minutes to a peak strain of 2%, for 8 hours/day for 2 weeks. The other half remained in an incubator (37°C, 5% CO_2, 95% RH) without stimulation for 2 weeks. All mechanically stimulated and non-mechanically stimulated constructs were fed three times weekly with high glucose DMEM, 5% ascorbic acid and 10% FBS. The stimulated (S) and non-stimulated (NS) constructs were implanted in full-length, central defects in the right and left PTs, respectively. The Institutional Animal Care and Use Committee (IACUC) approved all procedures.

Twelve weeks post surgery, animals were sacrificed and patellar tendon-tibia specimens were harvested. The average length, width, and thickness of the soft tissue were measured before and after removing the medial and lateral struts. The bone at each end was then fixed into special grips using PMMA cement. Each specimen was placed in a chamber of phosphate-buffered saline (pH 7.4, 37°C) mounted on a testing system (Model 8501, Instron, Inc., Canton, MA) and then failed in tension at a constant strain rate of 20%/s while monitoring grip-to-grip mechanical properties of the tissue. The force-elongation and stress-strain curves were plotted to determine structural biomechanical properties and mechanical properties of the material.

Tissues assigned for histology were placed in 10% neutral buffered formalin, fixed in paraffin and stained with hematoxylin and eosin. Adjacent sections were subjected to immunohistochemical staining for presence of collagen types III and V, decorin and fibronectin. Statistical analysis was performed using a paired Student t test. All conclusions regarding the significance of mechanical stimulation on biomechanical properties were made at the α = 0.05 experiment-wise level.

Results. Significant increases were found between the stimulated (S) and non-stimulated (NS) repairs for several load-related structural (p = 0.001) and mechanical properties (p = 0.01). Maximum forces for the S and NS repair groups averaged 339.3 ± 11.4 N vs. 271.5 ± 17.5 N and the linear stiffness averaged 141.6 ± 11.3 N/mm vs. 88.6 ± 9.6 N/mm, respectively (mean ± SEM). Maximum stresses for the S and NS repairs averaged 72.1 ± 11.1 MPa vs. 50.2 ± 9.2 MPa and linear moduli for the S and NS repairs averaged 441.2 ± 26.3 MPa vs. 343.2 ± 21.2 MPa, respectively (mean ± SEM).

The average maximum force and maximum stress of the stimulated repairs were approximately 70% of corresponding values for the normal central third PT [2]. Average linear stiffness and modulus were 85% and 50% of normal values, respectively. Tangent stiffness values for the stimulated repairs matched that for normal rabbit PT and 50% beyond (150N) the peak in vivo force recorded for inclined hopping in a previous study (Fig. 1) [7].

Histology for the stimulated and non-stimulated repairs showed similar biochemical staining and cellular alignment compared to normal. Sections from both repair types showed mild staining for collagen type V and decorin, and moderate staining for collagen type III and fibronectin. Both repairs showed excellent cellular alignment but the non-stimulated repairs showed moderately increased cellularity compared to the normal tendon.

Discussion. This study has demonstrated that mechanical stimulation of stem cell-collagen sponge constructs improves both the repair biomechanics and histological appearance of central patellar tendon defect injuries at twelve weeks post surgery. The repair tissue produced in this study exceeds the structural and mechanical properties found in previous studies [2-4], including one study in which stem cell-gel-collagen sponge repairs averaged 75% of normal tendon linear stiffness and 60% of the normal tendon maximum force [4]. Most importantly, the tangent stiffness of these repairs matched the values for the normal central third PT up to peak in vivo forces and displacements recorded in the rabbit PT [7]. The current repair tissue not only can withstand the in vivo forces and displacements recorded for inclined hopping [7] but matches the tangent stiffness of the normal tendon to 50% beyond peak in vivo force limits (i.e. 32% of normal PT failure force; Fig. 1). The results of this current study provide a promising first step towards the development of a tendon repair, but mechanical signals need to be optimized in culture so as to: 1) increase the safety factor to account for potentially more strenuous activities, and 2) shorten the repair time post surgery to satisfy principles of functional tissue engineering [6].


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