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. Tissue engineering using mesenchymal stem cells is attractive [2-4] but tissue stiffness and strength do not yet meet in vivo loading demands [5-6]. High cell densities (1, 4 and 8x10^6 cells/ml) also result in ectopic bone in 28% of all repairs when constructs are contracted around suture during incubation [4]. Lowering cell density and reducing construct contraction and thus cell-to-cell contact may improve repair biomechanics and eliminate ectopic bone formation.

The objective of this study was to test the hypothesis that lower cell-to-collagen ratios and permitting direct tensile strain to the cells during contraction will significantly increase repair biomechanics and eliminate ectopic bone in the patellar tendon model at 12 weeks post surgery.

Methods. Sixteen one-year-old female New Zealand White rabbits were assigned for biomechanical (n=13) and histological (n=3) evaluations at 12 weeks post surgery. Two levels of cell-to-collagen ratio (0.04 and 0.08 M/mg; hereafter labeled as L and H repairs, respectively) were investigated in each animal by seeding MSCs at one concentration (0.1 million cells/ml) in one of two concentrations of collagen gel (2.6 and 1.3 mg/ml, respectively). MSCs were isolated from the iliac crest of each rabbit and then culture expanded for five weeks until passage two for patellar tendon surgery. Cells were mixed with bovine gel (Vitrogen) and pipetted into specially designed dishes that permitted contraction around posts. Resulting constructs remained in an incubator (37°C, 5% CO2, 95% RH) for 14 days and were fed high glucose DMEM with ascorbic acid and 10% FBS twice weekly. At surgery, one construct was sutured into a 1 cm length defect in the central third of one patellar tendon. The construct containing the other cell-to-collagen ratio was placed in a matching contralateral defect. The Institutional Animal Care and Use Committee (IACUC) approved all procedures.

Twelve weeks post surgery, animals were sacrificed and patella-patellar tendon-tibia specimens were harvested. Radiographs were performed to determine presence of ectopic bone formation. Patella-patellar tendon-tibia specimens (including repair tissue) were harvested for biomechanical testing and total tissue dimensions (average length, width, and thickness) were measured. Adjacent tendon struts were removed and reduced tissue dimensions (repair tissue only) were measured. The bone at each end was 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 both grip and local tissue strain using optical strain analysis. The force-elongation and stress-strain curves were plotted to determine structural and material properties.

Tissues assigned for histology were fixed in 10% neutral buffered formalin, fixed in paraaffin and stained with hematoxylin and eosin. Adjacent sections were subjected to immunohistochemistry staining for presence of collagen types I, III and V and for fibronectin.

Statistical analysis was performed using a paired Student t test. All conclusions regarding the significance of cell-to-collagen ratio on biomechanical properties were made at the α = 0.05 experiment-wise level.

Results. No significant differences were found between the lower (L; 0.04 M/mg) and higher (H; 0.08 M/mg) cell-to-collagen ratios for any of the structural mechanical response measures (p > 0.05). Maximum forces for the lower and higher ratios averaged 117.6 ± 17.4 N and 130.8 ± 21 N (mean ± SEM), respectively. Stiffnesses for both repairs averaged 41.4 ± 11.5 N/mm and 40.6 ± 6.2 N/mm (mean ± SEM), respectively.

Similarly, no significant differences were found between the two cell-to-collagen ratios for any of the material properties of the repairs (p>0.05). Maximum stresses (Fig. 1) for the L and H repairs averaged 25.3 ± 3.1 MPa and 30 ± 3.6 MPa (mean ± SEM), respectively. Moduli (Fig. 2) for the L and H repairs averaged 113.9 ± 24 MPa and 107.4 ± 17.6 MPa (mean ± SEM).

The average maximum force and maximum stress of the repairs were approximately 30% of corresponding values for the normal central third of the PT [4]. Average stiffness and modulus were 30% and 20% of normal values, respectively.

Histologically the repairs showed strong staining for fibronectin and collagen type III and mild staining for collagen types I and V. There was no appreciable difference in staining intensity in the different samples. Radiographs revealed no ectopic bone in the soft tissue component of the repair sites.

Discussion. MSC-gel composites in a previous study significantly increased patellar and Achilles tendon repair biomechanics compared to natural repair between 4 and 26 weeks [2,4]. These cell-based repairs in the patellar tendon model [4] were 20% of normal maximum force and stress and 15% of normal stiffness and modulus. The two cell-to-collagen ratios in the current study yielded significantly greater moduli and maximum stresses than results from this prior study at 12 weeks although no differences were found between the two lower ratios in the current study. Normally high variability in the repair data likely contributed to the lack of significant cell-to-collagen ratio related differences. The absence of ectopic bone formation is encouraging and may reflect the need to maintain lower cell-to-collagen ratios in culture to control cell phenotype. Cell-to-collagen ratios and biomaterials must still be identified that will tolerate expected in vivo loads, one of the principles of functional tissue engineering [6].


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