Introduction. Injuries to tendons, ligaments and capsular structures represent almost 45% of the 32 million musculoskeletal injuries in the US each year [1]. Tissue engineered constructs containing mesenchymal stem cells (MSCs) seeded in type I collagen gels are one attractive alternative to direct surgical repair or reconstruction of defects in these structures. While introducing large numbers of cells in collagen gel-suture constructs accelerates repair of tendon defects ectopic bone forms in almost a third of the cases [2], in part due to excessive contraction during maturation in culture [3]. Cell density affects the kinetics of MSCs contracting collagen gels around suture [4], but cell-to-collagen ratio also affects contraction of MSC-gel constructs anchored to posts [5]. The current study sought to understand if cell-to-collagen ratio affects contraction kinetics of these cell-collagen constructs as they mature around posts in culture and which component of the ratio (cell density or collagen concentration) is more important in this process. Temporal changes in normalized area of the construct (ratio of current-to-initial area (A/Ao)) served as a single measure for kinetics of contraction with initial area being the area of the well excluding the post areas. In a two-part experiment using a silicone dish and posts, we hypothesized that: 1) increasing cell-to-collagen ratio would significantly increase contraction kinetics, and that 2) decreasing collagen concentration would affect normalized area more than increasing cell seeding density.

Methods. MSC’s from the iliac crest of the New Zealand White rabbit were culture expanded to second passage and mixed with type I purified bovine collagen gel (Vitrogen, Cohesion, CA) Experiment 1. Four cell-to-collagen ratios (0.04, 0.08, 0.4, 0.8 M/mg; labeled HK, LK, HM and LM, respectively) were contrasted with n=6 for each level by seeding MSCs at two concentrations (0.1 and 1 million cells/ml, respectively) in two different collagen concentrations (2.6 and 1.3 mg/ml, respectively). Each cell-gel mixture was pipetted into specially designed silicone dishes to allow contraction around posts. Contracting constructs were fed high glucose DMEM supplemented with 10% fetal bovine serum and ascorbic acid and incubated at 37°C, 5%CO₂ for 7 days. Experiment 2. Two identical cell-to-collagen ratios (0.4 M/mg) were contrasted, one using MSCs at 0.5 million cells/ml in a 1.3 mg/ml concentration of collagen (LSK) and the other using the same HM conditions in Experiment 1. All treatments in each experiment were compared using MSCs from the same animal and passage number. Digital images of the constructs were obtained at 4, 8, 16, 32, 72, 120 and 168 hours in culture. Images were analyzed using National Instrument’s Vision 7.0 software. Normalized areas were plotted against logarithmic time of contraction to create more equally-spaced time intervals on the graph. Hypotheses were tested via contrast analysis using least square means. All tests of contrasts were assumed to be one tailed and the p-values of t-tests were calculated by the LSMEANS option of the SAS program

Results. Hypothesis 1 was accepted (Fig. 1). At 32 hours of contraction, the construct with the highest cell-to-collagen ratio (LM: 0.8), contracted the most (to 13% of its initial area) while the construct with the lowest cell-to-collagen ratio (HK: 0.04) contracted the least (to 53% of its initial area). Beyond 32 hours, all LM constructs contracted even further and tore off the posts. High significant differences were found in normalized areas between HK and LK (p < 0.0001) and between HM and LM (p = 0.0033). Surprisingly, however, no significant difference was found in the contraction curves for two different cell-to-collagen ratios, LK (0.08) and HM (0.4) (p = 0.511) suggesting that other factors might also control contraction.

Hypothesis 2 was also accepted (Figure 2). Despite the same cell-to-collagen ratio (0.4), the construct with the lower collagen concentration (LSK: 1.3mg/ml) displayed significantly greater contraction kinetics than the construct with the higher cell density (HM: 1M cells/ml) (p = 0.0011). The fact that constructs with higher cell density but identical collagen concentration (LM vs LSK) showed no significant difference in normalized area up to 32 hours (p = 0.7082, Fig. 2) further supports this hypothesis. The nonlinear relationship between normalized area and log (time) also shows that rate of contraction is not exponential but more complex in nature for all treatments examined.

Discussion. The highest cell-to-collagen ratio (LM) contracted the constructs significantly more than the lowest ratio (HK), indicating that cell-to-collagen ratio plays a role in cell-mediated contraction (Fig. 1). These results are consistent with previous reports [2, 4]. However, the absence of differences between two intermediate ratios (LK: 0.08 and HM: 0.4) suggests that cell seeding density and collagen concentration may also be important. The more dominant role of collagen concentration vs. cell seeding density (Fig. 2) prioritizes the importance of these two parameters. Although contraction is generally viewed as a cell-mediated event where larger numbers of cells can produce more traction forces to facilitate contraction, increasing cell seeding density above a threshold value doesn’t further enhance the effect [4, 6]. Instead, regulating collagen concentration appears to better control contraction kinetics. Controlling MSC differentiation in vitro will be critical to creating desirable phenotypes that express appropriate ECM. Such in vitro approaches should ultimately improve repair outcome after surgery, a goal of functional tissue engineering [7-9].


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