Effects of Cell Density on Collagen Alignment in Mesenchymal Stem Cell-seeded Collagen Gels Used for Tendon Repair

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Introduction:

One therapeutic strategy for repair of musculo-skeletal soft-tissue injuries uses engineered cell-delivery constructs to actively supply repair cells to the wound site. In *in vivo* studies, mesenchymal stem cell(MSC)-seeded collagen gels improved repair biomechanics in comparison to natural healing in patellar and Achilles tendons of rabbits1,2. However, these improvements fell 10% short of the desired minimum biomechanical limits. Mechanical strain has been shown to modulate cell alignment3. MSC-seeded gels cultured with dynamic strain may provide better starting materials for the surgical implant. Before examining strain effects, quantitative response measures must be selected and tested.

Rationale/Hypothesis:

Collagen alignment and quantity of collagen are two factors predictive of tendon strength. We hypothesize that increased cell density applied to MSCs in collagen gel will improve collagen alignment and increase matrix synthesis.

Methods:

MSCs were obtained from fresh bone marrow aspirates taken from 1 yr. old female New Zealand white rabbits. MSCs were combined with a commercial bovine type-I collagen to form a cell-gel composite with an initial seeding density of 1x105 or 1x106 cells/mL. MSC-seeded gels were cultured for a period of two weeks in a culture dish with two restraining posts. Alignment was compared between the two concentrations of MSCs using the small angle light scattering(SALS) method. The SALS method quantitatively maps the magnitude of collagen fiber orientation and allows graphic representation. Collagen synthesis measurements for static cultures were collected using a calorimetric assay.

Results:

MSCs at a concentration of 1x106 cells/mL contracted and aligned the gel more than concentrations of 1x105 cells/mL (Figs. 1,2). MSCs at a concentration of 1x106 cells/mL also caused 15% of the gels to tear at the restraining posts. Total collagen measurements for MSC-seeded gels were not statistically different from acellular collagen gels.

Discussion:

Higher cell concentrations may be desirable to improve the alignment of collagen. However, higher cell density causes more gel contraction around the posts and a greater chance of tearing the gel (Fig 2). Measurements of total collagen did not detect a difference in collagen synthesis after 2 weeks of culture, an alternative method with greater sensitivity may be necessary. After testing response measures, the next step is to apply dynamic strains to gels in culture.

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