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Optimizing the Mechanical Stimulus in Culture to Improve Construct Biomechanics for Tendon Repair

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1 Introduction

Previous studies from our laboratory have demonstrated that in vitro mechanical stimulation of tendon tissue-engineered constructs significantly increases both construct stiffness and repair biomechanics after surgery [1-2]. In vitro mechanical stimulation has been reported to induce cell alignment [3-4], increase proliferation [3, 5] and collagen synthesis [3]. However, these studies have examined only a few mechanical stimulation profiles and these profiles contain multiple components (e.g. peak strain, frequency, duration, and number of cycles) whose effects are unknown. Studies are needed to understand how components of the mechanical stimulation profile influence construct mechanics and biology and ultimately which combination results in optimal repair stiffness after surgery. The purpose of this first study was to determine how altering three of these components affect the in vitro linear stiffness of these constructs and hence the repair biomechanics.

2 Materials and Methods

Constructs were created by seeding 0.14 x 10^6 MSCs (harvested from the iliac crest of 1 year-old female NZW rabbits; n = 10) into a type I collagen sponge (Kensey Nash Corporation, Exton, PA). Constructs were stimulated at a frequency of 1 Hz for 8 hours/day for 2 weeks in an incubator (37ºC, 5% CO_2, 95% RH), and a control group remained in the incubator without stimulation.

Iteration 1. Two levels of peak strain (1.2 or 2.4%), cycle number (100 or 3000 cycles/day), and cycle repetition (1 or 20) were examined. After 2 weeks of stimulation, constructs failed in tension at a strain rate of 10%/sec using an electromechanical testing system (TestResources Inc.; Shakopee, MN). The data was analyzed using a three-way ANOVA and Post hoc testing was conducted using Tukey's tests at a significance level of $\alpha = 0.05$.

Iteration 2. Intermediate levels of the three factors (peak strain 1.8%, 1550 cycles/day and 10 cycle repetitions) were then examined to produce a center point for a response surface to determine the next iterations using Response Surface Methodology [6].

Iteration 3. The response surface was extended for combinations of higher peak strains and cycle number/day.

3 Results

Iteration 1. Increasing peak strain produced the only significant change in construct linear stiffness (0.03 ± 0.002 N/mm (1.2%) to 0.05 ± 0.003 N/mm (2.4%;mean ± SEM; $p < 0.001$; **Fig. 1**). While having no effect at 1.2%, increasing cycle number at 2.4% strain increased construct stiffness from 0.05 ± 0.004 N/mm (100 cycles/day) to 0.06 ± 0.005 N/mm (3000 cycles/day; $p = 0.012$; **Fig. 2**).

Iteration 2. Stimulating the constructs with intermediate levels of the 3 factors (peak strain 1.8%, 1550 cycles/day and 10 cycle repetitions) produced lower stiffness compared to any other conditions (0.028 ± 0.003 N/mm).

Iteration 3. Higher peak strains (2.7% and 3.15%) and cycle numbers/day (4450 and 5900) lowered stiffness to 0.026-0.041 N/mm. The resulting response surface showed that constructs stimulated with 2.4% strain and 3000 cycles/day most stiffened the construct (0.068 N/mm; **Fig. 3**).

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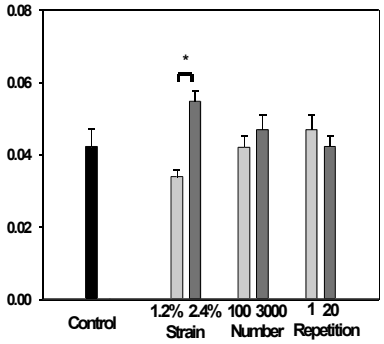

Figure 1 : Increasing peak strain produced the only significant increase in construct linear stiffness.

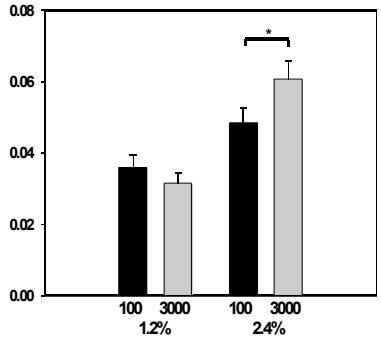

Figure 2 : At 2.4% strain, 3000 cycles/day produced significantly higher linear stiffness compared to 100 cycles/day.

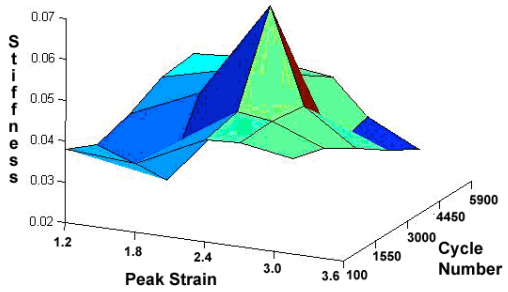

Figure 3 : Response surface indicates that the peak linear stiffness is produced by imposing 2.4% peak strain and 3000 cycles/day in culture.

4 Discussion

This study is the first in a series designed to optimize the mechanical stimulation signal needed to most improve tissue engineered tendon constructs and repair outcomes. The stiffness of constructs exposed to 2.4% strain, 3000 cycles/day constructs was larger than our previous results (0.05 N/mm). Given the significant positive correlations between construct and repair stiffness at 12 weeks post surgery [1, 7], these in vitro enhancements offer the prospects of further improving repair biomechanics. We are currently conducting an in vivo study in which we are implanting constructs exposed to 2.4% strain, 3000 cycles/day constructs in one knee and 2.4% strain, 100 cycles/day constructs in the contra lateral knee of 10 NZW rabbits. Mechanical stimulation is known to upregulate collagen types I and III gene expression [3]. Our results suggest that a signal composed of 2.4% strain and 3000 cycle/day might also upregulate these genes more than any other signal we have examined in this study. We are currently testing this hypothesis by evaluating these construct for expression of collagen type I, collagen type III, decorin, fibronectin and glyceraldehydes-3-phosphate dehydrogenase (GAPDH) genes using real time PCR. Optimizing the stimulation components to improve both construct stiffness and repair biomechanics offers the opportunity to speed the design and fabrication process, especially if in vitro predictors can be identified for in vivo repair outcome. Future studies will utilize Response Surface Methodology to optimize other components of the mechanical stimulus including cycle frequency, rise and fall times and total duration of mechanical signal.

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