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Functional Tissue Engineering to Repair Tendon & Other Musculoskeletal Tissues

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1 Introduction

Over the past 10 years, our group has been using functional tissue engineering (FTE) principles [1, 2] to repair rabbit tendon defect injuries. After computing functional design limits from normalized peak in vivo forces for activities of daily living [3-5], we tissue engineered constructs using autogenous mesenchymal stem cells (MSCs) and collagen scaffolds to try and improve linear stiffness in culture and repair tangent stiffness after surgery [6-8]. The objectives of this current set of studies were to evaluate: 1) how mechanical stimulation of these rabbit MSC-sponge constructs in culture affects repair stiffness and construct stiffness; 2) how these stimuli affect the construct's expression of two collagen structural genes responsible for stiffness (collagen type I) and repair (collagen type III); and 3) how stimulation of constructs with murine MSCs containing an intracellular fluorescent protein (GFP-T) influences collagen type I expression.

2 Materials and Methods

Study 1. Rabbit cell-sponge constructs were created by seeding 0.14 x 10^6 MSCs (from the iliac crest of 1 year-old female NZW rabbits; n = 10) into a type I collagen sponge (Kensey Nash Corporation, Exton, PA) and then placing them in an incubator (37ºC, 5% CO_2, 95% RH). Constructs received no stimulation (control) or were stimulated pneumatically (2.4% peak strain, 100 cycles / day) while controlling pulse frequency (1 Hz), total stimulation / day (8 hours/day) and total time of stimulation (2 weeks). Stimulated and non-stimulated control constructs were then implanted in bilateral central patellar tendon defects in 10 rabbits and examined at 12 weeks post surgery [9]. Using Response Surface Methodology [10] to optimize in vitro conditons, we then examined how two peak strains (1.2 or 2.4%), two cycle numbers (100 or 3000 cycles/day), and two cycle repetitions (1 or 20) affected construct stiffness during failure testing (TestResources Inc.; Shakopee, MN). We next measured stiffness both for intermediate levels (peak strain 1.8%, 1550 cycles/day, 10 cycle repetitions) and higher levels of two relevant factors (2.7% and 3.15% peak strains and 4450 and 5900 cycles / day).

Study 2. Similar constructs were created from 10 additional NZW rabbits and stimulated in culture (2.4% peak strain, 100 cycles/day, 1 cycle repetition). Half of the constructs were evaluated for gene expression (collagne type I, III, decorin, fibronectin) using real-time quantitative RT-PCR, and the other half were failed in tension to determine construct stiffness.

Study 3. Doubly transgenic mice were bred with transgenes pOBCol3.6GFPtpz (3.6 kb fragment of rat col1a1 promoter, enhancer sequence) and pCol2-ECFP. GFP-T expression was evident in skin, tendons and osseous tissues while ECFP expression was evident in cartilaginous tissues. MSCs were harvested from long bone marrow of 4 six-week old mice and inserted in collagen sponges for tensile stimulation (1.2% strain at 1 Hz for 5 hours/day). At days 0, 7, and 14, constructs were visualized in a fluorescence microscope after which cells were extracted and GFP-T fluorescence was quantified in

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relative fluorescence units (RFUs) in a micoplate spectrophometer.

3 Results

Study 1a. Tangent stiffness of the stimulated repair at 12 weeks matched normal tendon stiffness up to 50% greater than the largest in vivo forces recorded (**Fig. 1**) [3, 9].

Study 1b. A peak strain of 2.4% combined with 3000 cycles / day of stimulation and 1 cycle repetition produced a local maximum in construct linear stiffness (0.06 ± 0.005 N/mm; p = 0.012) (**Fig. 2**). Intermediate levels of the 3 factors as well as higher peak strains (2.7% and 3.15%) and cycle numbers/day (4450 and 5900) significantly lowered linear stiffness.

Study 2. Two weeks of in vitro mechanical stimulation significantly increased collagen gene expression for the stem cell-collagen sponge constructs. Stimulated rabbit MSC-sponge constructs showed 2.5- to 4-fold increases in collagen type I and III gene expression and linear stiffness (p =0.001; **Fig. 3**) without significant increases in either decorin or fibronectin expression.

Study 3. While tensile stimulation increased RFUs by 1.3 fold compared to controls at day 7 ($p < 0.05$; **Fig. 4**), no significant effect was observed at day 14 ($p > 0.05$). Time in culture also increased gene expression.

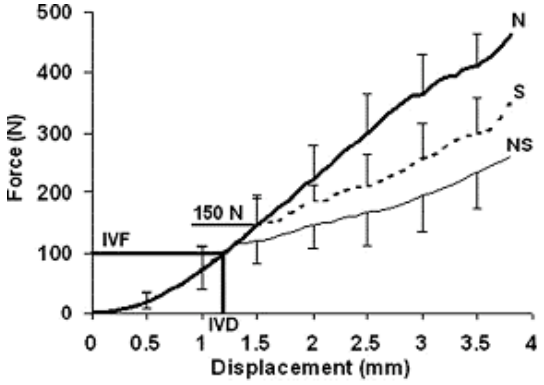

Figure 1 : Repairs containing mechanically stimulated MSC- sponge constructs (S) match the tangent stiffness of normal patellar tendon (N) up to 50% greater than the highest peak in vivo forces (IVF) recorded for inclined hopping activities. NS are non-stimulated constructs and IVD is the estimated in vivo displacement (IVD) threshold [3, 9].

4 Conclusion

Studies 1 and 2. The fact that the stiffness of rabbit MSC-collagen sponge constructs was largest for 2.4% strain is interesting since our estimated peak in vivo strains for rabbit patellar tendon were between 2% and 4% for the highest activities of daily living [3]. The positive correlations found between in vitro and in vivo response measures [9] also suggest that many less expensive in vitro experiments could be performed and results used to predict in vivo outcome. Response surface methodology offers an efficient method for optimizing multiple treatment conditions in culture. The proportional increases we observed between linear stiffness and collagen type I and III gene expression suggest the importance of these structural genes in functional outcome. Longer intervals or different stimuli may be required, however, to elicit increases from assembly genes like decorin and fibronectin.

Study 3. This study shows that collagen type I gene expression from harvested MSCs both increases with time in culture and with early tensile stimulation. Since the stimulation conditions for the rabbit and murine tissue engineered constructs differed, future studies will need to optimize and possibly tailor these mechanical signals in order to determine their specific effects on both in vitro and in vivo behavior.

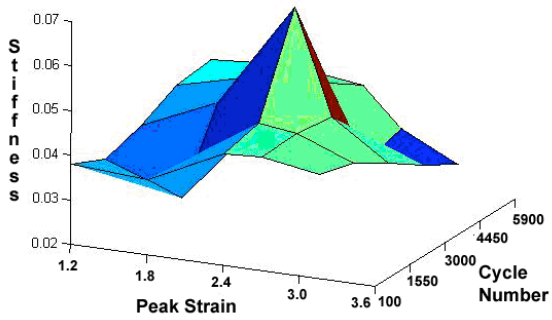

Figure 2 : Stimulating constructs with 2.4% peak strain and 3000 cycles/day in culture produces a local maximum in construct linear stiffness.

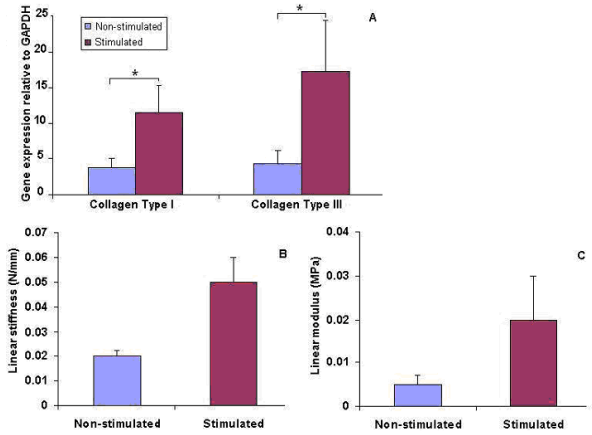

Figure 3 : Stimulated constructs showed higher collagen type I ($p = 0.0001$) and III ($p = 0.001$) expression vs. non-stimulated constructs after 14 days in culture ($n = 10$; mean SD). * Significantly different from non-stimulated (**A**). Stimulated constructs showed higher linear stiffness (**B**) and linear modulus (**C**) compared to non-stimulated after 14 days in culture ($n = 10$; $p = 0.002$)[11].

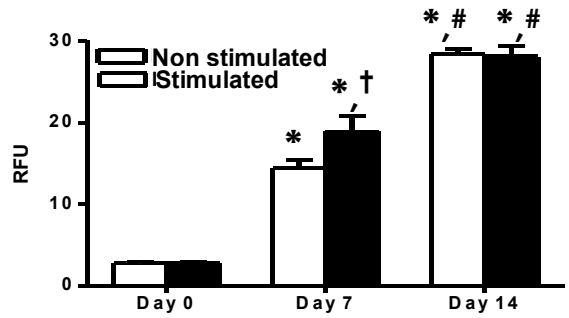

Figure 4 : GFP-T RFU changes with culture time and loading. *Significantly different from day 0 for the same treatment. †Different from non-stimulated controls at same interval. #Different from day 7 for the same treatment [12].

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