Effects of mechanical stimulation on the matrix synthesis of bone marrow derived stem cells in fibrous elastomeric scaffolds

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Statement of Purpose: Long term efficacy of tissue replacements or regenerative therapies relies on the critical processes of cell proliferation and differentiation, the production of organized matrix, and concurrent tissue remodeling or growth. Moreover, the development of tissue surrogates to perform critical load bearing functions necessitates the ability to manage cellular processes through controlled exogenous cues to direct the production of organized, functional extracellular matrix. In the current study, it is our goal to investigate the biosynthetic response of bone marrow derived stem cells microintegrated within a fibrous elastomeric scaffold. A cyclic tension bioreactor was employed to mechanically condition specimens in a controlled, reproducible manor.

Methods: Electrospinning produces continuous fiber scaffolds exhibiting a wide range of mechanical properties while providing a suitable environment for cell proliferation and growth [1-3]. Cell microintegrated constructs were prepared for the current study, via a concurrent electrospraying-electrospinning process [4]. After 30 minutes of electrospinning and electrospraying, the microintegrated tube was removed from the mandrel and cultured in a spinner flask containing D-MEM (Gibco™, Invitrogen Corporation) at a rotation rate of 15 rpm for 24-48 hours at 37 °C prior to mechanical conditioning.

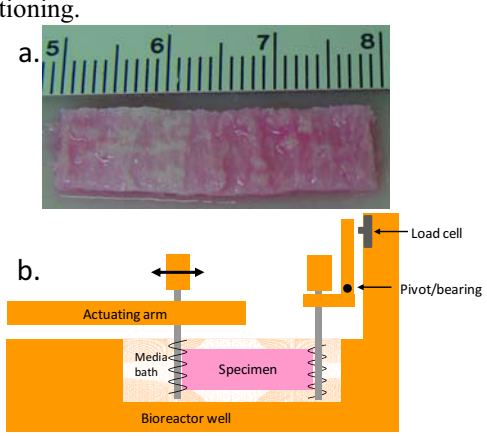

Figure 1. Once microintegrated constructs were produced and cultured for 24-48 hours, specimens were cut to size (a) and placed into the cyclic tension bioreactor wells for additional static or dynamic culture for 7 days (b).

Currently, 12 such specimens have been produced and designated into 3 testing groups. The first group was comprised of 4 control specimens obtained directly from the spinner flask with the remaining specimens divided equally between static and cyclic tension 7 day culture groups. Electrospun constructs were dynamically cultured via methods presented previously to a uni-directional strain of 15% (Fig. 1) [5]. Upon completion of dynamic culture, specimens were removed from the bioreactor for quantification of DNA (PicoGreen dsDNA, Molecular Probes) and collagen (Sircol™ Collagen Assay).

Results: Results indicated electrospun scaffolds behave in a highly elastic manner and exhibit no measurable creep when subjected to similar modes of deformation. Changes in DNA and collagen content between groups were not statistically significant. However, the trend of decreased collagen in static specimens and increased collagen levels induced by cyclic tension (Fig. 2) is consistent with that measured by Merryman et al. [5] for native valve interstitial cells exposed to cyclic tension.

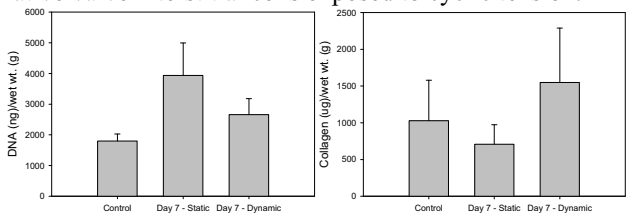

Figure 2. DNA and collagen quantification (n = 4 per group). Though differences between conditioning groups were not statistically significant, collagen production was seen to decrease in static specimens (abnormal state) while cyclic tension was observed to increase acid-soluble collagens.

Conclusions: This study is the first to quantify the effects of controlled mechanical cues on matrix synthesis in microintegrated elastomeric electrospun scaffolds. Building on previous work, where the relationship between cell deformation and macroscopic construct strain was quantified [4], we are able to dictate cellular deformation levels in order to optimize strain dependent tissue formation. In order to improve our understanding of the mechanical contribution of de-novo matrix proteins, force transducers are currently being incorporated into the bioreactor design to enable quantification of the time dependent matrix associated changes in construct mechanical properties (Fig. 1). Ultimately, this study will guide modeling efforts incorporating tissue accretion kinetics to predict evolving construct mechanical behavior.

References:

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