DYNAMIC LOADING IN TISSUE-ENGINEERED MITRAL VALVE CHORDAE TENDINEAE

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INTRODUCTION

The increase in mitral valve repair has stimulated an interest in developing alternative materials for artificial chordae. Currently, e-PTFE is the substitute that most closely mimics the characteristics of natural chordae, but the potential for continued elongation of the suture substitute may hinder the long-term function of the repair [1]. To develop better materials for use in chordal replacement, we have turned to tissue-engineering technologies. We have used the principle of directed collagen gel shrinkage to fabricate mitral valve chordae by combining cells and reconstituted type I collagen [2,3].

In an effort to continuously improve the mechanical properties of our constructs, we have begun to explore dynamic loading. It is well known that mechanical strain has a variety of effects on smooth muscle cells, including increased proliferation, matrix production and increased expression of phenotype-specific proteins and growth factors. It has also been shown that mechanical stimulation can have important effects on the development of collagen-based constructs [4]. To subject our constructs to mechanical stimuli, we designed a motorized dynamic loading system to cyclically stretch the constructs *in vitro*. It is speculated that such dynamic straining will encourage the cells to produce a greater amount of matrix, thus improving the mechanical properties of our collagen constructs.

MATERIALS AND METHODS

The cyclic device had four main parts -- motor housing, culture dish, cam race and cam follower. The culture dish is fabricated from plastic and has ports in the sidewalls that enable pull rods to pass through. These pull rods have cam followers at their distal ends and connect to the collagen constructs at their proximal inner ends. The culture dish is driven by a motor to slowly rotate, and the cam followers contact a static cam race (Fig. 1A). Displacement of the cam follower causes the pull rods to move in and out, and subject the collagen constructs to cyclic mechanical strain. The displacement waveform of the cam race was based on a simple sine wave transferred to polar coordinates (Fig. 2).

We used Pro-Engineer CAD/CAM software to design a motorized stretching bioreactor and built multiple copies of the system using stereolithography (SLA) (Fig.1B). The hole in the wall of the culture dish that passed the stretching rods was sealed with a mixture of vacuum grease and Neosporin. The cam follower consisted of a spinning bead that followed the cam race as the system rotated. A rubber band was used to pull the followers together against the track. The culture dish was firmly attached to the shaft of a DC Gear-head motor powered by a 0-25 V DC bench top power supply (both from Jameco Electronics, Belmont, CA). Because the entire apparatus, except the power supply, was placed in the incubator, the motor was enclosed within a sealed SLA box to protect it from humidity. Prior to use, the complete silicone-rubber-lined SLA culture dish with stretching rods was steam sterilized at 121 psi for 15 minutes. The collagen-cell suspension was then pipetted into the culture dish and incubated at 37?C for 2 weeks under static conditions to make the gel to set and undergo cell-mediated compaction. Once initial tendon-like structures formed, the motor was turned on and the constructs cultured dynamically for up to 6 weeks. The constructs were stretched up to 10% strain, at a rate of 2 Hz. The system was stopped periodically only for changing the culture media. All the constructs were cultured for up to 8 weeks. After culture, constructs were examined for mechanical properties, microstructure and cell and collagen content.

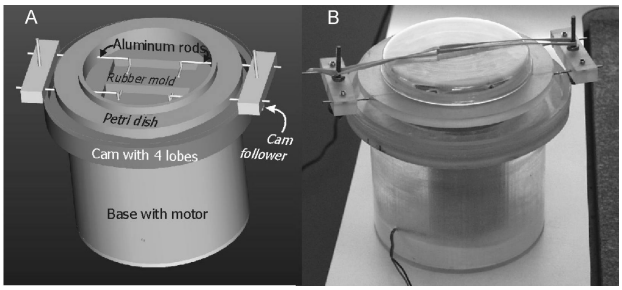

Figure 1. Pro-E model of dynamic loading bioreactor (A), prototype fabricated using SLA (B).

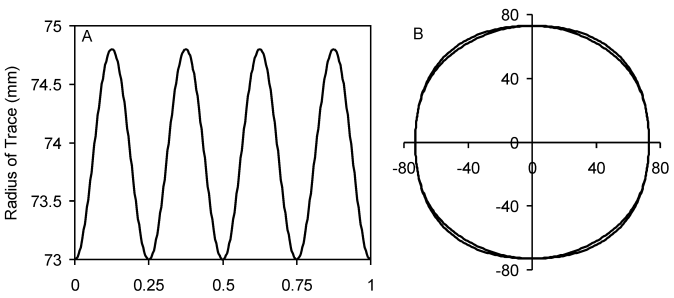

Figure 2. The four sinusoidal waves plotted in Cartesian (A) and polar (B) coordinates correspond to the four lobes of the cam race.

RESULTS

The mechanical properties of collagen constructs subjected to cyclic strain changed with time during the 8-week culture period. Modulus, failure stress and failure strain all increased with culture time. The constructs subjected to cyclic strain had greater mechanical strength and a higher elastic modulus relative to statically cultured controls (Fig. 3). Failure stress increased by 195% (to 3.25 MPa), tensile modulus increased by 188% (to 18.7 MPa) and extensibility increased by 12% (to 10.2%).

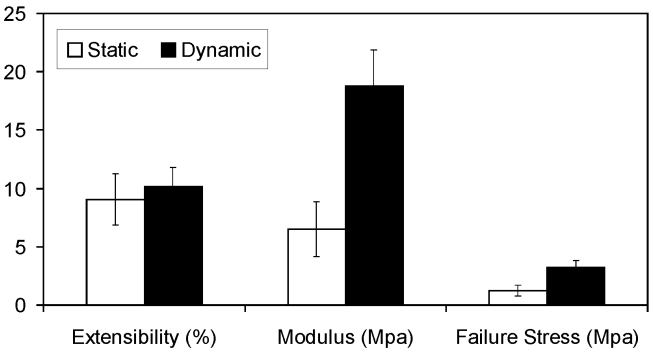

Figure 3. The constructs subjected to cyclic strain had greater mechanical strength and a higher elastic modulus relative to statically cultured controls.

During the first 4 weeks, DNA content per dry weight increased linearly, then remained steady for almost 2 weeks. From 6 weeks to 8 weeks, DNA content decreased. Overall, cyclic mechanical strain caused cell proliferation, resulting in a 155% increase in cell number during the whole culture period. Collagen content of native chordae was 66.9%. The collagen content of 8-week-old, dynamically cultured constructs was 60.3% and that of static controls was 56.6%. This suggests that cells in dynamically cultured constructs secreted more collagen than those in static controls. Transmission electron microscopy showed that the collagen fibers in the dynamically loaded constructs were longer, more aligned and more compacted (Fig. 4A). The elastin sheath that surrounded the collagen core of dynamically cultured constructs was 1-2 μm thicker than in statically cultured constructs (Fig. 4B). Proteoglycan filaments were evenly distributed along the surface of the collagen fibrils in the collagen core (Fig. 4C), similar to native chordae.

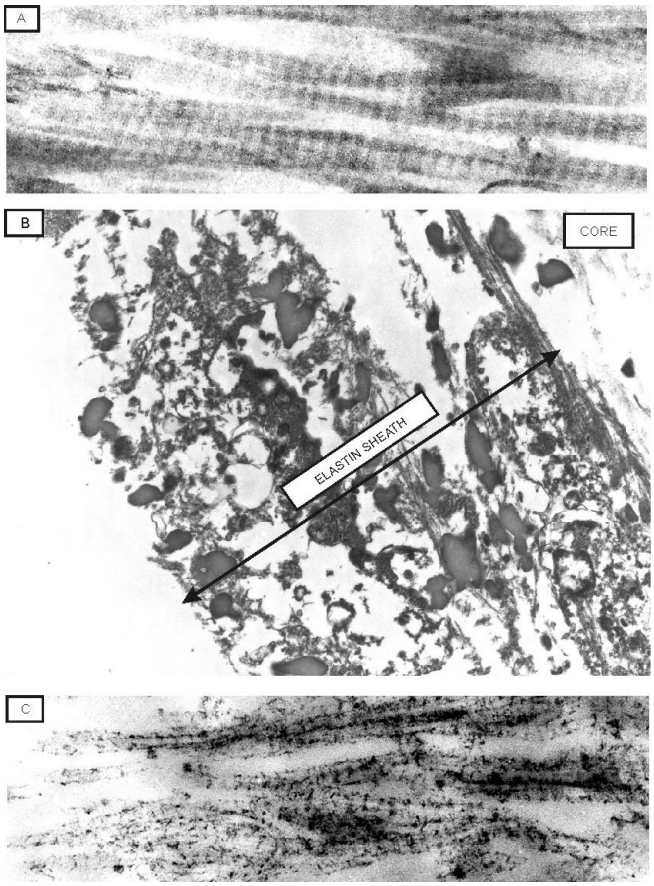

Figure 4. TEM images of dynamically cultured constructs (A, 35,000X). TEM images showed that the elastin sheath surrounded the collagen core (B, 5,000X). Cuprolinic blue staining demonstrated that proteoglycans wrapped around the collagen fibrils at regular intervals (C, 35,000X).

CONCLUSIONS

This study has demonstrated that mechanical stimulation offers a means of producing a denser matrix with more cells. Increasing cell content and stimulating cell metabolism increases matrix production and improves structural integrity, ultimately leading to stronger constructs.

REFERENCES

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