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Fibroblast-seeded collagen gels in response to dynamic equibiaxial mechanical stimuli: A biomechanical study

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ABSTRACT

The remodeling of fibroblast-seeded collagen gels in response to dynamic mechanical stimuli was investigated by using a newly developed biaxial culture system capable of cyclically stretching planar soft tissues. Fibroblast-seeded collagen gels were subjected to three distinct dynamic mechanical conditions for six days: Cyclic Equibiaxial Stretching at two constant strain magnitudes (CES-7% and CES-20%), and Cyclic Equibiaxial Stretching with incrementally Increasing stain magnitude (ICES, 7% → 15% → 20% each for two days). The frequency of cyclic stretching was set at 1 Hz. At the end of culture, mechanical properties of the gels were examined by biaxial mechanical testing and checked again upon the removal of seeded cells. Collagen microstructure within the gels was illustrated by multiphoton microscopy. The mRNA levels of collagen type I and type III and fibronectin in the cells were examined by reverse transcription PCR. The protein expression of α-smooth muscle actin was detected by immunohistochemistry. We found that the gels cultured under cyclic stretching were stiffer than those cultured under static stretching. Particularly, the stiffness appeared to be significantly enhanced when the ICES was employed. The enhancement of mechanical properties by cyclic stretching appeared to persist upon cell removal, suggesting an irreversible remodeling of extracellular matrix. Second harmonic generation images showed that collagen fibers became thicker and more compact in the gels cultured under cyclic stretching, which may explain the mechanical findings. The mRNA expression of collagen type I in the cells of the ICES was significantly greater than that of the other groups except for the CES-20%. This study suggests that when cyclic stretching is to be used in engineering soft tissues, incrementally increasing strain magnitude may prove useful in the development of the tissue.

1. Introduction

Many biological tissues have the ability to grow and remodel in response to an altered mechanical environment. For example, arterial walls thicken in hypertension and a few musculoskeletal tissues such as bone, tendon, and ligament deteriorate under immobilization. Mechanical stimuli have been shown to influence gene expression directly associated with extracellular matrix (ECM) turnover, resulting in changes in the structural and mechanical properties of tissues (Gupta and Grande-Allen, 2006; Sarasa-Renedo and Chiquet, 2005). The stiffness of the ECM *per se* is found to regulate cell phenotype and behavior in many tissues or organs (Rozario and DeSimone, 2010; Wells, 2008).

Many *in vitro* culture systems have been developed to study mechanobiology in defined biochemical and biomechanical environments. In most of these systems, including the Flexcell Tension System, cells are grown on a two-dimensional (2D) elastic membrane and statically or dynamically stretched by deforming the membrane. These systems have provided valuable insights into the mechanotransduction of cells residing on 2D surfaces *in vivo* such as endothelial cells. Cell behaviors can be very different in 2D and three-dimensional (3D) environments, however (see, for example, Edmondson et al. (2014) for details). For cells residing in the 3D matrix *in vivo* such as fibroblasts, more physiologically relevant information can be obtained if the cells are studied in a 3D environment. To advance the knowledge of mechanobiology, there is a need for a culture system that is capable of stretching native tissues or tissue equivalents in which cells are embedded in a 3D environment.

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Fibroblasts are able to produce, reorganize, and degrade components of the ECM, in particular, collagen, and hence play important roles in the development, repair, and remodeling of various tissues. Fibroblast-seeded collagen gels prepared by gelling a neutralized, cell-containing collagen solution have been used as a 3D cell culture model to study cell-matrix interactions in tissue morphogenesis and wound healing (Bell et al., 1979). Owing to the recognized importance of mechano-stimulation in tissue behavior, the model were also used to investigate growth and remodeling of the gel under mechanical loadings (Knezevic et al., 2002; Kural and Billiar, 2013). Our previous investigation demonstrated that manipulation of the microstructure of fibroblast-seeded collagen gels was possible by culturing the gel under static biaxial mechanical constraints (Hu et al., 2009). Further, the microstructural findings were correlated well with the bulk mechanical properties (Hu et al., 2013). The static mechanical constraints exerted on the gel, however, represent a single stepwise stretching. The effects of the stretching may decay over time during a long-term culture.

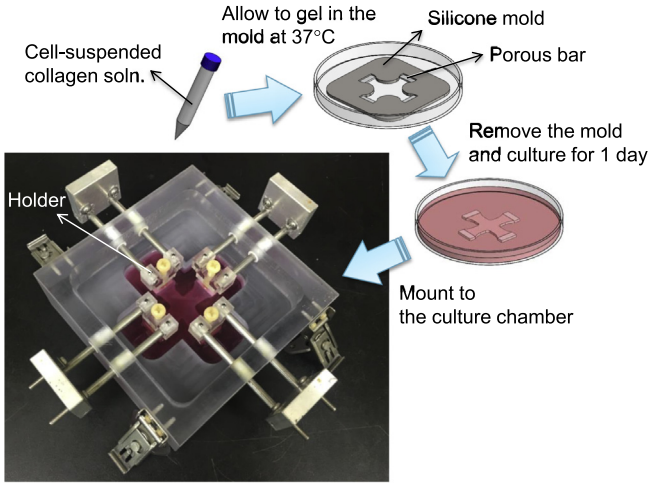

Fig. 1. Preparation of cruciform-shaped cell-seeded collagen gels and placement of the gel to the culture chamber. The embedded porous bar at each arm of the gel permitted the arm to be clamped on the holder.

Here we made a step further and designed a novel biaxial actuator system to be incorporated into the previous biaxial culture chamber, creating a dynamic biaxial culture system capable of cyclically stretching collagen gels. There are few reports on how fibroblast-seeded collagen gels react in response to cyclic biaxial stretching. Tranquillo's group investigated how cyclic (uniaxial) distension improves the development of tissue-engineered blood vessels and showed that cells adapt to cyclic stretching of constant strain magnitude, limiting their collagen production (Paxton et al., 2012; Schmidt et al., 2016). The influence of stepwise increasing strain magnitude during culture, particularly in the case of biaxial loading, has not been investigated. In this study, the dynamic biaxial culture system was used to study growth and remodeling of fibroblast-seeded collagen gels in response to dynamic mechanical stimuli.

2. Methods

2.1. Preparation of cruciform-shaped fibroblast-seeded collagen gels

NIH 3T3 fibroblasts were cultured in DMEM containing 10% calf serum and antibiotics, and passaged every 3–4 days. The preparation of cell-seeded collagen gels was performed on ice to avoid premature gelation. Specifically, 0.45 mL of reconstituted buffer (10×) was mixed thoroughly with 0.9 mL of DMEM solution (5×) and 1.2 mL of concentrated rat tail type I collagen (9.37 mg/mL; BD Biosciences, San Jose, CA), and then neutralized with ~110 μL of 0.1 N sodium hydroxide before adding 1.95 mL of the cell

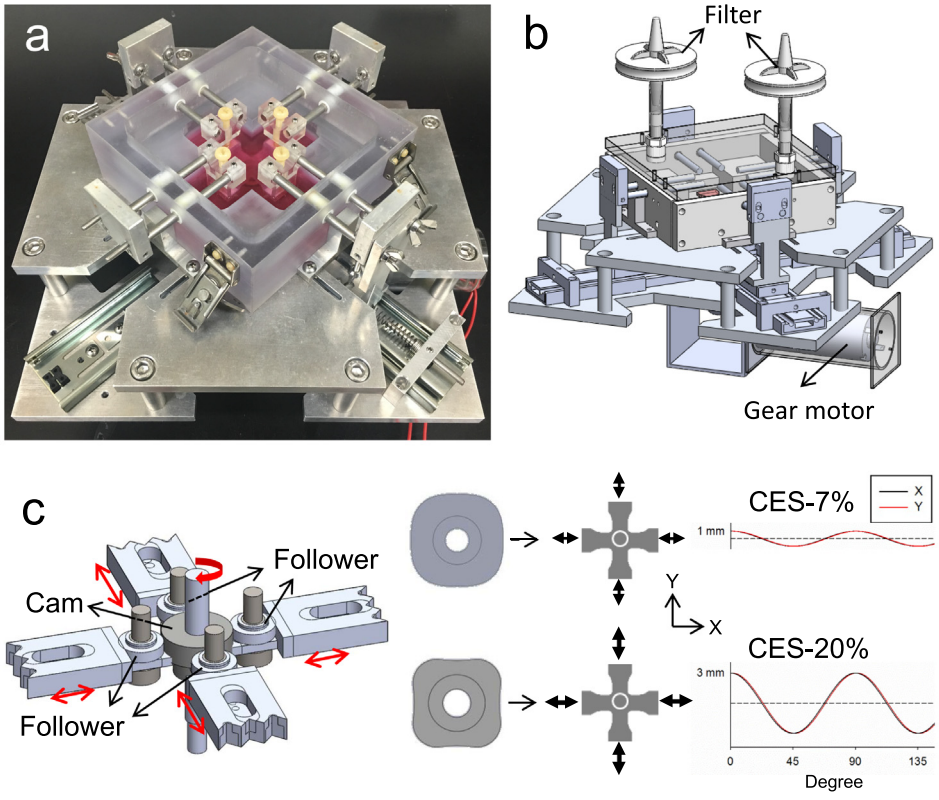

Fig. 2. Photograph (a) and schematic (b) of the culture chamber mounted on the dynamic actuator system, and design of the cam and followers (c). Three different cams were used to provide three strain magnitudes (7%, 15%, and 20%). The displacement diagrams of the followers for cyclic equibiaxial stretching at two constant strain magnitudes (CES-7% and CES-20%) are shown.

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