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Effect of cyclic mechanical strain on glycosaminoglycan and proteoglycan synthesis by heart valve cells

Vishal Gupta [a], Hubert Tseng [a], Brian D. Lawrence [b], K. Jane Grande-Allen [a,*]

[a] Department of Bioengineering, Rice University, Houston, TX 77251-1892, USA
[b] Department of Biomedical Engineering, University of Toledo, Toledo, OH 43606-3390, USA

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Abstract

Heart valves are presumed to remodel their extracellular matrix upon application of mechanical strains. In this study, we investigated the effect of cyclic tensile strain on valvular interstitial cells' synthesis of glycosaminoglycans (GAGs) and proteoglycans (PGs), which are altered during myxomatous degeneration. Interstitial cells were isolated from mitral valve leaflets and chordate, and seeded separately within three-dimensional collagen gels. Cell-seeded collagen gels were then subjected to cyclic strains of 2%, 5% or 10% at 1.16 Hz for 48 h using a custom-built stretching device. The application of cyclic strains reduced the total GAGs retained within collagen gels in a magnitude-dependent manner for both leaflet and chordal cells. With increasing strain magnitude, however, secretion of total GAGs into the medium was reduced for leaflet cells and elevated for chordal cells. Retention of 4-sulfated GAGs increased with increasing strain magnitude for both cell types; for the chordal samples, retention of 6-sulfated GAGs was reduced at higher strain magnitudes. Compared to statically constrained or unconstrained conditions, the application of cyclic strain reduced the secretion of 6-sulfated GAGs by both cell types, and elevated secretion of 4-sulfated GAGs by leaflet cells only. Retention of the PG biglycan and secretion of the PG decorin was significantly reduced at 10% strain compared to 2% strain. In addition, there were numerous differences in the strain-dependent retention and secretion of GAGs and PGS within the leaflet and chordal groups. These results demonstrate that GAG and PG synthesis by VICs is regulated by cyclic stretching conditions.

Keywords: Glycosaminoglycan; Proteoglycan; Mechanical strain; Collagen gels; Valvular interstitial cells

1. Introduction

The application of mechanical strains has been shown to induce remodeling of the extracellular matrix (ECM) in many tissues, including heart valves [1,2]. Heart valves are maintained in vivo under a range of strain magnitudes during opening and closing of the valve and during relaxed or stressed conditions of the heart, such as exercise or pathological conditions. Correspondingly, the valvular interstitial cells (VICs) within heart valves are mechanoresponsive to tensile strains in two-dimensional (2-D) and three-dimensional (3-D) cell cultures [3,4] and to pressure and shear forces in organ culture [5,6], resulting in altered ECM synthesis. However, the effect of varying magnitudes of strain and frequencies, such as those found in vivo, has not been widely investigated in the context of VICs in 3-D cultures. One study of VICs did examine the effect of strain magnitudes on ECM synthesis, but in 2-D culture [3].

Nevertheless, investigations of other types of cardiovascular cells have shown that their synthesis of ECM is significantly influenced by strain. To provide several examples, cardiac fibroblasts subjected to either uniaxial or equibiaxial strains tend to express more collagen III mRNA at lower strains and less at higher strains compared to unstretched controls [7]. Smooth muscle cells (SMCs) have also been shown to synthesize more collagen when subjected to 10% or higher magnitudes of strain [8]. Moreover, the secretion of transforming growth factor-β [9] and fibroblast growth factor-2 [10] by SMCs was found to be proportional to

* Corresponding author. Tel.: +1 713 348 3704; fax: +1 713 348 5877.
E-mail address: grande@rice.edu (K.J. Grande-Allen).

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strain. The release of heparan sulfate glycosaminoglycans (GAGs) and fibronectin by vascular endothelial cells was decreased when shear rates [11] and biaxial strains [12], respectively, were elevated. Therefore, strain demonstrably but variably affects ECM synthesis in different types of cardiovascular cells and its effect on VICs should be investigated in order to improve our understanding of valve mechanobiology.

GAGs and proteoglycans (PGs) are integral ECM components of valve tissues and play an important role in defining the material and structural behavior of valves. PGs consist of GAG chains (linear molecules of repeating disaccharides) attached to a core protein; these molecules perform many biological functions in tissues [13]. The sulfation pattern of GAG chains, i.e. whether they are 4-sulfated or 6-sulfated, characteristically varies according to the biological and biomechanical needs of the tissue [14]. Dermatan sulfate PGs such as decorin and biglycan, which contain mostly 4-sulfated GAGs, regulate collagen fibril diameter and fibrous tissue organization [15,16]. In contrast, the GAG hyaluronan (HA), which does not bind to a protein core but tends to aggregate with large PGs such as versican (mainly containing 6-sulfated GAGs), entraps large amounts of aqueous solvent to provide compression resistance [17]. Previous studies of mitral valves from our laboratory showed that 4-sulfated GAGs and the PGs decorin and biglycan were abundant in tensile loading regions such as chordae tendineae, while regions experiencing compression, such as the leaflet free edges, contained more hyaluronan, 6-sulfated GAGs, and versican [18]. In pathological conditions such as myxomatous mitral valve disease, the valve tissues experience altered tissue loading, along with an overabundance of GAGs and PGs [19,20].

GAG and PG synthesis has recently been shown to be regulated by static mechanical strains applied to VICs grown in 3-D cell cultures [4,21]. Although VICs have been investigated under cyclic straining conditions (in 2-D culture), no study has examined the effect of strain on GAG and PG synthesis by VICs [3,22]. Determining how GAG and PG synthesis responds to different strains is important since the patterns of strain and distribution of GAGs and PGs are evidently altered during myxomatous valve degeneration. In this study, VICs seeded within 3-D collagen gels were stretched at different strains within a custom-designed bioreactor. We chose to use a 3-D collagen matrix, as opposed to a 2-D cell culture approach, in order to provide a more biologically and anatomically appropriate model, since a 3-D environment for the cells is closer to in vivo conditions [23]. Furthermore, previous studies have shown that VICs seeded on top of or within 3-D collagen scaffolds retain their native phenotype and secrete GAGs and PGs comparable to those found in vivo [24–27]. Therefore, the purpose of this study was to determine if the GAG and PG synthesis by VICs isolated from distinct regions of the mitral valve is regulated by different cyclic strain magnitudes.

2. Materials and methods

2.1. Cell culture and seeding in collagen gels

Porcine mitral valves were obtained from an abattoir and VICs were isolated using a previously described protocol [4]. Briefly, the tissues were first soaked in Dulbecco's modified Eagle's medium (DMEM; Mediatech, Herndon, VA), containing 2 mg ml^{-1} collagenase type II (Worthington, Lakewood, NJ), within an incubated shaker for 20 min (140 rpm, 37 °C). The endothelial cells were then brushed from the valve surface using cotton swabs and all chordae tendineae were dissected from the leaflet. The separated leaflet and chordal tissues were minced and dissociated with DMEM containing 1 mg ml^{-1} collagenase type III and 0.1 mg ml^{-1} hyaluronidase (both from Worthington) for 4 h in an incubated shaker. Leaflet and chordal VICs were cultured separately in DMEM:F12 medium (1:1, containing low glucose with HEPES, Mediatech) with 10% bovine growth serum (BGS; HyClone, Logan, UT) and 1% antibiotic–antimycotic solution (Mediatech). Our laboratory has previously shown that the resulting cell populations demonstrate phenotypic characteristics that are non-endothelial, and that are slightly different between leaflet VICs and chordal VICs [28].

VICs were seeded within collagen gel scaffolds to provide them with an in vivo-like 3-D environment. The collagen gels were prepared using a protocol from Eastwood et al. [29]. Briefly, 8 parts rat-tail collagen type I at 2.28 mg ml^{-1} (BD Biosciences, Bedford, MA) in 0.02 M acetic acid, 1 part 10× DMEM and 1 part cells suspended in 1× DMEM (1 million cells per ml of gel) were mixed together and brought to physiological pH using 5 M NaOH dropwise. The resulting gel solution was immediately poured into the mold (with or without anchors) within the cyclic stretching device. To minimize batch-to-batch variability, the same type of cells (from the same primary culture harvest date and passage number) and collagen lot number were used in the various samples of engineered tissues. VICs from passage numbers 6–7 were used to prepare all the collagen gels; VICs cultured in tissue culture flasks have been shown to maintain a consistent phenotype until late passage number [30].

2.2. Stretching device

A stretching device developed in our laboratory was used to apply cyclic mechanical strains to collagen gels seeded with VICs [31]. The details of this device have been published previously [31], but briefly, the main components of the device consisted of an aluminum base, a stretching cam and a culture lid. The collagen gel anchors were connected to roller bearings, which were displaced by the stretching cam. The stretching cam was shaped as a 4-cycle sinusoidal waveform superimposed around the circumference of a circle and caused the collagen gel to be stretched