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Influence of strain on proteoglycan synthesis by valvular interstitial cells in three-dimensional culture

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Abstract

Differently loaded regions of the mitral valve contain distinct amounts and types of proteoglycans (PGs); these PG profiles are altered in abnormal loading and disease conditions. We developed an *in vitro* three-dimensional model to analyze PGs secreted by valvular interstitial cells (VICs) isolated from distinct regions of porcine mitral valves (leaflet or chordae) and subjected to either biaxial or uniaxial mechanical constraints. In addition, the PGs, DNA and collagen content of the collagen gels was monitored over time. All three PGs previously found in heart valves (decorin, biglycan and versican) were present in the collagen gels and the conditioned medium. Compared to unconstrained gels, the constrained collagen gels (whether biaxially or uniaxially loaded) retained more decorin and biglycan but less versican. However, the conditioned medium from constrained collagen gels contained higher amounts of all three PGs than did medium from unconstrained gels. Constrained collagen gels containing leaflet cells retained more decorin and biglycan than did those containing chordal cells. DNA content was maintained early in the culture period but was reduced by 55–80% after 7 days, whereas PG synthesis increased over time. At the end of the culture period, the cell density was highest in the biaxial region of gels seeded with leaflet cells. In contrast, collagen content in both constrained and unconstrained gels remained consistent over culture duration. This study provides valuable information about the role of applied loading on proteoglycan segregation, which should aid in tissue engineering applications and for understanding valve biology and pathology.
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Keywords: Proteoglycan; Extracellular matrix; Mechanical constraint; Collagen gels; Valvular interstitial cells

1. Introduction

Mechanical loading is well known to regulate the production and organization of extracellular matrix (ECM) [1]. Valvular interstitial cells (VICs), in particular, have been recently demonstrated to be mechanoresponsive to pressure and shear forces in organ culture [2,3] and to tensile strains in two-dimensional (2D) cell cultures [4], resulting in altered production of matrix collagen and glycosaminoglycans. The effect of mechanical stimulation on VICs' production of specific proteoglycans (PGs), however, has not yet been investigated, even though PG production is modulated by mechanical strains in other cardiovascular cell types [5–7]. Moreover, the direction of loading itself is influential; fibroblasts exposed to biaxial strains in 2D culture responded different from those experiencing uniaxial strains [8,9].

PGs, which are composed of a core protein and various glycosaminoglycan (GAG) chains [10], are one of the major ECM components in soft tissues and perform many biological and structural functions [11,12]. The chondroitin/dermatan sulfate PGs decorin, biglycan and versican are the most commonly found PGs in heart valves [13]. Decorin and biglycan are small PGs that are known to aid in collagen fibril formation [14,15], whereas the large PG versican aggregates with the GAG hyaluronan, together accumulating large volumes of water that provides compressive resistance to the tissues [12]. Previous research on mitral valves has shown that valve regions in tension

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(chordae and center of anterior leaflet) contain abundant quantities of decorin and biglycan, while regions experiencing compression (posterior leaflet and free edge of anterior leaflet) contain more of the PG versican [13]. Furthermore, mitral valves with myxomatous degeneration, which are subjected to altered tissue loads, contain more GAGs and PGs than normal [16].

3D collagen scaffolds have been used widely to provide cells with a more natural culture environment [17]. Several studies have shown that VICs seeded on top of or within collagen matrices retain their native phenotype and secrete the GAGs and PGs normally present in heart valves [18–21]. As noted earlier, VICs have been shown to synthesize ECM in response to mechanical strains [2–4]. However, most previous investigations of the mechanically stimulated responses of VICs utilized either monolayer cell culture or organ culture approaches. Recently, we reported the use of a 3D tissue-engineered model to determine the effect of biaxial vs. uniaxial loading on GAG synthesis [21]. In this paper, we describe the continuation of that study to analyze the effect of different loading conditions on the production of distinct PGs; this information is relevant to heart valves as well as to other tissues. The collagen and DNA contents of these engineered tissues were also monitored, as their concentrations reportedly affect collagen gel contraction and hence the tensile strains experienced by VICs [22]. Studies on other cell types, such as fibroblasts seeded within collagen gels, have also shown that cells can become quiescent and undergo apoptosis in collagen gels depending upon their anchoring conditions [23,24]. As in our previous report, we utilized a 3D model to study the effects of biaxial or uniaxial loading on VICs isolated from leaflets and chordae of mitral valves.

2. Materials and methods

2.1. Cell culture

Porcine mitral valves obtained from an abattoir were used to develop primary cultures of VICs. Mitral valve leaflets and chordae were dissociated separately in a two-step collagenase digest as described previously [21]. Isolated VICs were then cultured in DMEM:F12 medium (1:1, containing low glucose with HEPES, Mediatech, Herndon, VA) with 10% bovine growth serum (BGS, HyClone, Logan, UT) and 1% antibiotic/antimycotic/antifungal solution (Mediatech). The culture was incubated in a humidified atmosphere of 95% air/5% CO_2 at 37 °C with changes of medium every 48 h. The cells were split in a 1:3 ratio when they became 90% confluent. Primary leaflet and chordal cell cultures of passage 5–10 were used to prepare the collagen gels.

2.2. Mold preparation

A mold was designed to apply static loading to collagen gels [21]. Briefly, due to the shape of this mold, the collagen gels anchored within were regionally subjected to either uniaxial or biaxial strains. Different regions of the mitral valve experience biaxial and uniaxial strains, so this 3D structure was chosen to provide more *in vivo*-like conditions. Holders for anchoring the collagen gel were prepared using stainless steel tubing and polyester mesh (Fig. 1). Several collagen gels were prepared without anchors to test the engineered tissue formation in an unconstrained environment (Fig. 1, inset).

2.3. Cell seeding in collagen gels

Mitral valve leaflet and chordal VICs were seeded in collagen gels using a protocol adapted from Eastwood et al. [25]. These collagen gels were prepared using 8 parts rat-tail collagen type I in 0.02 M acetic acid, 1 part 10 × DMEM and 1 part cells suspended in 1 × DMEM. Briefly, rat-tail tendon collagen (BD Biosciences, Bedford, MA) at 2.28 mg ml^{-1} was brought to physiological pH by the addition of 5 M NaOH and kept over ice to delay gel formation. Cells (1×10^6 ml^{-1}), suspended in 1 × DMEM, were added to the collagen solution and immediately poured into the mold (with or without anchors). A number of constrained (8 leaflet, 6 chordal) and unconstrained (4 leaflet, 3 chordal) collagen gels were prepared for direct comparison. Every other day the conditioned medium from the collagen gels was collected and replaced with fresh medium. The medium collected throughout the 7 day period for the 3D culture was later combined for PG analysis. After 7 days,

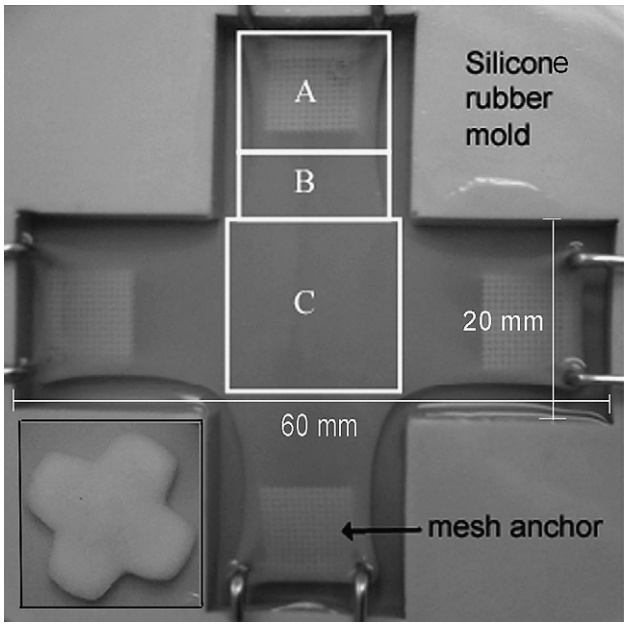

Fig. 1. Differently loaded regions of collagen gels seeded with valvular interstitial cells. Constrained collagen gel: (a) mesh, (b) uniaxial and (c) biaxial region. The dimensions of the cross were chosen as length 60 mm, width 20 mm and depth 7 mm). Unconstrained collagen gel is shown in the inset (prepared in the same mold as constrained but without anchors). Adapted from Gupta et al., *Tissue Eng.* 13(1), 41–49, 2007 (with permission).

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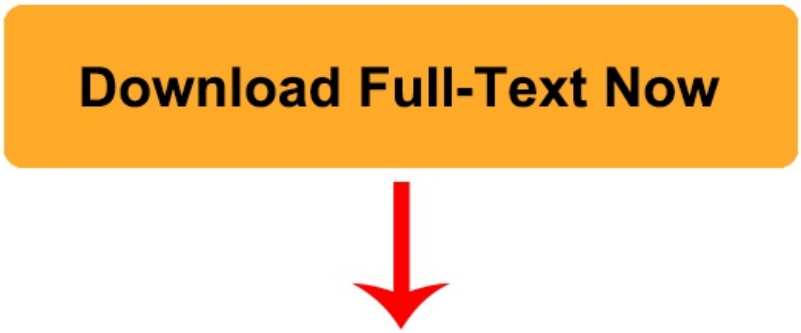

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