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Valve interstitial cell tensional homeostasis directs calcification and extracellular matrix remodeling processes via RhoA signaling

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ABSTRACT

Aims: Valve interstitial cells are active and aggressive players in aortic valve calcification, but their dynamic mediation of mechanically-induced calcific remodeling is not well understood. The goal of this study was to elucidate the feedback loop between valve interstitial cell and calcification mechanics using a novel three-dimensional culture system that allows investigation of the active interplay between cells, disease, and the mechanical valve environment.

Methods & results: We designed and characterized a novel bioreactor system for quantifying aortic valve interstitial cell contractility in 3-D hydrogels in control and osteogenic conditions over 14 days. Interstitial cells demonstrated a marked ability to exert contractile force on their environment and to align collagen fibers with the direction of tension. Osteogenic environment disrupted interstitial cell contractility and led to disorganization of the collagen matrix, concurrent with increased αSMA, TGF-β, Runx2 and calcific nodule formation. Interestingly, RhoA was also increased in osteogenic condition, pointing to an aberrant hyperactivation of valve interstitial cells mechanical activity in disease. This was confirmed by inhibition of RhoA experiments. Inhibition of RhoA concurrent with osteogenic treatment reduced pro-osteogenic signaling and calcific nodule formation. Time-course correlation analysis indicated a significant correlation between interstitial cell remodeling of collagen fibers and calcification events.

Conclusions: Interstitial cell contractility mediates internal stress state and organization of the aortic valve extracellular matrix. Osteogenesis disrupts interstitial cell mechanical phenotype and drives disorganization, nodule formation, and pro-calcific signaling via a RhoA-dependent mechanism.

1. Introduction

Aortic valve disease (AVD) is a leading cause of cardiovascular morbidity resulting in approximately 15,000 deaths per year in the USA. It is primarily a disease of the elderly, occurring in 2.8% of Americans over the age of 75 [1]. It is an active degenerative process driven by complex cell-cell and cell-matrix interactions [2]. Despite sharing risk factors with atherosclerosis [3], it is a pathobiologically unique disease that has no clinically proven biologically-based intervention strategies outside of cardiothoracic surgery [4].

As the aortic valve becomes diseased, its mechanics change in a number of ways. The leaflets stiffen as calcific nodules form [5], the extracellular matrix becomes disorganized [6] and altered in composition [7], and the strain experienced by valvular interstitial cells (VIC) increases [8]. These changes influence VIC phenotype and modulate VIC interactions with their environs [9,10]. Strain state regulates VIC cytokine secretion [11,12], calcification [13], collagen synthesis [14], alignment [15], and proliferation [16]. Extracellular matrix composition influences VIC homeostasis [17], in part through modulation of leaflet [18] and cellular [19] stiffness. VIC become activated in response to changes in their mechanical [20] or biochemical [21] situation, initiating a complex feedback loop between cellular decisions and changes in the extracellular

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environment. The temporal dynamics of this loop, as well as the role of disease progression on signaling, are not well understood. Understanding the time and phase governance of the mechanical-biological interplay in AVD is critical to the design of effective interventions that could capitalize on biological signaling cascades, rather than drug- or surgical-based strategies.

In this study, we have designed a novel system to culture VIC in 3D hydrogels with a controlled and sustained level of tension. The system allows measurement of dynamic tissue stresses developing within a 3D VIC and collagen environment, as well as imaging of changes to collagen and cell morphology via longitudinal confocal microscopy. This system allowed us to uniquely investigate the interplay between collagen fibers, cell morphology, activity, signaling, and osteogenesis in order to shed light on the complex mechanopathology of aortic valve disease. We identified a distinct tri-phasic temporal mechanobiological response of VIC to osteogenic environment. We further show that the tri-phasic remodeling and mechanobiological response is disrupted by RhoA inhibition.

2. Materials and methods

2.1. Bioreactor system fabrication

The systems were created using a custom mold, polydimethylsiloxane (PDMS) at a 15:1 base to curing agent ratio, and steel springs. A collagen hydrogel seeded with porcine aortic valve interstitial cells (PAVIC) was pipetted into the system with the use of a PDMS plug to help the hydrogel stay in place. The plug was then removed in 30 min, after the hydrogel had set (Fig. 1). PAVIC used in the system were harvested from fresh porcine aortic valves from a local abattoir, cultured up to a maximum of six passages, and seeded at 1 million cells per milliliter into a 1 mg/mL type I collagen hydrogel (rat-tail collagen, Corning, 354236), as described previously [15]. Hydrogels in the spring system were cultured in either control medium or osteogenic medium. Control medium was made from Dulbecco's Modified Eagle Medium (ThermoFisher Scientific, 12100061) with 10% fetal bovine serum (ThermoFisher Scientific, 10437028) and 1% penicillin-streptomycin (ThermoFisher Scientific, 15140122). Osteogenic medium was made from control medium supplemented with 10 mMol/L β-glycerophosphate (Sigma, G9422), sterile filtered 50 mg/mL ascorbic acid (Sigma, 1043003 USP), and 10 nMol/L dexa-methasone (Sigma, D4902) for 14 days with media changed every 48 h.

2.2. Effect of varying spring parameters

Two different springs (Table 1) were tested for their ability to recapitulate physiological tissue stress levels in the hydrogels over 14 days of culture. Both springs were made of SS316 stainless steel and obtained from Lee Spring Company.

Each spring was cut to 9 cm length and inserted into the system. The load required to cause deflection of the end of the springs between zero and 1 mm was measured for each spring system by downward translation of the spring onto a high-precision scale (Fig. 2A, B). This process was repeated using five different systems for each type of spring, with all five springs obtained from the same batch from Lee Spring Company, to estimate variation between springs. A cantilevered beam approximation was used to calculate the effective modulus (EI) of each type of spring according to equation (1).

$$\delta_{max} = \frac{PL^3}{3EI} \tag{1}$$

Table 1
Spring parameters.

Spring parameter	Spring 1	Spring 2
Wire diameter	0.008 in	0.01 in
Outside diameter	0.088 in	0.088 in
Hole diameter	0.094 in	0.094 in
Load at solid length	0.333 lb	0.666 lb
Rate	0.70 lb/in	1.10 lb/in
Part ID	CI 008B 09	CI 010B 13

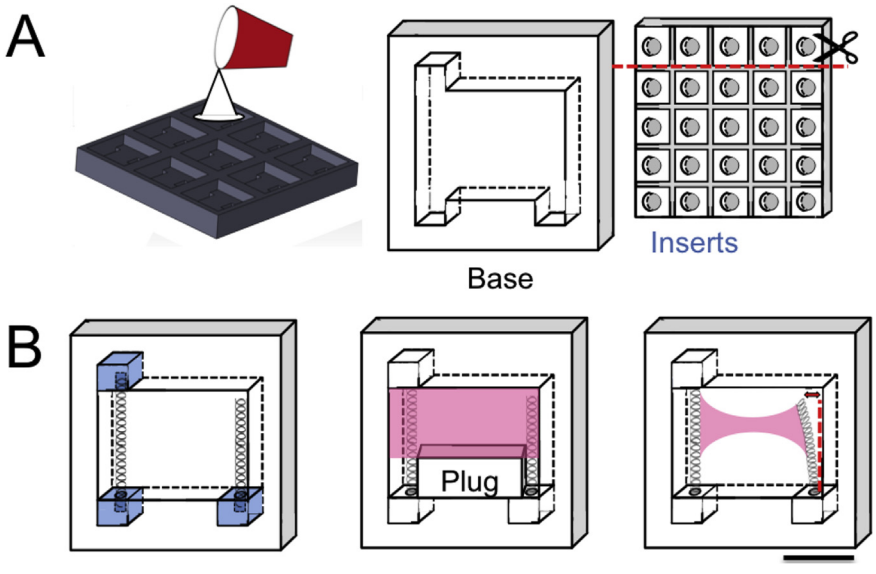

Fig. 1. Design and manufacturing of system. A. Custom molds machined from polycarbonate. B. Inserts and springs were inserted into device base to form complete system (left). Temporary plug prevented downward flow of collagen hydrogel (pink) as VIC gels were applied to upper region of device (center). The plug was removed once the gel polymerized. Over 14 days, hydrogel + VIC contracted, deflecting cantilevered spring (right) by a measurable amount. Scale bar is 1 cm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)