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Regional structure–function relationships in mouse aortic valve tissue

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ABSTRACT

Site-specific biomechanical properties of the aortic valve play an important role in native valve function, and alterations in these properties may reflect mechanisms of degeneration and disease. Small animals such as targeted mutagenesis mice provide a powerful approach to model human valve disease pathogenesis; however, physical mechanical testing in small animals is limited by valve tissue size. Aortic valves are comprised of highly organized extracellular matrix compartmentalized in cusp and annulus regions, which have different functions. The objective of this study was to measure regional mechanical properties of mouse aortic valve tissue using a modified micropipette aspiration technique. Aortic valves were isolated from juvenile, adult and aged adult C57BL/6 wild type mice. Tissue tensile stiffness was determined for annulus and cusp regions using a half-space punch model. Stiffness for the annulus region was significantly higher compared to the cusp region at all stages. Further, aged adult valve tissue had decreased stiffness in both the cusp and annulus. Quantitative histochemical analysis revealed a collagen-rich annulus and a proteoglycan-rich cusp at all stages. In aged adult valves, there was proteoglycan infiltration of the annulus hinge, consistent with the observed mechanical differences over time. These findings indicate that valve tissue biomechanical properties vary in wild type mice in a region-specific and age-related manner. The micropipette aspiration technique provides a promising approach for studies of valve structure and function in small animal models, such as transgenic mouse models of valve disease.

1. Introduction

Aortic valve disease (AVD) is a significant cause of cardiovascular morbidity in humans and occurs in greater than 2% of people in the US (Nkomo et al., 2006; Otto, 2006). There is increasing interest in the development of durable aortic valve bioprostheses for the treatment of AVD (Vesely, 2005; Gallegos, 2006; Sacks et al., 2009b). Aortic valve malformation (AVM) is a heritable condition that underlies the majority of AVD cases, suggesting a developmental etiology (Hoffman and Kaplan, 2002; Cripe et al., 2004; Roberts and Ko, 2005). AVD is associated with valve cell-matrix abnormalities (Otto et al., 1994; Fedak et al., 2003; Hinton et al., 2006) and consequently, extracellular matrix (ECM) disorganization may alter the mechanical microenvironment of valves (Matsumoto et al., 2002; Yip et al., 2009). Aging is a significant risk-factor for AVD (Otto, 2006; Tzemos et al., 2008), and recent studies have demonstrated that aging induces alterations in ECM material properties of porcine valves (Stephens and Grande-Allen, 2007; Stephens et al., 2009). Small animal models, including targeted mutagenesis mouse models of valve disease, provide an effective tool to study genetic factors underlying valve disease pathogenesis (Yutzey and Robbins, 2007). However, the mechanical properties of normal mouse valve tissue remain largely unknown.

Positioned between the left ventricle and aorta, the aortic valve functions to maintain forward blood flow throughout the cardiac cycle. Late embryonic valvulogenesis is characterized by ECM remodeling and organization that continues postnatally (Armstrong and Bischoff, 2004; Aikawa et al., 2006; Hinton et al., 2006). The aortic valve is situated within the aortic root and is composed of discrete cusp and annulus regions (Fig. 1). Cusps consist of highly organized connective tissue hinged to a crown-shaped fibrous annulus (Yacoub et al., 1999; Anderson, 2000). The valve annulus functions as a supporting structure for valve cusps; however, regional valve structure–function relationships have not been clearly defined. Importantly, changes in valve anatomy and underlying matrix composition may lead to alterations in valve mechanical properties and therefore affect its function.

To date, mechanical studies of aortic valves as a viscoelastic tissue have focused predominantly on cusps in valves from large animals or humans (Christie and Barratt-Boyes, 1995a; Billiar and Sacks, 2000; Sacks et al., 2009a). Previous studies have identified

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differences between different heart valves such as aortic and pulmonary valves (Christie and Barratt-Boyes, 1995b; Merryman et al., 2007; Merryman, 2010) and reported the mechanical properties of the aortic root in large animal models (Grande et al., 1998; Nicosia et al., 2002; Yap et al., 2010). However, regional differences in valve biomechanics and the mechanical properties of the annulus region in particular remain largely unexplored.

Physical interrogation strategies to determine biomechanical properties of valve tissue have been developed for large animals. However, conventional uniaxial or biaxial mechanical testing of valves in smaller animals such as mice is complicated due to its tissue size. Micropipette aspiration systems have been used previously for measuring mechanical properties of chondrocytes and their pericellular matrix (Guilak et al., 1999; Alexopoulos et al., 2003), valve interstitial cells (Merryman et al., 2006b; Zhao et al., 2009), and embryonic chicken atrioventricular endocardial cushions (Butcher et al., 2007). The objective of this study was to use a modified micropipette aspiration technique coupled with quantitative histology to measure structure–function relationships of regional mouse aortic valve tissue and to determine how these properties change with aging. Based on previous findings in humans (Roberts, 1970; Otto et al., 1994), the hypothesis was that the aortic valve annulus region would demonstrate significantly higher tissue stiffness compared to the cusp region, and the entire valve would stiffen with age.

In summary, tensile stiffness in the annulus region is significantly greater than the cusp region at all stages, and interestingly, tissue stiffness in both valve regions decreases with advanced age in mice. Histochemical analyses were consistent with the biomechanical findings both spatially and temporally. These results establish the feasibility of micropipette aspiration in mouse valve tissue and provide a basis to evaluate the mechanical and structural properties of targeted mutagenesis mouse models of valve disease. This approach may improve our fundamental understanding of age-related phenomena in valve tissue and ultimately contribute to the development of new therapies for AVD.

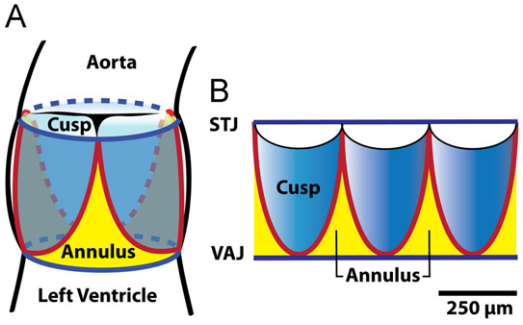

Fig. 1. Anatomy of the mature aortic valve. The aortic valve consists of three cusps (blue), hinged (red lines) to the supporting annulus (yellow). The aortic root extends from the ventriculo-arterial junction (VAJ, bottom blue ring) proximally to the sinotubular junction (STJ, top blue ring) distally and is composed of fibrous valve annulus tissue and the arterial sinuses of Valsalva (A). When the aortic root is transected, the valve cusp and annulus regions are revealed (B).

2. Materials and methods

Aortic valves were isolated in situ from C57BL/6 wild type mice at three postnatal developmental stages: juvenile (1-month old (mo)), adult (4 mo), and aged adult (12 mo). Ten mice per stage were used for mechanical studies and three mice per stage were used for histochemistry. All protocols were approved by the Institutional Animal Care and Use Committee at Cincinnati Children's Hospital Medical Center.

2.1. Valve tissue isolation and preservation

The heart was excised after the mice were euthanized. The dissection extended superiorly from the left ventricular apex to the interface between the anteriorly oriented pulmonary artery and posteriorly oriented aorta. Further dissection around the proximal aspect of the circumference of the aortic valve was performed. The tubular aortic valve structure within the aortic root from the ventriculo-arterial junction to the sinotubular junction was removed intact (Fig. 1A). The tissue was washed and frozen for less than 72 h in Dulbecco's Phosphate Buffered Saline (PBS, Hyclone) and supplemented with protease inhibitor (1:100, Sigma) for biomechanical testing. Previous studies demonstrate that short-term freezing does not adversely affect tissue biomechanical properties (Woo et al., 1986). Tissue was processed for histochemical analyses as previously described (Hinton et al., 2006).

2.2. Valve tissue preparation for mechanical testing

Glass slides coated with the silicone polymer polydimethylsiloxane (PDMS) were mounted on the microscope chamber, and a notch exposing the right aspect of the chamber was created (Fig. 2A). The isolated aortic valve wheel was dissected open to facilitate accessibility (Fig. 2B) such that the ventricular surface of the valve cusp or annulus region was oriented perpendicular to the plane of the micropipette. Tissue hydration was maintained for the duration of testing.

2.3. Mechanical testing of mouse valve tissue using micropipette aspiration

Micropipettes with an inner radius of 25–30 μm were used to ensure that the geometric influences on the measured mechanical properties were negligible (Aoki et al., 1997; Ohashi et al., 2005). Upon contact, aspiration pressure (ΔP) was applied using small incremental steps of less than 0.5 kPa to the tissue surface through the micropipette via a custom-built syringe water reservoir, resulting in a small portion of the tissue surface aspirating into the pipette. Seal formation was observed when resistance to aspiration increased sharply and tissue was

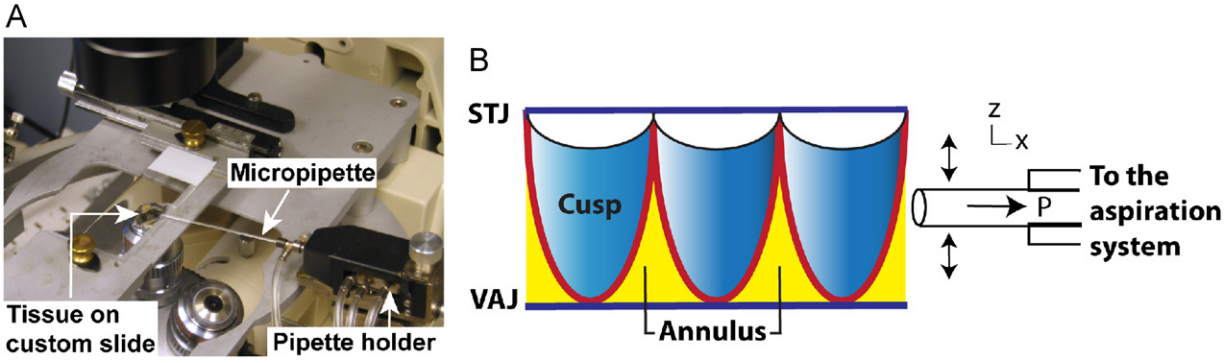

Fig. 2. Valve tissue orientation for micropipette aspiration. The microscope stage holds a PDMS-coated glass slide that is designed to accommodate the lateral introduction of a micropipette (A). Valve tissue is secured in the slide with micropins. The micropipette is attached to the pipette holder and accesses the tissue surface in an orthogonal plane; upon contact, a suction pressure is applied (B).