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Determination of the tensile mechanical properties of the segmented mitral valve annulus

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ABSTRACT

The mitral valve annulus is a complex and irregular component of the mitral valve apparatus, serving both a structural and sphincteric role. We have sought to determine the mechanical properties of the mitral valve annulus segmentally. Twenty porcine hearts were dissected to isolate the annulus. The annulus was segmented into four sections: anterior, posterior, and left and right commissural sections. Ten of these were tensile tested to failure as control samples. The remaining ten were digested in order to fully isolate the annulus from the myocardium, and subsequently tensile tested to failure. Histological samples of each segment were analysed to determine collagen/annular content. Whole segments of muscular annulus were tensile tested to failure; the stress and strain at failure and location of failure were determined in these larger specimens. Our results demonstrated that the anterior annulus is stiffer than the posterior segment by a factor of approximately 27 at a 2% strain level, and approximately 13 at a 6% strain. There is a trend in the results that identifies that the muscular annulus is stiffest at the right commissural segment, while the posterior segment tends to be the least stiff. The stiffness of the samples can be correlated with the area associated with the dense collagen annulus using histological analysis. Finally, the weakest section of the mitral valve annulus was identified as the intersection of the right commissural segment and the posterior segment.

1. Introduction

Mitral valve (MV) disease is the most prevalent form of heart valve disease among the US population (Roger et al., 2012), and in moderate and severe cases, the disease can lead to arrhythmia and ultimately heart failure. MV disease can affect any one of the four components of the MV apparatus: the leaflets, the chordae tendinae, the papillary muscles (PMs) and/or the annulus (MVA). Mechanisms of mitral valve disease can be subdivided into two categories: (1) functional, where the structure of the valve is normal and the disease results from remodelling, or (2) organic, which is characterised by the presence valvular lesions (Enriquez-Sarano et al., 2009). Further classification can be made according to the movement of the leaflets, following Carpentier's functional classification of three main types of regurgitation (Carpentier, 1983). According to this classification, valves exhibiting a normal structure but which leak due to annular dilation or leaflet perforation are classified as Type I. Type II dysfunction is characterised by increased leaflet motion (such as in prolapse), and Type III by restricted leaflet movement (specifically; IIIa – restricted motion during both diastole and systole, and IIIb – motion is predominantly restricted during systole). The causes of mitral valve disease are many and varied, including rheumatic fever, myxomatous degeneration, endocarditis and ischaemic MV disease. In many cases, MV disease can lead to dilation of the annulus, thus aggravating mitral regurgitation (MR). It has been speculated that the underlying structure of the mitral valve annulus has a role to play in this dilation (Fedak et al., 2008).

Definitions of the annulus vary from discipline to discipline. In echocardiography, the annulus is defined as the hinge point of the leaflets; surgically it is identified as the apparent transition between the left atrial tissue and the MV leaflet (Rahman et al., 2010). Angelini et al. studied the structure of the MVA histologically in 13 hearts and found it to be a non-continuous collagenous structure, chord-like in some sections and curtain-like in others (Angelini et al., 1988). It is in fact generally agreed that the annulus is not a continuous well defined chord, but rather an incomplete and irregular fibrous structure (Angelini et al., 1988; Ho, 2002; Rahman et al., 2010; Walmsley, 1978).

Properties of the MV annulus have been studied *in vitro* previously. He and Bhattacharya analysed the phenomenon of

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annular tension by pressurising a valve attached to a series of radially arranged strain gauges (He and Bhattacharya, 2008). This study examined the forces in play when the annulus is closed and contracted, rather than during the open, expanded phase. However, it is during the expanded phase that the annulus experiences the highest strains, and therefore it may be argued that it is the tensile force present during diastolic relaxation that is the cause of annular dilation. A number of *in vivo* studies have also been conducted. In their study, Siefert et al. investigated the *in vivo* forces generated by the closing mitral valve in an ovine model (Siefert et al., 2012). Jensen et al. (2008) investigated the vertical (*i.e.* base-apical) forces experienced by the mitral valve annulus in 16 porcine models using strain gauges bonded to annuloplasty rings, demonstrating the saddle-shaped nature of the mitral valve. However, to date there has been no quantification of the mechanical properties of the mitral valve annulus.

This study aims to quantify the tensile properties of the segmented mitral annulus and correlate these with histological findings. Potentially the findings of this study may enhance our understanding of MV annulus enlargement, and may subsequently enlighten new transcatheter annuloplasty techniques or lead to the design of better surgical MV repair devices.

2. Methods and materials

2.1. Mitral valve annulus isolation

Twenty porcine hearts weighing between 300 g and 400 g were frozen when fresh, and defrosted individually on the day of testing. These specimens were divided into two groups: a control group ($n=10$), and a digested group ($n=10$).

The ten control specimens were dissected and prepared on the day of testing. The annulus was isolated from the heart in the following manner: the right side of the heart was removed, leaving the left ventricle (LV) and left atrium (LA). From the remaining tissue, the LA was removed along with the bulk of the LV. The specimen was then cleaned of fat (particularly at the coronary sulcus), and the coronary sinus and circumflex branch were removed. The MV leaflets were then excised, and the remaining ring of tissue was trimmed to a width of 8 mm and a depth of 4 mm (approximately). This ring was then divided into four sections; the anterior section (A), the posterior section (P) and two commissural sections (left commissural (Cl) and right commissural (Cr)), each about 30 mm long (see Fig. 1). As the thickness of the anterior section is predetermined by the anatomy of the heart, the A specimen was cut to a height of 8 mm, in order to match the dimensions of the other three sections as closely as possible.

Ten additional samples (prepared in the same way as described for the control samples) were digested using a detergent in order to fully isolate the annulus from the myocardium. The digestion protocol is as follows: 24 h in ddH_20, 3 days in TritonX-100 solution (490 ml ddH_20, 10 ml TritonX-100, 3.4 ml ammonium hydroxide (Sigma-Aldrich, Ireland) followed by 48 h rinsing in ddH_20 (Sheridan et al., 2012). For all stages of the digestion, samples are stored at 4 °C, with gentle agitation (see Fig. 1).

2.2. Mechanical testing

The control and the digested samples were tested under identical mechanical and environmental conditions. The tests were conducted in a water bath at 37 °C. Each test consisted of 5 preconditioning cycles from 0.5 N to 1.5 N at a speed of 2 mm/min (approximately 2% to 4% strain), followed by a tensile test at 2 mm/min to the first sign of failure using a Zwick Universal testing machine (Zwick Z2.5, Roell, Germany), fitted with a 50 N load cell. The samples were clamped at each end using a pair of customised, toothed grips lined with emery paper. A needle was inserted transversely into both ends of each sample, before being bolted into the grips (Fig. 2). The needle helped prevent slipping between the sample and the grip by anchoring between the grip teeth. On average, between 7 mm and 8 mm of sample was held within each grip, leaving a grip to grip sample length of approximately 15 mm. The dimensions of each sample were recorded when the samples were mounted within the grips. To determine the strain, each sample was

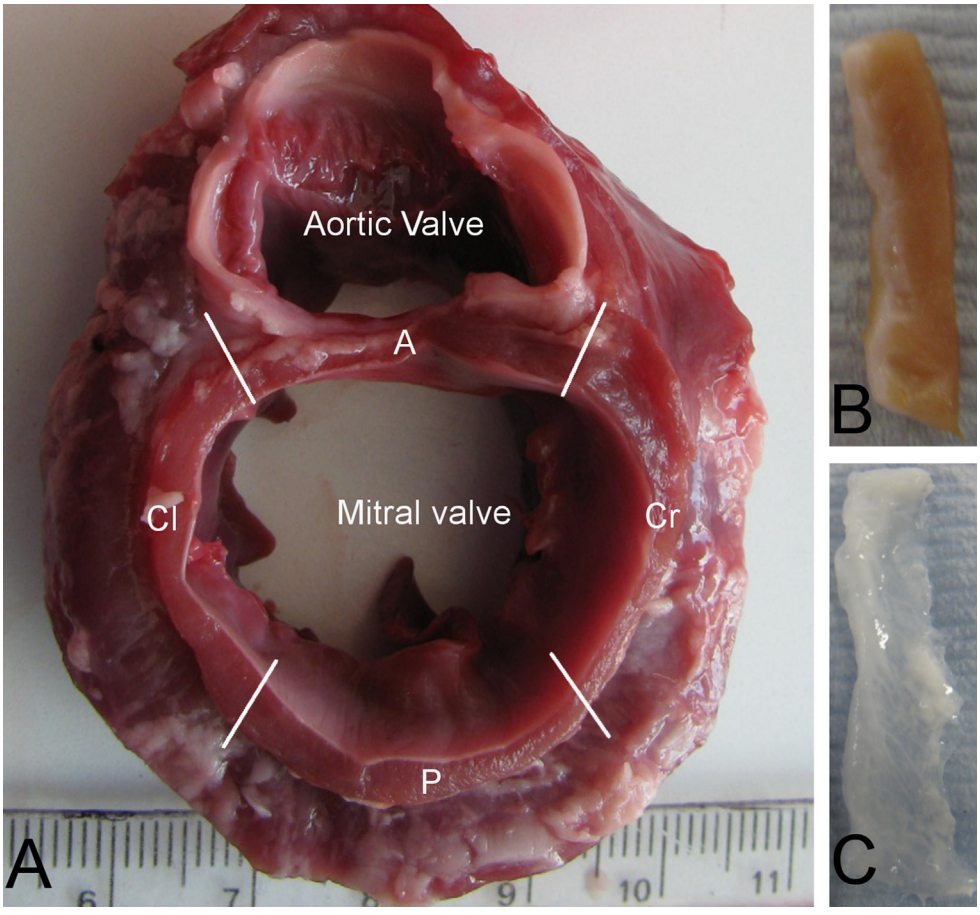

Fig. 1. (A) Partially dissected MV showing anterior [A], posterior [P], left commissural [Cl] and right commissural (Cr) segments; (B) Cl section of a control sample; (C) Cl section of a digested sample.