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The effects of collagen fiber orientation on the flexural properties of pericardial heterograft biomaterials

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Abstract

Improving cardiac valve bioprostheses (BHV) utilizing heterograft biomaterials requires a better understanding of their mechanical behavior. Flexure is a major mode of deformation for BHV leaflets during valve operation, inducing more complex deformation patterns within the tissue compared to tensile loads. In this study, we investigated the relation between collagen fiber preferred direction and the resulting flexural properties of native and glutaraldehyde-treated bovine pericardium. 20 mm × 4 mm strips were cut from the presorted sheets of bovine pericardium and divided into four groups: two directions of collagen fiber orientation in two groups of native and chemically treated specimens. Specimens were flexed in two different directions using a three-point bending technique (ASAIO J. 45(1999)59) and their flexural mechanical response compared. Results indicated that: (1) the relationship between the applied flexing moment and change of curvature of specimens was non-linear in both native and chemically fixed groups, (2) there were no directional differences in flexural properties when the bovine pericardium is flexed towards either the epi-pericardial or visceral surfaces in both native and chemically fixed specimens, (3) native and chemically fixed bovine pericardium were stiffer when flexed perpendicular to local preferred collagen fiber direction, and (4) chemical fixation increased the flexural rigidity of bovine pericardium. Results of this study indicate that the flexural properties of bovine pericardium are dominated by inter-fiber cross-links as opposed to the stiffness of the collagen fibers themselves. These findings can be used to guide the development of novel chemical treatment methods that seek to optimize biomechanical properties of heterograft biomaterials.

Keywords: Pericardium; Bioprosthetic heart valves; Collagen orientation; Flexural properties; Heterograft biomaterials

1. Introduction

The main advantages of bioprosthetic heart valves (BHV) continue to be their excellent hemodynamic performance and low thrombogenicity [1,2]. Currently, porcine aortic valve and bovine pericardium are the two preferred soft tissue sources for heterograft biomaterials used in commercially available BHV. Of these two types, second generation pericardial BHV generally display better durability than their discontinued predecessors, and appear to be as durable as the best porcine BHV [3]. An advantage of pericardial heterograft biomaterials is their amenability to design, as there are no anatomic restrictions associated with the native porcine aortic valve geometry. Irrespective of the specific heterograft biomaterials used, BHV continue to suffer from limited long-term durability, with an average lifespan of 10–15 years [3]. Calcification [4] and mechanically induced fatigue damage [5–7] are known to be the major causes of failure.

Heterograft biomaterials will continue to be utilized in fabricating replacement prosthetic valves for the foreseeable future. There have been many attempts to improve heterograft biomaterial durability through novel chemical treatments. For example, dye-mediated photo-oxidation has been shown to be resistant to mineralization and in vitro chemical and enzymatic degradation, supportive to endothelial cell growth, and to be stable in vivo [8–11]. However, pericardial BHV made utilizing this chemical treatment have met with poor clinical outcomes exclusively due to structural failures, a result of both faulty valve design and poor tissue durability [12].

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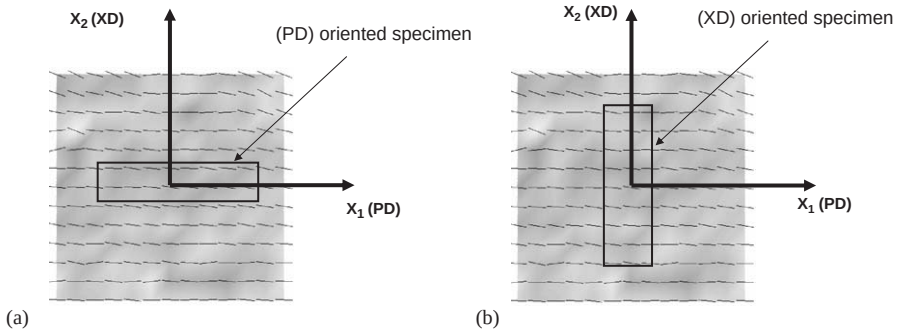

Fig. 1. A schematic of the specimen's orientation chosen either (a) aligned to the preferred direction of collagen fiber orientation (PD) or (b) aligned to the cross-preferred direction of collagen fiber orientation (XD).

Thus, for novel heterograft biomaterials to be optimally utilized in replacement of heart valves, an improved understanding of the effects of chemical modification on their mechanical behavior is clearly a critical step. Native bovine pericardium is known to be mechanically anisotropic [13–16]. Moreover, we [13,17] have quantitatively demonstrated that the direction and degree of mechanical anisotropy is dictated by the local collagen fiber architecture.

However, the above-cited studies have been limited to tensile mechanical stress states. Flexure is also a major mode of deformation in the native valve [18] and for pericardial BHV [19] leaflets during valve operation. Flexure induces more complex deformation patterns within the tissue; with one portion of tissue in tension while the remaining portion in compression. Thus, unlike tensile tests both the compressive and tensile material behaviors are factors in determining the flexural rigidity. Flexural deformation is also a sensitive method of evaluation of mechanical properties in the low strain range, which is often very difficult to quantify for soft tissues using tensile testing approaches. Moreover, the advent of tissue engineering has placed additional needs for accurate material models for tissue design [20]. Clearly, the development of heterograft and potentially tissue engineered heart valve biomaterials will clearly require knowledge of their flexural behavior.

In the present study, the effective stiffness of native and chemically modified bovine pericardium under flexure was determined and correlated to the local collagen fiber architecture. Specifically, native and glutaraldehyde-treated specimens were presorted into preferred collagen fiber direction (PD) and cross-preferred collagen fiber direction (XD) groups, and the flexural behavior quantified in both opposing flexural directions. The resulting flexural rigidity results were compared in terms of fiber orientation, presence of cross linking, direction and degree of flexure (i.e. epipericardial-EPI and visceral-VIS). In addition, we compared our results to the flexural properties native and chemically modified porcine aortic valve [21].

2. Methods

2.1. Specimen preparation

Details of the specimen selection procedure have been previously presented [14]. Briefly, native and glutaraldehyde treated bovine pericardium (GLBP) were cut into 60 mm × 60 mm sheets. GLBP sheets were prepared from the native tissue using 0.625% aqueous glutaraldehyde solution at 4°C in a stress free state. The sheets were then mounted in the SALS device and scanned. Sections exhibiting a relatively uniform fiber orientation were identified and 25 mm × 25 mm specimens were excised with their collagen fiber preferred direction aligned to either PD or XD fiber direction (Fig. 1). From these square sections, thirty four 3 mm × 20 mm flexural test specimens were prepared as follows: native PD ($n=7$), native XD ($n=7$), GLBP PD ($n=10$), and GLBP XD ($n=10$). The specimens were cut with parallel razor blades with a 3 mm spacer, and the specimen thickness was measured using a Mitutoyo gauge (resolution 25.4 μm) immediately prior to testing. The average of readings taken from four equally spaced locations along the specimen length was used. Specimens were then placed in normal saline solution at 4°C and tested within 24 h.

2.2. Testing methods

The device and initial analysis methods used in this study have been described previously [22,23], which were extended to deal with the unique properties of the pericardium (see surface strain measurements below and results). Briefly, the device includes computer-controlled stepper motors, a calibrated bending bar, a high-resolution CCD camera, and a PC with frame grabber (Truevision. Targa+, 640 × 480 pixel), and a time code generator (Burst Electronics, TC-3). Along one edge of the specimen small (~0.3 mm) black graphite markers were attached (Fig. 2). The specimens were then subjected to three point bending while submerged in