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Research Paper

Tear and decohesion of bovine pericardial tissue

Almudena Tobaruela[a,b,*], Manuel Elices[a,b], Jean Yves Bourges[a,b], Francisco Javier Rojo[a,b], José Miguel Atienza[a,b], Gustavo Guinea[a,b]

[a]Centro de Tecnología Biomédica, Universidad Politécnica de Madrid, 28223 Pozuelo de Alarcón (Madrid), Spain
[b]Departamento de Ciencia de Materiales, ETSI Caminos, Canales y Puertos, Universidad Politécnica de Madrid, 28040 Madrid, Spain

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ABSTRACT

The aim of this study was to evaluate quantitatively the fracture—by tear and delamination—of bovine pericardium tissues which are usually employed for the manufacture of bioprosthetic valves. A large number of samples (77) were tested in root-to-apex and circumferential directions, according to a standardised tear test (ASTM D 1938). Before performing the tear test, some samples were subjected to 1000 cycles of fatigue to a maximum stress of 3 MPa. Fracture toughness of tearing and delamination were computed by following a simple fracture model. The study showed significantly lower values of delamination toughness compared with tear delamination. Moreover, tear forces were different in each test direction, revealing a clear orthotropic behaviour. All these results, as well as the testing procedure, could be of value for future research in the physiological function of pericardium tissues and clinical applications.

1. Introduction

Bovine pericardium (BP), due to its outstanding biological and mechanical properties, is a source of natural biomaterials with a wide range of clinical applications. BP tissue are used, after treatment with different cross-linking methods, for the manufacture of bioprosthetic heart valves and for structural repair of several soft tissue deficiencies.

The pericardium is a tough fibro-serous sac which covers the heart. It has two layers: the outermost fibrous pericardium and the inner serous pericardium. The fibrous pericardium is the layer nearest the surface. It is made up of dense and loose connective tissue. The serous pericardium, in turn, is divided into two layers: the parietal pericardium, which is fused to and inseparable from the fibrous pericardium, and the visceral pericardium, which is part of the epicardium. The space between the two serous layers is the pericardial cavity filled by the serous fluid. Currently, only the adjoined fibrous and parietal layers are considered for bioprosthetic purposes.

BP can be characterised mechanically as a non-linear, anisotropic, multilaminate pliable material. Clinical complications have been reported, especially for bioprosthetic heart valves, due to large deformations during its physiological function and calcification (Bruck, 1983; Schoen and Levy,

*Corresponding author at: Departamento de Ciencia de Materiales, ETSI Caminos, Canales y Puertos, Universidad Politécnica de Madrid, 28040 Madrid, Spain.
E-mail address: almudena.tobaruela@upm.es (A. Tobaruela).

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1999; Aguiari et al., 2015, among others). Clearly, a full understanding of the mechanical behaviour of BP is needed to advance in the use as heterograft biomaterial.

Static tensile experiments have shown that the fibrous components are the main load-bearing elements (Mavrilas and Missirlis, 1991; Sacks and Chuong, 1998). In creep, stress relaxation, and forced vibration experiments the viscoelastic nature of BP has been explored (Naimark et al., 1992; García Sestafe et al., 1994; Duncan and Boughner, 1998). Most recently, a study of the dynamic behaviour of BP in two modes, fresh intact tissue and after selective enzymatic degradation of glycosaminoglycans (GAGs), was performed by Mavrilas et al. (2005).

At the time of writing, to the authors' knowledge, no detailed quantitative research has been done on tear and delamination of BP tissues. Qualitative results on tearing strength were reported by Trowbridge and Crofts (1989). In this paper it was mentioned that rupture of the tissue occurred following laminate debonding by shear and fibre slippage through the matrix. García Páez et al. (2006, 2010) measured the tearing resistance of BP and the influence of the suture in stitched samples. They concluded that sutures produced a significant loss of resistance to tearing.

Fracture of BP tissues is quantitatively studied in this work. 77 samples were tested according to a standardised tear test and fracture toughness of tearing and delamination were computed. Results have shown lower values of delamination toughness compared to tear ones, as well as a clear orthotropic behaviour was revealed. These facts are most relevant for the design of biological valve leaflets since the lower resistance to delamination and the noticeable orthotropy of pericardium membranes should be taken into account in the mechanical design to minimise risk of failure.

2. Materials and methods

2.1. Material

Calf pericardium was obtained from a local abattoir. The livestock was bred in Spain and had an age of between nine and 12 months at the time of slaughter. Pericardial sacs were immediately removed after death from the parietal anterior region of the heart and transported in a cold (4 °C) isotonic saline solution (0.9% sodium chloride, pH 5.5) to the laboratory. The pericardial sacs were opened, leaving the diaphragmatic ligament in the centre and the breastbone pericardial ligaments at the circumference, as described elsewhere (Purinya et al., 1994).

Glutaraldehyde fixing by Sellaro et al. (2007) was used because it is a widely applied protocol in pericardium membranes for using in bioprosthesis. Briefly, the pericardium was treated for 24 h with 0.625% glutaraldehyde prepared from a commercially available solution of 25% (Merck, Darmstadt, Germany) at a ratio of 1/50 (w/v), in 0.1 M sodium phosphate buffer with a pH 7.4.

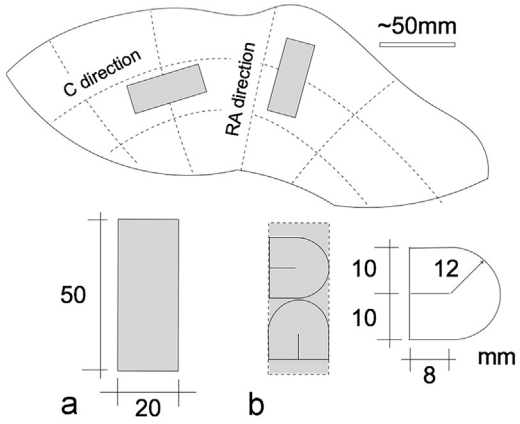

Fig. 1 – a) Rectangular samples. b) Tear geometry. Cut length: 8mm. Radius: 12 mm. RA (root-to-apex), C (circumferential).

2.2. Samples

Thirty-nine rectangular pericardial pieces 50mm high x 20mm wide were cut in both, root-to-apex (RA, 26 samples) and circumferential (C, 13 samples) directions, following the main anatomical axes (Fig. 1a). Sample thickness was measured at six points, uniformly distributed over each sample, with a micrometre Mitutoyo (Elecount E/A33/8) with an accuracy of better than ±3 μm at 20 °C.

For improved homogenisation, as well as a careful manual selection of the membranes where non-homogeneous, stiff and fibrous regions were discarded, the following two exclusion criteria were established:

- A minimum thickness criterion, to ensure that no sample is locally too thin; samples with an absolute difference between their minimum thickness and the mean value of the series exceeding one standard deviation were rejected.
- A homogeneous thickness criterion, to discard those samples that were not homogenous enough; samples with a difference between their mean and minimum thickness exceeding one standard deviation of the series were rejected.

From each rectangular sample, two tear specimens with orthogonal orientations were cut, as depicted in Fig. 1b. Dimensions of tear samples were adapted from the ASTM D 1938 Standard: pre-cut length 8 mm, radius 12 mm and 20 mm width.

Prior to cutting the tearing specimens,13 RA-direction rectangular samples, and 13 C-direction samples were subjected to fatigue preconditioning as described below. The remaining 13 RA-direction samples were left as obtained (control).

2.3. Testing methods

Tear and delamination fracture toughness of BP were measured by using standardised methods. Two types of tear samples were used; control samples (N), without any kind of mechanical preconditioning, and pre-fatigued samples