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Research Paper

Indentation hardness: A simple test that correlates with the dissipated-energy predictor for fatigue-life in bovine pericardium membranes for bioprosthetic heart valves

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ABSTRACT

The aim of this study was to evaluate the variation of hardness with fatigue in calf pericardium, a biomaterial commonly used in bioprosthetic heart valves, and its relationship with the energy dissipated during the first fatigue cycle that has been shown to be a predictor of fatigue-life (García Páez et al., 2006, 2007; Rojo et al., 2010). Fatigue tests were performed *in vitro* on 24 pericardium specimens cut in a root-to-apex direction. The specimens were subjected to a maximum stress of 1 MPa in blocks of 10, 25, 50, 100, 250, 500, 1000 and 1500 cycles. By means of a modified Shore A hardness test procedure, the hardness of the specimen was measured before and after fatigue tests. Results showed a significant correlation of such hardness with fatigue performance and with the energy dissipated in the first cycle of fatigue, a predictor of pericardium durability. The study showed indentation hardness as a simple and reliable indicator of mechanical performance, one which could be easily implemented in improving tissue selection.

1. Introduction

Bioprosthetic heart valves have been gaining recognition in recent years as hemodynamically superior alternatives to mechanical ones that do not require anticoagulation (Butany and Leask, 2001; Vesely et al., 1995; Sun et al., 2004). In 2013, bioprosthetic heart valves accounted for more than 40% of all heart-valve replacements in Spain (Bustamante-Munguira et al., 2014) with their use increasing due to new catheter-based delivery systems that avoid major surgery (Vesely, 2003;

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Flecher et al., 2008). Nevertheless, a wider use is hampered by their lower and less predictable durability and is not recommended for young patients (Tillquist and Maddox, 2011).

Failure of bioprosthetic valves – whether associated or not to calcification – is ordinarily due to fatigue of valve leaflets (Butany and Leask, 2001; Schoen et al., 1987; Vongpatanasin et al., 1996; Tyers et al., 1995; Kent et al., 1998). Thinner leaflets, like those used for catheter-implantable biosprostheses lead to both greater uncertainty in their durability and a major risk of failure (Vesely, 2003; Flecher et al., 2008).

The mechanical behavior of calf pericardium – the major source of bioprosthetic heart valve leaflets – depends on the organization and properties of collagen and elastic fibers and is naturally variable (Sacks et al., 1994; Sacks and Schoen, 2002; Sellaro et al., 2007). The manufacturing of durable and reliable leaflets for heart valve bioprostheses entails a good tissue selection and the techniques for the non-destructive assessment of its expected mechanical behavior (Sacks et al., 1994). Although optical methods may resolve the local fibrillar microstructure of a pericardium membrane, the assessment of durability remains challenging due to a lack of reliable parameters and models that comprehensively link microstructural features and fatigue behavior. While other non-destructive techniques – such as the measurement of the energy dissipated during the first hysteresis cycle – have proved useful for predicting fatigue performance of pericardium membranes (García Páez et al., 2006, 2007; Rojo et al., 2010), they require accurate and complex stress–strain measurements that are difficult to implement in a production line.

In order to help material selection, this study focuses on indentation hardness as a non-destructive parameter related to fatigue performance. Hardness measurement is a simple, inexpensive and non-destructive technique that may be applied to pericardium samples of a variable size. Indentation hardness measures the penetration depth of an indenter pressed onto the sample. The value is sensitive to the thickness, stiffness and fibrillar configuration of the biomaterial, gathering most of the parameters relevant for fatigue performance.

This work examines the close relationship between the indentation hardness and fatigue behavior of the biomaterial and, more specifically, with the energy dissipated in the first hysteresis cycles which was shown to be a reliable predictor of the mechanical performance of pericardium in previous works carried out by the authors (García Páez et al., 2006, 2007; Rojo et al., 2010).

Pericardium membranes, similar to those used in bioprothesis, were used in this study with 24 samples being subjected to a reduced fatigue test procedure where they were uniaxially loaded up to a maximum stress of 1 MPa in blocks of 10, 25, 50, 100, 250, 500, 1000 and 1500 cycles. The aim of these tests was not to reproduce the actual loading in real leaflets (which is about 30-40 million cycles per year at stresses up to 0.5 MPa (Butcher et al., 2011), but explore the relationship between the two chosen fatigue indicators.

After fatigue, hardness was measured by a modified Shore A test procedure. The results show that the loss of hardness is closely related with the number of cycles of fatigue and with the dissipated energy. Consequently, indentation hardness, which is both reliable and simple, is expected to offer help in better selection of biomaterials for biomedical purposes.

2. Materials and methods

2.1. Materials

2.1.1. Pericardium membranes

Calf pericardium was obtained from a local slaughterhouse. 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). The samples were carefully cleaned and cut into pieces with an approximate 15 cm length (root-to-apex) and 10 cm width (transverse).

2.1.2. Chemical treatment

Pericardium was treated by following the standard procedure for pericardial membranes (García Páez et al., 2001). The tissue was treated with 0.625% glutaraldehyde, prepared from a commercial solution of 25% glutaraldehyde (Merck) at a ratio of 1/50 (w/v), in a 0.1 M sodium phosphate buffer with a pH 7.4 for 24 h. After treatment, the pericardium samples were stored in glycerol at 4 °C.

2.1.3. Fatigue specimens

Twenty-four specimens for the fatigue tests were cut in a root-to-apex direction. The nominal size was 8 cm × 4 cm. Thickness was measured at 10 points, uniformly distributed over each sample with a micrometer Mitutoyo (Elecount E/A33/8) with an accuracy of better than ± 3 μm at 20 °C.

For improved homogenization, 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.

In the case of tests used for the measurement of energy dissipated in the first fatigue cycle, and with the objective of guaranteeing a greater uniformity in the mass and volume of all samples, only those with a mean thickness between 0.35 mm and 0.45 mm were used. Of the samples, 19 out of 24 (79.2%) satisfied this requisite.

2.2. Methods

2.2.1. Hardness tests

A Shore A hardness tester (Baxlo 53505/A, Instrumentos de Medida y Precisión, S.L. Polinyà, Barcelona) was used to measure