available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/jmbbm

Technical note

The micromechanical behavior of lyophilized glutaraldehyde-treated bovine pericardium under uniaxial tension

F.M. Sánchez-Arévalo[a,*], M. Farfán[b], D. Covarrubias[c], R. Zenit[d], G. Pulos[d]

[a] Universidad Iberoamericana, Prol. Paseo de la Reforma 880. Lomas de Santa Fe, C.P. 01219, Mexico
[b] Universidad Politécnica de Pachuca, Ex-Hacienda de Santa Bárbara, Zempoala, Hidalgo, C.P. 43830, Mexico
[c] Departamento de medicina, cirugía y zootecnia para especias pequeñas, Facultad de Veterinaria, Universidad Nacional Autónoma de México, D.F. 04510, Mexico
[d] Instituto de Investigaciones en Materiales, Universidad Nacional Autónoma de México, Apdo. Postal 70-360, Cd. Universitaria, D.F. 04510, Mexico

ARTICLE INFO

Article history:
Received 14 November 2009
Received in revised form
1 June 2010
Accepted 9 June 2010
Published online 8 July 2010

Keywords:
Biomaterial
Bovine pericardium
Cardiac valves
Micromechanical behavior
Lyophilization
Digital image correlation

ABSTRACT

The micromechanical behavior of lyophilized glutaraldehyde bovine pericardium undergoing uniaxial tension was studied by digital image correlation. The experiments were conducted simultaneously at macromechanical and micromechanical levels, to correlate the mechanical response of this biomaterial at different scales. From the experiments, displacement and force data were acquired; in addition, an image sequence of each sample surface was registered with a high-definition camera. With the images, it was possible to obtain the vector displacement field between pairs of images and then the in-plane strain was calculated. The secant and final moduli of this material were obtained at macromechanical and micromechanical levels. A good agreement between the micro and macro moduli was observed. This analysis is a useful alternative technique for studying this biomaterial when local properties are needed for medical applications.

1. Introduction

The life expectancy of human beings has increased in recent years thanks to medical innovations; undoubtedly, material science and engineering have contributed significantly, in particular the study and development of biomaterials which must be compatible with human tissue and homeostatic processes. One commonly used biomaterial is the bovine pericardium (BP); it has been employed in clinical applications to repair diaphragmatic, abdominal and chest wall defects and to replace heart valves, blood vessels and tracheal segments (Mansberger et al., 1973; Gallo et al., 1982; Jasso-Victoria et al., 1995; Golomb et al., 1987).

The prosthetic heart valve is one of the most important applications of BP since it was first employed by Carpentier et al. (1969). Since then, several investigations have been conducted

* *Corresponding author.* Tel.: +52 55 5950 4000.
E-mail address: francisco.sanchez@uia.mx (F.M. Sánchez-Arévalo).

1751-6161/$ - see front matter
doi:10.1016/j.jmbbm.2010.06.010

to understand the behavior of bovine pericardium to replace the leaflets of the heart valves successfully. Previous investigations have studied the structure and composition of this material and they revealed that bovine pericardium was composed of layers of elastin and collagen fibers (Holt, 1970; Elias and Boyd, 1960; Ishira et al., 1980). The layers of elastin and collagens fibers make the bovine pericardium a biocomposite material with an intrinsic structural variability and high degree of nonlinearity in response to large strain. This mechanical behavior has been studied under uniaxial and biaxial tension, flexure, and fatigue (Adja et al., 1985; Zioupos et al., 1992; Zioupos and Barbenel, 1994; Sacks and Choung, 1998; Sacks, 2000; Mirnajafi et al., 2005); in addition, simulations and mathematical models, to predict the mechanical behavior of BP, have been conducted as well (Arcidiacono et al., 2005). Although the mechanical behavior of BP has been investigated, there are only few studies concerning the micromechanical behavior of this material (Zhang et al., 2002; Sánchez-Arévalo and Pulos, 2008). The purpose of this work is to study the micromechanical behavior of lyophilized glutaraldehyde-treated BP under uniaxial load using digital image correlation. To our knowledge, the micromechanical behavior of BP has not been addressed fully in the existing literature.

2. Material and methods

Bovine hearts from 12 to 18 month calves with intact pericardium were collected fresh from a local slaughterhouse. The hearts were transported to the laboratory in cold saline solution and were processed within the following four hours. The pericardium sacs were removed from the hearts; subsequently, fat and excess of tissues were removed from the BP sacs. Then the sacs were selected and cut into sheets. The sheets were hung in custom-built frames and then they were cross-linked with glutaraldehyde (GA). The fixation process was carried out with 0.5% GA in 0.1 M phosphate-buffered saline solution, pH 7.4, for 24 h at 4 °C. After that, the sheets were washed in distilled water and finally stored in distilled water until the lyophilization process took place.

2.1. *Lyophilization process*

Lyophilization is a multistage operation that stabilizes biomaterials through four main operations: freezing, sublimation (primary drying), desorption (secondary drying) and storage. Lyophilization is commonly known as freeze-drying; it allows one to dry delicate materials without damage in their structure. This process is frequently used to facilitate the conservation, transport, manipulation and sterilization of BP (Maizato et al., 2003; Aimoli et al., 2007; Maizato et al., 2008). In this work, the BP treated with glutaraldehyde was washed three times in saline solution (NaCl 0.9%) for 30 min, changing the saline solution after 15 min. Then the BP was placed into a container and it was immediately frozen at −70 °C for 24 h; subsequently, the container was located inside the lyophilazator (Labcono) at −55 °C and 10 μmHg of pressure until the BP was completely dehydrated; finally, it was stored in a pack with a special gas (Sterrad from Johnson & Johnson Medical) to keep it sterilized.

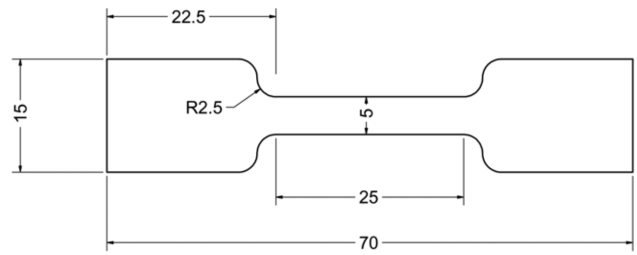

Fig. 1 – Dimensions of the uniaxial tensile sample in mm.

Before the uniaxial tension tests were performed, the lyophilized glutaraldehyde-treated BP was rehydrated with physiologic solution (at 0.9%) and then it was dyed with methylene blue ($C_{16}H_{18}ClN_3S \cdot 3H_2O$) to obtain an adequate optical texture on the surface (Sánchez-Arévalo and Pulos, 2008).

2.2. *Experimental procedure*

From the lyophilized glutaraldehyde-treated BP rehydrated sheets, samples were cut with a special jig to obtain specimens with the same dimensions. The dimensions, in mm, of the sample are shown in Fig. 1.

The tensile tests were conducted in a servohydraulic loading device (MTS 858 MiniBionix axial) with a strain rate of 0.02 mm/s. The grips were specially designed and manufactured using stainless steel to hold the specimens firmly; additionally, special soft marks were machined between two plates of the grips to press the samples, thereby avoiding any sliding of the samples between plates. For the digital image correlation an optical microscope was adapted to a high-resolution digital camera (Nikon D2X, 12 Mpix), as shown in Fig. 2. The modular microscope works as an infinity-corrected compound microscope with magnifications of 5×. To control the MiniBionix MTS, a 407 MTS controller was used, while data and image acquisition was supported by National Instruments PXI-1002 chassis and PXI-boards (6281, 8331 and 4220) and a PC. A virtual instrument (VI) was programmed in LabVIEW in order to synchronize and record the data and images. With the displacement, strain and force data, the macroscopic stress–strain curve was obtained along with images were associated to them. The extension was measured using an LVDT sensor which was previously calibrated using a digital indicator (IFD150HE Mitutoyo). A gage length compensation was used in order to minimize the effects caused by the wider parts at the ends of the specimens. From the images, the displacement vector fields were obtained, and the micromechanical behavior was determined for each type of pericardium. These measurements were done at the central region of the sample with a field of view of 5mm × 3 mm approximately.

The in-plane strain was determined using a six-parameter model (Sánchez-Arévalo, 2007; Sánchez-Arévalo and Pulos, 2008; Sánchez-Arévalo et al., 2009):

$$u_k(x_k, y_k) = A_1 x_k + B_1 y_k + C_1 + \delta u(x_k, y_k) \quad (1a)$$

$$v_k(x_k, y_k) = A_2 x_k + B_2 y_k + C_2 + \delta v(x_k, y_k). \quad (1b)$$