# MICROMECHANICAL CHARACTERIZATION OF BOVINE PERICARDIUM FOR CARDIAC IMPLANTS

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## Introduction

Biological materials are largely adopted for the fabrication of bioprosthetic heart valves (BHVs). BHVs currently available on the market are fabricated using porcine aortic valves, sheets of porcine (PP) or bovine pericardium (BP). BP is an extremely heterogeneous soft tissue, and the process of characterization of its mechanical properties has not yet been defined through a standard protocol [1]. This lack of standardization hampers the optimization of the BHVs manufacturing process, causing a large waste of processed BP. Thus, the identification of tools to test affordably the mechanical behavior of BP samples for BHVs fabrication has become essential. In this context, nanoindentation is one of the most effective techniques for an in-depth characterization of the mechanical response of heterogeneous soft tissues like BP. In a wider perspective, integrating nanoindentation measurements with scanning electron microscopy (SEM) analysis could allow to link the local mechanical response of the tissue to its microstructure. In this study, the local BP mechanical properties were characterized by nanoindentation with the final aim of establishing an affordable pass/fail criterion of BP patches for BHVs fabrication before their treatment and usage.

## Materials and Methods

The samples were cut from a BP patch (provided by Epygon Italie S.r.l) and their thickness was measured with a digital caliper (0.51± 0.01 mm). Stress relaxation tests (Piuma nanoindenter, Optics11) were performed in wet conditions, at 37 °C, controlling the indentation depth ($\delta$). After contact detection, the tests started with a loading phase, where $\delta$ = 2500 nm was reached in 1 s. Then, the set $\delta$ was held constant for 8 s while recording the load, after which the probe was retracted from the sample. For each sample, two matrices of 10x10 indentations (with a distance step size equal to 500 µm) were recorded. The two matrices were spatially overlapped of 250 µm both in the horizontal (x) and vertical (y) direction. The loading phases of the acquired curves were analyzed applying the Hertz model to obtain the local Effective Modulus ($E_{eff}$), thus estimating the elastic properties of the samples. Data recorded during the holding phase of the tests allowed to estimate the local time-dependent response of the BP samples. The indentation curves were analyzed by applying the Prony series [2], obtaining the relaxation modulus, from which the instantaneous G$(t=0)$ and equilibrium G$(t=\infty)$ moduli were calculated.Then the samples were analyzed through a scanning electron microscope.

## Results

A representative relaxation curve is showed in Fig.1A, where the model fits the experimental data. An example of $E_{eff}$ distribution on a BP sample is presented in Fig. 1B: the $E_{eff}$ value varies locally in the range 1 - 76 kPa, with an average value equal to 20.21 (± 12.16) kPa. The dispersion of the estimated values of the elastic modulus $E_{eff}$ and of the relaxation moduli are presented in Fig. 1C. Average values of $G(0)$ and $G(\infty)$ were 38.3 (± 23.5) kPa and 33.6 (± 21.8) kPa, respectively, indicating a reduction of 4.73 (± 3.26) kPa. Figure 1D shows a SEM image of the tested sample.

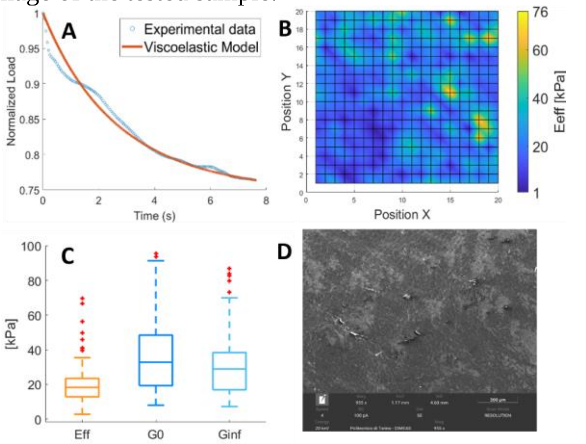


*Figure 1: A) Representative experimental and fitted curve; B) Map of the effective modulus $E_{ff}$ of the tested area; C) Box plot of Eff, G(0), and G(∞); D) SEM image of the BP sample.*

## Discussion

Combining the nanoindentation map and the surface morphology at the microscale, the proposed approach allowed a proper mapping of the mechanical properties of a representative BP sample. The BP sample behavior is affected by viscous phenomena (Fig. 1A); indeed, the viscoelastic properties are subjected to a reduction of about 13%, on average. The comparison of $E_{eff}$, $G(0)$ and $G(\infty)$ indicates that the analysis of the recorded curves performed adopting the Hertz Model, i.e., without considering the viscous effects, could introduce errors in the evaluation of the elastic properties of a BP sample. Furthermore, the high variability of the results (Fig. 1C) is reasonable in relation to the pronounced heterogeneity of the sample, which is also evident at the microscale (Fig. 1D). As a matter of fact, it appears that there is a correlation between the clear spots in the SEM image and the higher $E_{ff}$ values in the nanoindentation map.

## References

1. Stieglmeier et al, J Mech Beh Biomed Mat,119,10443, 2021
2. Mattice et al, J Mater Res, 21(8), 2003-2010, 2006