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# Mechanical response of the crosslinked pericardial tissue

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**Abstract.** The significance of studying of human pericardial mechanics arises from a wide range of possible applications. For instance, tissue engineers study potential use of a human pericardium in a design of homograft valve bioprosthesis. In cooperation with the Institute of the Physiology of Academy of Sciences of the Czech Republic, human pericardial tissue has been crosslinked by glutaraldehyde and genipin. The function and performance of the potential future bioprostheses depends closely on sources material. Thus characterization of the mechanical properties of the human pericardial tissue is an important problem. Several samples of native pericardial tissue as well as crosslinked tissue underwent uniaxial loading and unloading. Their nonlinear mechanical response was modeled using hyperelastic theory, assuming incompressible isotropic deformation. Considering future possible tissue application, mechanical properties of the material under stress corresponding to the loading in aortic valve were within the statistical analysis evaluated. Results of our study indicate that glutaraldehyde may be more appropriate crosslinking agent than genipin from the mechanical point of view.

## Introduction

Tissue engineered heart valve should be implant able to behave in the same way as the native aortic valve. The porcine xenograft valve consist of an entire decellularized intact pig aortic valve while bovine pericardial valve is fabricated from up to 3 separate pieces of pericardium, affixed to a supporting stent in a configuration very similar to that of the porcine xenograft [1]. Both the porcine and bovine valve tissues are fixed in low-concentration glutaraldehyde solution to reduce their antigenicity and to stabilize the tissue against the proteolytic degradation. Despite the chemical treatment of the prostheses some complications associated with immune reaction may occur. Therefore there is a need of the autologous material which could be used for the purpose of heart valve tissue engineering.

The goal of this study is to determine whether the crosslinking agent treated pericardial tissue reflects similar mechanical response to the raw material. In cooperation with the Institute of the Physiology of Academy of Sciences of the Czech Republic, human pericardium fixed with the most common crosslinking agent glutaraldehyde and naturally occurring crosslinking agent genipin was tested as a suitable autologous material. The function and performance of the bioprosthesis depends closely of the sources material. Thus characterization of the mechanical properties of the with different crosslinking agents fixed human pericardial tissue is an important task.

## Materials and methods

**Crosslinking.** Fresh pieces of human pericardial tissue from 7 donors, dissected during the surgeries realized in Institute for Clinical and Experimental Medicine in Prague (IKEM) were used as raw material. The procured pericardia were transported from the IKEM in the Dulbecco's Modified Eagle's Medium to the Laboratory of the Institute of the Physiology of Academy of Sciences of the Czech Republic, where the chemical treatment was realized. Altogether 50 specific samples (of different orientation and subsequent processing) were prepared for mechanical testing. Samples could be categorized in following groups: 11 specimens of fresh pericardium and corresponding 23 samples of pericardium fixed with 4% glutaraldehyde solution, 8 specimens of fresh pericardium and corresponding 8 samples of pericardium fixed with 0,5% genipin solution. Since pericardial tissue has been shown to be mechanically anisotropic [2,3], specimens were cut in the meridian and circumferential direction (Fig.1).

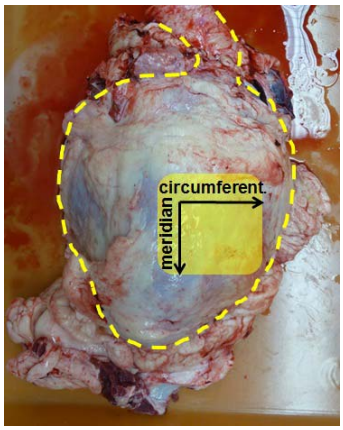


Figure 1. Circumferential and meridian orientation of the pericardial samples.

**Mechanical testing.** The specimen dimensions were measured by Laser profile sensor ScanControl 2800 (Micro-Epsilon, Ortenburg, Germany). Uniaxial tensile tests were performed on the customer specific biaxial testing machine (Zwick/Roell). Experiments were recorded by a videoextensometer which provides for online detection of the deformation. The tests were controlled according following scheme: four cycles of loading with a constant limit deformation (preconditioning of a material) followed by stress relaxation were completed by fifth loading cycle till a failure.

**Hyperelastic model.** The mechanical response was modeled as incompressible and hyperelastic. Using Fung´s exponential form of the strain energy density function and assuming isotropic deformation, Cauchy stress is expressed [4]:

$$\sigma = \mu \cdot e^{C\cdot\left(\lambda^2+\frac{2}{\lambda}-3\right)} \cdot \left(\lambda^2 - \frac{1}{\lambda}\right), \quad (1)$$

where $\mu$ represents stress-like parameter (corresponding to initial infinitesimal shear modulus), $c$ is dimensionless parameter and $\lambda$ is stretch (defined as ratio of actual and referential length) in the direction of loading.

The model (1) was differentiated with respect to stretch so as to obtain formula representing Young´s modulus of the material. Considering possible future application of the pericardial tissue, Young´s modulus under stress corresponding to the loading in the aortic valve $E_{av}$ (stress ~ 130 kPa) was evaluated.

**Statistical analysis.** Statistical analysis for determination of significance of the difference between the mean values of $E_{av}$ (ratio of sample variances) of the raw and crosslinked material was accomplished using *Two Sample T-Test* (*F-ratio test)*, performed with Maple (Maplesoft, Waterloo, Canada). Significance level of this study was $\alpha = 0.05$. No attention was paid to the sample orientation.

## Results

At first, $\sigma$–$\lambda$ graphs of each test were evaluated (valid mechanical response is assumed after for cycles of the preconditioning). Typical experimental nonlinear response of the tested samples with numerical model is depicted in the Fig. 2 (left panel).

Mechanical responses obtained during 50 specific experiments were subsequently modeled using hyperelastic exponential model (1) and $E_{av}$ was finally determined.

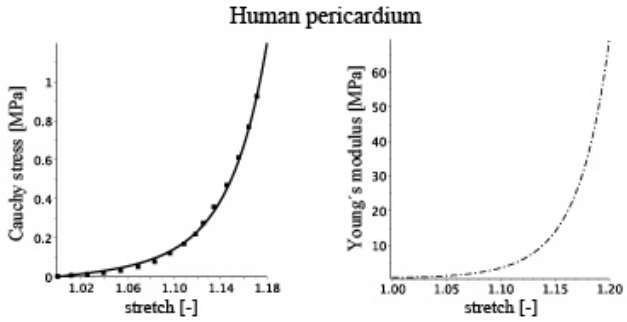


Figure 2. Typical mechanical response of the human pericardial tissue, black points represent experimental data while solid curve represents hyperelastic model (on the left). Dash – dot line (on the right) represents model values of the Young´s modulus (the same donor for both panels).

Table 1. Mean value and standard deviation of the $E_{av}$ of each sample category. P-GL denotes fresh Pericardium from which are Crosslinked samples by GLutaraldehyde (CP-GL); P-GN denotes fresh Pericardium from which are Crosslinked samples by GeNipin (CP-GN)

| Sample category | Number.of samples | Mean value $\overline{Eav}$ [MPa] | Standard deviation $S_{Eav}$ [MPa] | *T-Test* $\overline{Eav}(P) \sim \overline{Eav}(CP)$ | *F-Test* $S_{Eav}(P)/S_{Eav}(CP)$ |
|---|---|---|---|---|---|
| P-GL | 12 | 9.1 | 9.4 | Accepted | Accepted |
| CP-GL | 23 | 4.5 | 5.3 | | |
| P-GN | 8 | 11.0 | 9.8 | Rejected | Rejected |
| CP-GN | 8 | 2.4 | 2.6 | | |

Calculated values of $E_{av}$ were finally subjected to statistical evaluation. Data were analyzed within 4 sample categories (P-GL, CP-GL, P-GN, CP-GN). Results of *Standard T-Test* and *F-Ratio Test* are summarized in the Table 1.

The *Two Sample T-Test (F-ratio Test)* has revealed that the hypothesis of agreement in the mean values (variances) of $E_{av}$ could be accepted for the raw pericardium and glutaraldehyde modified pericardium. The statistical analysis of the raw pericardium and genipin fixed pericardium suggested the rejection of the null hypothesis

## Discussion and conclusion

The stress stretch mechanical responses of the fresh and glutaraldehyde/genipin fixed pericardial samples were examined in both the meridian and circumferential direction. Although references [5,6] note material anisotropy, our preliminary statistical analysis of samples with respect to orientation (circumferential versus meridian ) did not confirm significant differences in $E_{av}$ and so we classified samples only according their fixation process. The reason of this difference could be generated by the procedure of sample resection where the orthogonality was guaranteed only within one donor.

With regard to future possible application $E_{av}$ was set as a meaningful parameter. While glutaraldehyde has been tested extensively as a crosslinking agent, the genipin impact is still not understood in detail at present [6]. Conclusions of our study are following: statistical analysis based on *Two Sample T-Test* and *F-ratio test* of the results obtained in uniaxial tensile testing has indicated that the mechanical response of the raw pericardium and glutaraldehyde fixed pericardium is coincident (on the significance level $\alpha$=0.05). The agreement of the mechanical responses of the raw pericardium and genipin fixed pericardium was rejected in the statistical analysis. This study recommends refusal of the usage of the genipin as a crosslinking agent for the purpose of tissue engineering. However, the weak point of our results is small amount of tested samples which were undergone statistical testing. For more significant results, additional experiments are required.

## Acknowledgments

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