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INFLUENCE OF HDI FIXATION ON THE MECHANICAL PROPERTIES OF PORCINE PERICARDIAL TISSUE FOR HEART VALVE ENGINEERING

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Abstract: *In this study, biomechanical properties of porcine pericardium and aortic valvular leaflet tissue were comparatively investigated in tensile tests. Fresh porcine pericardium and aortic valves were harvested from domestic pig cadavers. Specimens were prepared from either native or HDI-fixed tissue. Uniaxial tensile testing was conducted in physiological saline solution at 37°C. The results show a clear distinction regarding the biomechanical behaviour of native pericardium and aortic valvular leaflet tissue. It could be demonstrated that the concentration of HDI and the fixation time influence the properties of native pericardium towards the biomechanical behaviour of aortic valvular leaflet tissue.*

Keywords: *porcine pericardium, HDI fixation, tensile tests, mechanical properties.*

Introduction

For the development of biological heart valve prostheses, different approaches towards the optimal material resource have been investigated. Recently, porcine pericardial tissue has been increasingly applied in percutaneous heart valve prostheses. Stabilization of collagen-based biomaterials by chemical methods is necessary in order to decrease the susceptibility towards enzymatic degradation and to adjust the mechanical properties of heart valve prostheses. The bifunctional, aliphatic reagent hexamethylene diisocyanate (HDI) has been used for the crosslinking of soft tissue [1]. Treatment of collagen-based biomaterials with HDI mainly involves the formation of crosslinks containing stable urea groups resulting from the reaction of isocyanate groups with amine groups [2]. In this study, biomechanical properties of native and HDI-fixed porcine pericardial and native aortic valvular leaflet tissues were comparatively investigated in uniaxial tensile tests.

Methods

2.1 Sample preparation

Native porcine pericardium and aortic valves were harvested from domestic pig cadavers at the Leibniz Institute for Farm Animal Biology (Dummerstorf, Germany) and transported to the laboratory stored in DMEM with 10% antibiotics. The specimens were processed within 1 hour after the tissue was harvested. Pericardial sacs and aortic valves from female animals (age 6-8 months, weight 100-120 kg) were used. The sacs were divided into two groups. In the first group the sac was split in 5 patches and the samples were fixed in 0.2wt% HDI, commonly dissolved in aprotic organic solvents, such as ethyl acetate. After 2, 5 and 9 days individual patches were removed from the HDI solution and washed 3 times in physiological saline solution. After rinsing, strips with a width of 3 mm and a length of 30 mm were prepared from the tissue. In the second group the sac was split in 4 patches and the samples were fixed in 0.1wt% HDI, prepared in ethyl acetate. After 2 and 5 days individual patches were removed from the HDI solution and washed 3 times in physiological saline solution. After rinsing, strips with a width of 3 mm and a length of 30 mm were prepared from the tissue.

2.2 Uniaxial tensile tests

Uniaxial tensile testing was accomplished on a Zwicki ZN 2.5 (Zwick GmbH & Co. KG, Ulm, Germany). During the tensile tests the samples were immersed in physiologic salt solution at a temperature of 37°C. The experimental procedure was set up according to Lim and Boughner (1975) [3]. The data were analyzed with respect to elongation at 0.5 MPa tensile stress, secant and final modulus [3], ultimate tensile strength and elongation at break.

Results

The results for 0.2% HDI fixation are shown in Fig 1, the black graph refers to native aortic valvular tissue, the blue graph to native pericardium, and the other graphs correspond to 0.2% HDI-fixed pericardium (1, 2, 5 and 9 days fixation). All graphs were averaged over at least n = 5 samples.

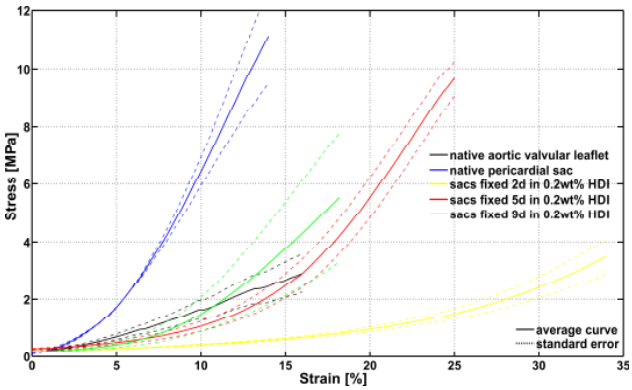

Figure 1: Stress-strain response of native porcine aortic valvular leaflets (black), native porcine pericardium (blue) and 0.2% HDI-fixed (2, 5 and 9 days) porcine pericardium. Dotted lines indicate standard error.

Native porcine aortic valvular leaflet samples show an almost linear behavior until sample failure at approx. 16% strain. However, the native pericardium shows a distinctly different stress-strain response. The curve increases much faster, and the ultimate tensile strength is four times higher than for the valvular tissue. The stress-strain behavior of the 2 days fixed pericardium (yellow) shows a strong increase in elasticity, which is exhibited in a very high elongation at break, and a low elastic modulus. After 5 days of fixation (red curve) the stress-strain behavior is shifted towards the behavior of native aortic valve leaflets and shows no further changes after 9 days fixation (green curve).

The results for 0.1% HDI fixation are shown in Fig 2, the black graph refers to native aortic valvular tissue, the blue graph to native pericardium, and the other graphs corresponds to 0.1% HDI-fixed pericardium (2 and 5 days fixation). All graphs were averaged over at least n = 5 samples.

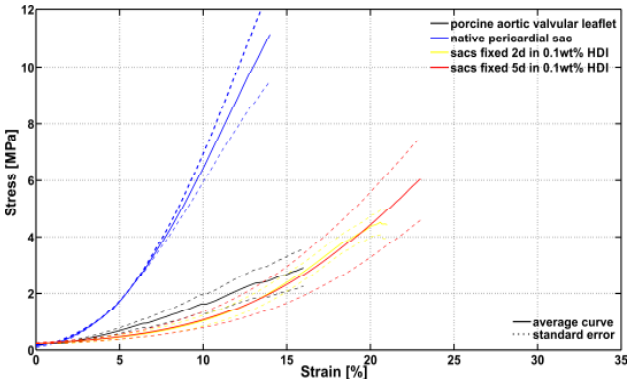

Figure 2: Stress-strain response of native porcine aortic valvular leaflets (black), native porcine pericardium (blue) and 0.1% HDI-fixed (2 and 5 days) porcine pericardium. Dotted lines indicate standard error.

The 0.1% HDI-fixed pericardium shows no different stress-strain response after 2 days of fixation (yellow curve). After 5 days of fixation the stress-strain behavior is similar to after 2 days fixation (red curve). For a quantitative comparison of material properties, elongation at 0.5 MPa tensile stress, secant und final modulus, ultimate tensile strength, as well as elongation at break were extracted from the curves, see Table 1. The thickness of all fixed pericardial samples is smaller than the aortic valvular tissue. The secant modulus of the fixed pericardial samples is smaller compared to the native aortic valvular leaflets. The final modulus of all pericardial samples is much higher than for the native aortic valvular tissue. The 0.2% HDI-fixed pericardium shows a three times higher final modulus. The strain at 0.5 MPa shows for all samples the same behavior. Both fixed pericardium show similar mechanical behavior as native aortic valve leaflets.

Table 1: Mechanical properties of native porcine aortic valvular leaflets and HDI-fixed porcine pericardium.

	Native porcine Aortic valve	**0.1% HDI-fixed pericardium (2 days)**	**0.2% HDI-fixed pericardium (5 days)**
Thickness [mm]	0.28 ± 0.07	0.16 ± 0.05	0.17 ± 0.03
Secant modulus [MPa]	6.0 ± 2.7	3.6 ± 1.8	3.7 ± 1.3
Final modulus [MPa]	28 ± 4	43 ± 21	87 ± 14
Strain at 0,5 MPa stress [%]	5.4 ± 2.6	6 ± 3	5.2 ± 2.8
Elongation at break [%]	23 ± 13	22 ± 3	27.9 ± 2.9
Stress at break [MPa]	4.1 ± 2.5	5.1 ± 2.3	11.8 ± 2.0
Ultimate tensile strength [MPa]	4.0 ± 2.6	2.1 ± 1.3	4.4 ± 1.7

Discussion

Identification of the biomechanical properties of native porcine aortic valvular leaflet and HDI-fixed pericardial samples could be accomplished in uniaxial tensile tests. Native pericardium appeared stiffer as compared to the mechanical properties of the native aortic valvular tissue. However, by fixing the native pericardium with 0.1% and 0.2% HDI the mechanical properties could be shifted to a more elastic behavior. The presented preliminary tests could demonstrate that porcine pericardial tissue can exhibit biomechanical properties in the range of aortic valvular leaflet tissue. In order to further match the mechanical behavior of HDI-fixed pericardial tissue, the fixation process will have to be adjusted in consideration of the fixation time and the concentration of the HDI solution. Altogether, the results indicate the suitability of HDI-fixed porcine pericardial tissue for application in biological heart valve prostheses.

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