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INFLUENCE OF PRESERVATIVE ON THE TENSILE STRENGTH OF THE TISSUE OF PORCINE CIRCULATORY SYSTEM

ABSTRACT

There are many biomaterials that can substitute pathologically altered tissue, however, none of them is as perfect as a native tissue. Currently, scientists are looking for new biomaterials that can be successfully implanted without exposing the patient to reoperation. Each material introduced into an organism must afford sufficient mechanical and biochemical properties and meet the criteria of the biomaterial. Materials intended to take over the function of natural tissue materials should be characterized to the greatest extent by similar mechanical properties. The authors of many publications describing the results of strength tests of biological tissues show different ways of researching them. In many cases, the form of the test material preparation is different because of anisotropy of biological tissue. This study provides an overview of selected methods for the tensile tests characterizing the mechanical properties of the heart valves, pericardium and porcine aortas. We also present results of our study of mechanical properties of the natural porcine tissues.

Keywords: *tensile test, heart valves, pericardium, aorta, mechanical properties*

THEORETICAL CONSIDERATIONS ON HEART VALVE

The authors of many publications describing the results of strength tests of biological tissues show different ways of researching them. In many cases, the form of the test material preparation is different which turns out to influence the test results as demonstrated by the doctoral dissertation [4] studying the effect of the storage on tensile properties of natural tissue heart valve showing the effect of preservation method of the research model before attempting the tensile test on change of its mechanical properties. It turns out that the monthly glutaraldehyde preservation may yield up to 15 times decrease in biological tissue strength, and in the case of long-term preservation the stiffness increases up to 25 times. The scientists take into account the anisotropy of biological tissue by making investigations, both in circumferential and radial direction. Tests carried out in Paul's Stradins Clinical University Hospital and Riga Stradins University [3] show the variability of mechanical properties of the aortic valve leaflets, determining Young's modulus in the presence of different stress values. The maximum difference reaches up to 50%.

In order to obtain reliable results of tensile test valve leaflet we should provide a storage environment which should be close to the natural conditions of the valve operation to ensure constancy of the mechanical properties of the tissue. Due to the influence of preserving agents on the results the 0.9% sodium chloride solution is popularly used and it seems to be the least invasive.

It is recommended to take a sample from the location subjected to the approximate stress values in all its volume. Analysis of the nature of valve functioning allows distinguishing four anatomical leaflet areas which are discerned by the presence of various stress values: leaflet coaptation area, commissures and the nearby area of valve anulus. For simplicity, it is recommended to perform research only on a sample taken from the area of the occurrence of the greatest stress (commissures) determining the same value of maximum stress. Determining the mechanical properties of the valve leaflet require consideration of the anisotropic nature, therefore, it is important for the definition of test sample. As the leaf valve is exposed to various stress values in both the circumferential and radial research, the model should take into account the specimens tested in both directions.

Table 1. *Acquisition and storage conditions of test samples including summary of the measured values during tensile tests; p - porcine, h - human*

Type of valve	Number of tested valves	Storage	Direction of sample	Sample dimensions	Thickness [mm]	Maximum stress [MPa]	Strain	Young's modulus [MPa]
Human [1]	18	Hartman's solution, for 14 days, 8ºC	no definition	10x10 mm	no definition	no definition	0,6 mm	no definition
human, porcine and St Jude Epic prosthesis [2]	3	glutaraldehyde	circumferential and radial	3,5x20 mm	St: 0.59 ± 0.15 p: 0.92 ± 0.17 h: 0.57 ± 0.16	circumferential St: 5,77 ± 1,94 p: 1,58 ± 0,26 h:1,74 ± 0,29 radial St: 0,7 ± 0,21 p: 0,55 ± 0,11 h: 0,32 ± 0,04	circumferential St: 5,95% ± 1,54 p: 7,26% ± 0,69 h:18,35% ± 7,61 radial St: 7,92%±1,74 p: 8,57% ± 0,80 h: 23,92% ± 4,87	Circumferential St: 101,99 ± 58 p: 42,3 ± 4,96 h: 15,34 ± 3,84 Radial St: 9,18 ± 1,81 p: 5,33 ± 0,61 h: 1,98 ± 0,15
Porcine [5]	11	sodium chloride, temp.20ºC	circumferential and radial	3x20 mm	0,606 ± 0,196	circumferential 1,74 ± 0,29 radial: 0,32 ± 0,04	circumferential ~18,5% radial: ~23,3%	Commissures: 13.80 ± 3.16 Fibrous ring: 12.50 ± 2.98 STJ: 7.41 ± 2.34 Sinuses: 10.53 ± 3.22
Human [6]	19	sodium chloride, temp.4ºC, time: 48 h	circumferential and radial	no definition	circumferential 0,67 radial: 0,64	circumferential 1,197 radial: 0,19	circumferential 10% radial: 23%	no definition
Porcine [6]	12	sodium chloride, temp.4ºC, time: 48 h	circumferential and radial	no definition	circumferential 0,59 radial: 0,51	circumferential 4,218 radial: 0,477	circumferential 12% radial: 26%	no definition

THEORETICAL CONSIDERATIONS ON PERIACARDIUM

Pericardium is a tough double layered membrane which covers the heart. Serous membrane closely adjacent to the myocardium consists of three layers: an outer epicardium, middle and inner - endocardium. Epicardium is a visceral lamina epicardium of serous pericardium and is a plate made of connective tissues that are rich in collagen and elastic fibers. The middle layer is the thickest layer of the heart wall, which is built by the muscle tissue of the heart. Endocarditis covers the entire inner surface of the heart, together with valves and its thickness is 0.2-0.5 mm. It smoothes the inner surface of the heart and prevents blood from clotting during contact with the wall [7].

The mechanical properties of the pericardium can be different in various places, depending on the arrangement of collagen fibers in it. In the experiment of Tom Lavrijsen [8] pericardium obtained from pig hearts previously frozen and cut from the outer portion connected to the diaphragm has been studied, because it was considered to have the most homogeneous structure. In order to verify the anisotropic properties, the samples were cut in relation to the diaphragm: parallel, perpendicular and at angles other than 0 and 90^0. It was observed that the samples taken from the pericardium in a parallel direction to the diaphragm were characterized by the highest values of Young's modulus (110 MPa), while the samples from the pericardium cut in a perpendicular direction to the membrane – the lowest values (82 MPa). The samples from the pericardium oriented at angles other than 0 and 90^0 received the intermediate values. Average tensile stress rupture was obtained at around 10 MPa. On the other hand, J. M. Garcia Paa Ez and others [9] checked the tensile strength of samples taken from the pericardial sac surrounding the left and right ventricle of the heart. Samples were taken from symmetrical locations and tested using a hydraulic simulator. The results showed that the pericardium surrounding the right ventricle has a higher tensile strength (64.38 MPa) than the pericardium surrounding the left ventricle (60.63 MPa). However, these values do not differ significantly. The results of the tests described below are shown in Table 2.

Table 2. *Tensile stress, strain and Young's modulus for h-human and p-porcine pericardial*

Type of sample	Number of tested samples	Storage	Direction of sample	Sample dimensions	Maximum stress [MPa]	Strain [%]	Young's modulus [MPa]
Porcine [9]	6	0,625M glutaraldehyde	up from the left and from the right ventricle	circular samples with undefined diameter	left ventricular area: 60,63 ± 16,95	no definition	no definition
					right ventricular area: 64,38 ± 17,59		
Porcine [8]	24	fluorescent sensor collagen, time: ~ 10 h	in relation to the diaphragm: parallel	30 x 5mm	parallel ~10 perpendicular – no tests	13	parallel 110
			perpendicular				perpendicularly 82

In order to obtain reliable results of the pericardium tensile test it is necessary to provide a storage environment (from the time of obtaining from the donor to the start of testing) close to the natural conditions (the same as in the heart valves -0,9% sodium chloride) of the pericardium to ensure constancy of the mechanical properties of the tissue. To improve the tensile strength samples can be preserved in glutaraldehyd which forms a bond with collagen and causes strengthening of the material. Then, it is recommended to perform research only on a sample taken from the area of occurrence of the greatest stress determining the same value of maximum stress. Determination of the mechanical properties of the pericardium requires consideration of the anisotropic nature, therefore, it is important to the definition of test sample. As the pericardium is exposed to various stress values simultaneously in parallel and perpendicular relation to the diaphragm, the research model should take into account the specimens tested in both directions

THEORETICAL CONSIDERATIONS ON AORTA

Aorta is a large arterial trunk. Its branches bring arterial blood to all the tissues. It is the largest human artery [10]. The most important structural components of the aortic wall are elastin and collagen. Elastin and collagen condition reversible deformation ability and mechanical strength of the aorta. Elastin is synthesized only in childhood, and the protein half-life is 70 years. Collagen is synthesized individually throughout the whole life. The collagen fiber is characterized by about 20 times more tensile strength than elastin while the resiliency of collagen is 300 - fold less than elastin. This means that the collagen may be only slightly stretched without damaging its structure. Greater extension of collagen fibers causes irreversible changes in the structure and loss of the mechanical strength [11,12,13,14]. Therefore, the role of aorta is of extreme importance. Mechanical testing of healthy aorta and its various forms of disease states (e.g. aortic aneurysm) may be useful in the treatment processes in which it is necessary to replace a damaged artery.
The paper entitled "Mechanical properties of the aorta" [15] discusses the tensile test of increasing thoracic aortic aneurysm performed in order to determine the maximum stress and the maximum tensile elastic modulus (Young's modulus) for its greater and lesser curvature. The tests were performed in the longitudinal direction (L) and a circumferential (C) direction. Assuming that the wall of the aorta is incompressible, the maximum values of the elastic modulus were as follows: the greater curvature stretched circumferentially aortic aneurysm GC - 14.36 MPa, for an aortic aneurysm the lesser curve LC of the stretched circumferentially - 14.95 MPa, for the greater curvature aortic aneurysm stretched longitudinally GL - 6.68 MPa, and the lesser curvature of the aortic aneurysm stretched longitudinally LL - 3.44 MPa.
On the other hand, the research conducted by Lanzo [16] consisted of examining both pathologically altered human aortas and healthy porcine aortas. The samples were cut with a metal dyne providing a dogbone shape. In order to determine the anisotropy of the samples, they were collected in the circumferential and the axial direction. Studies have shown that tissues from porcine peripheral orientation were characterized by better mechanical properties, and their tensile stresses are maximum at around 1.7 MPa, and for axially direction tissues tensile stresses are at a level of 0.4 MPa, which is a significant difference. On the other hand, pathology changes of human aorta are responsible for its smaller tensile strength. The values obtained for the samples in the circumferential direction (1 MPa) were smaller than the values obtained for the samples in the axial direction (1.1 MPa). Young's modulus was determined only for the diseased human aorta, because other studies [17] showed that in pathologies of the aorta the stiffness is greater. Thus, a higher Young's modulus was obtained for the samples cut in the circumferential direction (17.13 MPa) than for the samples taken in the longitudinal direction (10.87 MPa). The results of tests described below are shown in Table 3.

The mechanical properties of the aorta are mainly determined by the structural components of the wall. These are mainly elastin, collagen and smooth muscle cells. Smooth muscle cells are capable to contract and relax, which supports the cyclic deformation of the vessel wall. However, these collagen and elastin fibers are the most important determinants of the mechanics of the aortic wall. Both mechanical strength and Young's modulus of the conventional aortic wall are smaller in the longitudinal direction than in the circumferential direction and are strongly dependent on the structure of the vessel wall [17]. Lesions in various stages of disease affect the degree of the elasticity of the aorta. The influence of a disease of the aorta causes the reduction of the mechanical strength and rigidity of the wall. In order to perform the tests with high accuracy class it is recommended to prepare a sample of the dogbone shape. The shape of the surface of the sample with a range of length-measuring, minimizes the damage or rupture in the vicinity of the surface of the jaws.

Table 3. *Tensile stress, strain and Young's modulus of the human and porcine aortas*

Type of sample	Number of tested samples	Storage	Direction of sample	Sample dimensions	Maximum stress [MPa]	Strain [%]	Young's modulus [MPa]
human and porcine [16]	no definition	Ringer's solution, temp. 4ºC	circumferential and radial	p: dogbone shape 50 x 4 mm	circumferential p: 1,7 h: 1	circumferential p: 0,65 h: 0,4	circumferential p: no definition h: 17,13
				h: 3 ÷ 4 mm x variable length	longitudinal p: 1,7 h: 1,1	longitudinal p: 0,13 h: 0,21	longitudinal p: no definition h: 10,87
Human [15]	109	0,9% sodium chloride, temp.4ºC, time: 48 h	circumferential and radial	3x20 mm and 3x30 mm	circumferential 1,6	circumferential 0,58	circumferential GC max 14,36 LC max 14.95
					longitudinal no definition	longitudinal no definition	longitudinal GL max 6,68 LL max 3,44

THE SUMMARY OF THE THEORETICAL PART

The authors of many publications describing the results of strength tests of biological tissues show different ways of researching them. In many cases, the form of the test material preparation is different because of anisotropy of biological tissue. Some authors show results their tests when the tissue are storage in saline solution, the other one- in glutaraldehyde. Literature shows influence of preservative on the tensile strength of the tissue that are build from collagen. We want to confirm that because natural tissues are our reference material. These result will be compared with bionanocellulose material which would be an interesting alternative for the currently used biomaterials in cardiac and vascular surgery.

AIM OF THE STUDY

The aim of the study was to compare the tensile strength of the porcine tissues of the circulatory system that were stored in saline solution, with the same tissue-curing in a solution of glutaraldehyde. Subsequently, the applicability of Polish bionanocellulose produced by BoWil as implanting material used in cardiac surgery and vascular surgery will be checked. Next the tensile strength of the porcine tissues will be compared with tensile strength of bionanocellulose.

The study used flakes of the aortic valve (left coronary petal, right coronary petal and coronaryless petal), pericardium and porcine aortic patches (lobe).
The tissues were divided into two groups. Before testing, both groups of tissues were frozen in 20% glycerol at -80^{o}C. On the day of the study, all tissues were thawed and immersed for a period of about 2 hours in 0.95% sodium chloride solution. Test of the tension of the first part of the samples was carried out after the immediate removal from the sodium chloride, and the tension test of the second part was performed after keeping them in 0.05% glutaraldehyde solution for 10 min.

Tensile test was carried out at the company testing machine INSTRON model 1112. Tension tests of the flakes were done transversely, and the pericardium and the aortic patches in the longitudinal direction. Tensile speed was different for each tissue, and so for the petals was 5 mm/s, pericardium: 10 mm/s and patches of the aorta: 15 mm/s.
All tissues had rectangular cross-sections. The distance between the jaws was minimal, so as to ensure secure fastening of tissues eliminating the possibility of slipping out.
Three samples of each type of material were made. Each sample came from another heart.

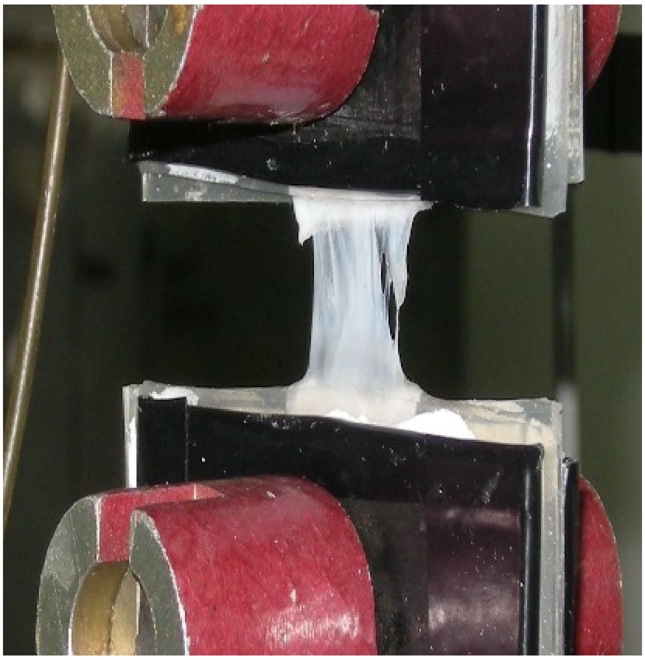

Fig. 1. Tensile leaflet of aortic valve

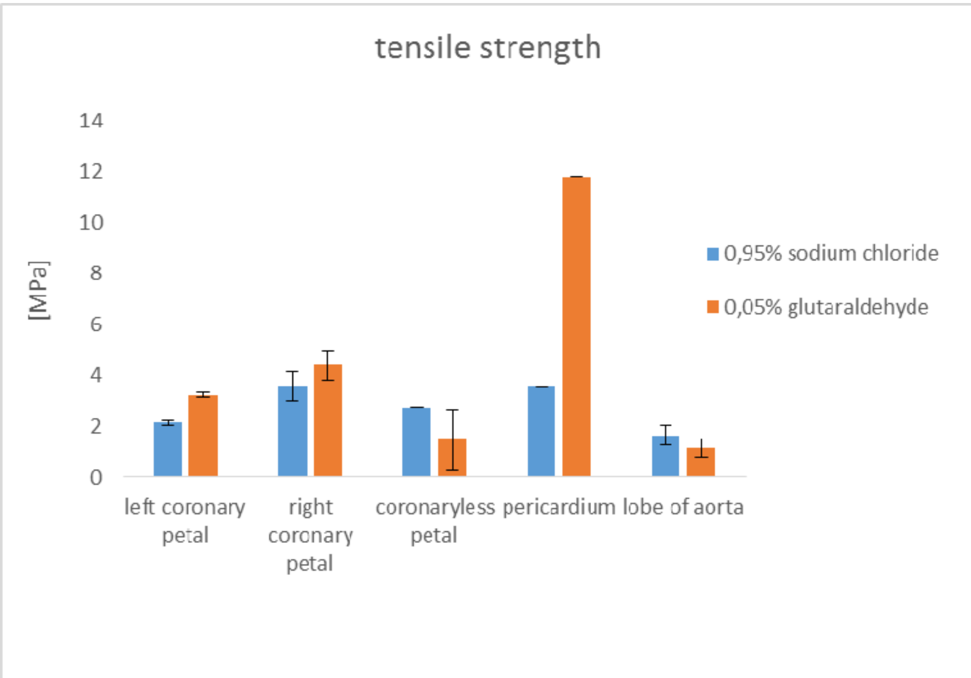

Fig. 2. Tensile strength of tissue stored in 0.95% sodium chloride and in 0.05% glutaraldehyde

DISSCUSION

The studies show that the tensile strength of the left and right coronary petal and pericardium which were fixed in glutaraldehyde is characterized by higher strength properties relative to the same tissue stored in saline solution. The first application of this type of fixation of implantation material has been introduced by Carpentier. Fixing the biological material with an aldehyde results in the mechanical reinforcement by the binding of the so-called collagen cross-linking, loss of susceptibility to proteolytic enzymes and a significant reduction of antigenic properties.

In our study only the maximum tensile strength was identified. Coronaryless petal and lobe of aorta fixed with aldehyde obtained slightly lower tensile strength values, relative to the same tissues stored in the saline, which may be due to errors resulting for example from a very fusing material and thus insufficient reagent bound collagen. The thickness of the tissue affects the binding of collagen. In the case of thinner tissue, for example pericardium, a significant increase in tensile strength was observed in comparison with the aorta, whose thickness is several times higher. In the case of application of the materials of various thickness at the same time of fixation, results consistent with expectations were obtained, because the thicker tissue requires longer time to total collagen cross-linking. The big standard deviation of the results for coronaryless petal could be due to the fact that the tissues were taken from a slaughterhouse, which does not guarantee the same conditions of rearing pigs.

However, studies show that the impact of the reagent coming into contact with the test material affects its mechanical strength.

FORECASTING THE FUTURE

The results discussed in this article allow further studies on the comparison of the mechanical properties of natural tissues with bionanocellulose material, which is listed in the program for the development of the Pomeranian province to 2030. Thanks to the program within which a grant project called “Preclinical studies on the possibility of the application of the genuine, Polish bionanocellulose (BNC) in regenerative medicine in terms of bioimplants in cardiac and vascular surgery” was created, it is possible that a new biomaterial derived from plants will be developed in the near future. Such a material would be an interesting alternative for the currently used biomaterials in medicine, which, unfortunately, in spite of their numerous advantages also have some disadvantages.

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