THE INFLUENCE OF DIMETHYL SUBERIMIDATE AND PENICILLIN ON STRUCTURE OF PORCINE PERICARDIUM

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Abstract: Porcine pericardium (PP) tissues used for bioprostheses manufacturing are stabilized due to cross-linking of proteins, mainly of collagen in the tissue. Dimethyl suberimidate (DMS) may be used for this purpose. Antibiotics including penicillin (P) are commonly used for biomaterials sterilization. In the present work, changes in stability of the PP tissues (native, DMS-modified, P-treated) have been investigated using electrophoretic and histological methods. Results of electrophoretic studies show that DMS causes stabilization of the porcine pericardium structure. This effect is reflected by decrease in number of peptides extracted from the modified tissue as well as by its higher resistance to enzymatic digestion. Thus, electrophoretic studies are useful for reveal effects of the tissue modification and its stabilization. The PP tissues susceptibilities to the peptides extraction from them were higher in case of the P- or the P- and DMS-treated tissues as compared with the native tissues. It may be stated that the tissue pre-treatment with penicillin (P) resulted in reduction of the DMS cross-linking action. Differences between structure of native and DMS-treated tissues have been shown in histological studies. The porcine pericardium tissue pre-treatment with penicillin did not cause significant changes in the tissue morphology.

Introduction

Some collagen-rich connective tissues including the pericardium are used as biomaterials in bioprostheses manufacture [1]. Various chemical compounds are involved in stabilization of the tissue structure, mainly of their extracellular matrix. These substances include many cross-linking reagents such as glutaraldehyde [1] or dimethyl suberimidate (DMS) [2] as well as antibiotics commonly used for biomaterials sterilization [3]. The aim of this study was to determine the influence of DMS and penicillin on structure of the porcine pericardium tissue.

Materials and Methods

Pericardium tissues: Experiments have been carried out using the porcine pericardium (PP) tissues obtained from the local abattoir directly after animal slaughtering. The tissues were immediately rinsed in cooled phosphate-buffered saline (PBS; pH 7.4) at 4°C. Tissular fat, heavy vasculatures and ligaments were removed before modification. Any significant changes in the tissue structure did not observed after this pre-treatment procedure. The PP tissues were treated with 0.5% DMS (Tris, pH 9) and/or penicillin (1000 U/ 1 ml of NaCl), at temperature 4˚C. The tissue resistance to biodegradation was determined on the basis of its enzymatic digestion (1.5 g pancreatin [PA] in 100 ml of water, 3 h) and/or the SDS/NaCl extraction method [4]. Changes in structure were investigated using SDS-PAGE electrophoresis [5].

Electrophoresis: Before electrophoresis, native and modified samples (1 g) were homogenised in 50 ml of water (Polytron ® PT 2100 - Kinematica AG). Aliquots of 1.5 ml of tissular homogenates were collected and concentrated by centrifugation (14000 x g) for 10 minutes to obtain samples of 0.5 ml volume. Native and modified tissues were subjected to the SDS/NaCl extraction procedure performed according to Laemmli [5]. The tissue samples (15µl) were stiffened in 4% gel (voltage 70 V) and then peptides were separated in 10% gel (140 V) (Minipol, Kucharczyk T.E. Co). Peptides in electrophoregrams stained with Coomassie Brillant Blue R250 were analyzed using the software package Scangel 1.45 program (Kucharczyk T.E.). For destaining, gels were incubated in the same solution without dye. The qualitative analyses of the peptides were performed using Biotec Fischer System.

Histology: Structural changes were examined under optical microscope. Tissue samples for histological studies were dehydrated in absolute ethanol, and then embedded in paraffin wax. Six micron samples were stained routinely with Harris hematoxylin (background stained with 1% erythrosine solution). Histological observations were carried out under optical microscope Polyvar 2 (Leica), at magnitude 400×. Preparations were documented in Quantimet 500 Plus system.

Results and discussion

Changes in stability of structure of the collagen-rich tissue can be reflected by changes in number of peptides of various molecular weights, which are released from modified tissues as compared with the native one.

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The electrophoretic profiles of proteins extracted from the pericardium tissues: native (N), treated with penicillin (P) for 24 h, modified with dimethyl suberimidate (DMS) for 3 h, digested with pancreatin (PA) for 3 h have been shown in Figure 1 (gel stained with the Coomassie Brillant Blue).

The molecular weights of proteins extracted from the tissues being investigated have been presented in Table 1.

The results of electrophoretic studies revealed significant differences between the peptide patterns of native tissues and tissues treated with DMS alone, as well as tissues pre-treated with DMS and then sterilized with penicillin (P). It has been demonstrated that the peptides extraction efficiencies were the most significantly reduced in the DMS-modified tissues as compared with other tissues being examined (Fig. 1, Table 1).

The electrophoretic patterns of peptides originating from the P- or P- and DMS-treated tissues after their SDS/NaCl extraction were almost the same as those representing the native tissues (Fig. 1, Tab. 1).

Histological studies revealed differences between native and pancreatin-digested tissues (Fig. 2-5). Loosed structure of the tissue and small cracks of extracellular matrix were observed in images of PA-treated tissues (Fig. 3 and 5). No significant changes in the tissue morphology were observed in samples of the porcine pericardium treated with penicillin (Fig. 4).

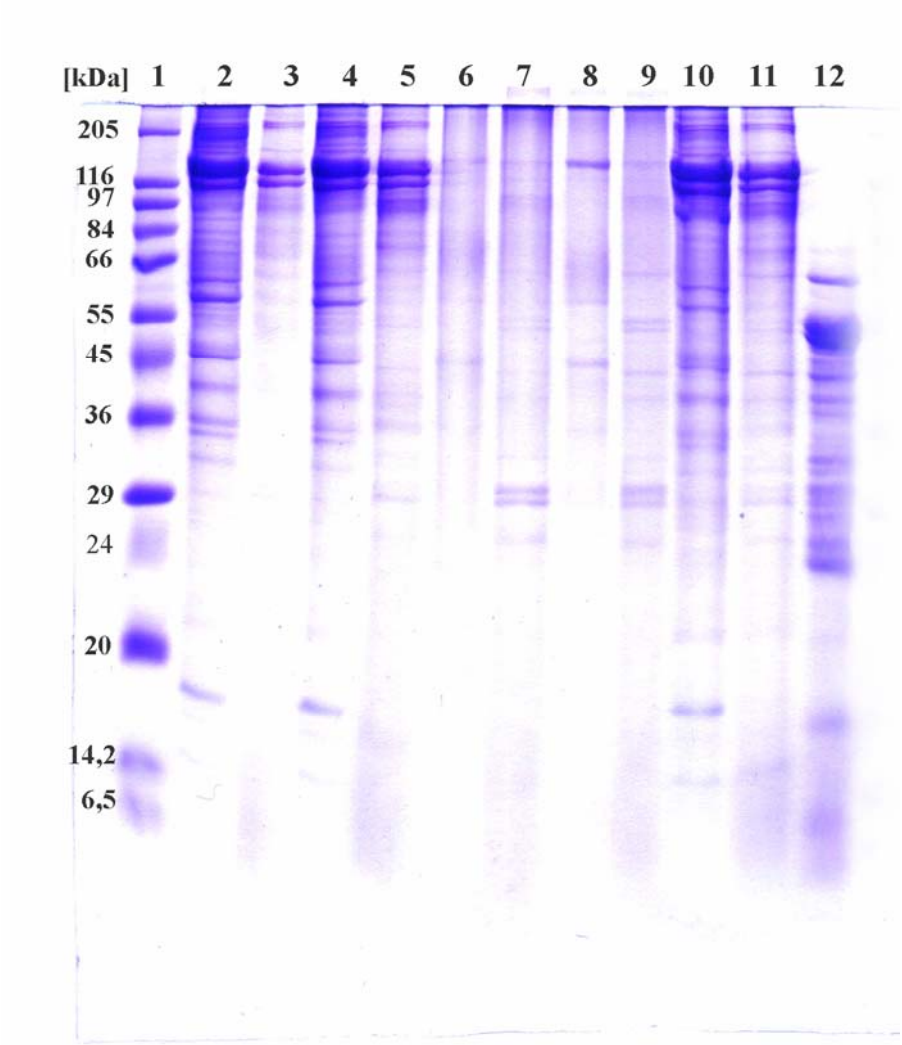

Figure 1: Electrophoretic profiles of peptides extracted from porcine pericardium tissue. Line 1 – molecular weight standards. Line 2 – native tissue (N). Line 3 – N digested with pancreatin (PA) for 3 h (N+PA). Line 4 – tissue treated with penicillin (P) for 24 h. Lane 5 – tissue (N+P+PA). Lane 6 – tissue modified with dimethyl suberimidate (DMS) for 3 h (N+DMS). Lane 7 – tissue (N+DM+PA). Lane 8 – tissue (N+DMS+P). Lane 9 - tissue (N+DMS+P+PA). Lane 10 – tissue N treated with P for 24 h, modified with DMS for 3 h (N+P+DMS). Lane 11 – tissue (N+P+DMS+PA). Lane 12 – pancreatin (PA).

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Table 1: Molecular weights of proteins extracted from the porcine pericardium tissues: native (N), treated with penicillin (P), modified with dimethyl suberimidate (DMS), digested with pancreatin (PA).

Molecular weights [kDa] of peptides extracted from porcine pericardium tissues										
N	**N+PA**	**N+P**	**N+P +PA**	**N+DMS**	**N+DMS +PA**	**N+DMS +P**	**N+DMS +P+PA**	**N+P +DMS**	**N+P +DMS+PA**	**PA**
133,34	133,34	133,34	133,34					133,34	133,34	
114,73	115,97	117,22	117,22	117,22	117,22	117,22	117,22	117,22	118,49	
107,58	109,91	107,58	111,10	108,74				111,10	112,30	
101,95	101,95	101,95	101,95		101,95		101,95	100,88	105,29	
93,56	93,72	94,57	94,57					91,58	91,58	
82,25	83,14	78,80	84,04			82,25		82,25	82,25	
73,09	70,02	73,09			73,09	73,09	73,09	73,88	73,88	77,12
68,53		67,80					62,89	68,53		
										59,60
54,11		53,54			52,11	54,11	52,11	54,70	54,70	51,84
47,57		46,56		46,56		50,20	46,56	46,56	46,56	47,06
41,38		40,50						40,93	40,06	
35,22		35,99						34,85	34,85	44,13
										36,77
					31,64		31,64			32,32
					29,66		29,66			29,03
					25,52		25,52			23,68
										18,30
13,12		12,43						12,70		14,92
11,91		11,29						11,66	11,66	12,43
10,25		9,61						10,14	10,36	
										8,54

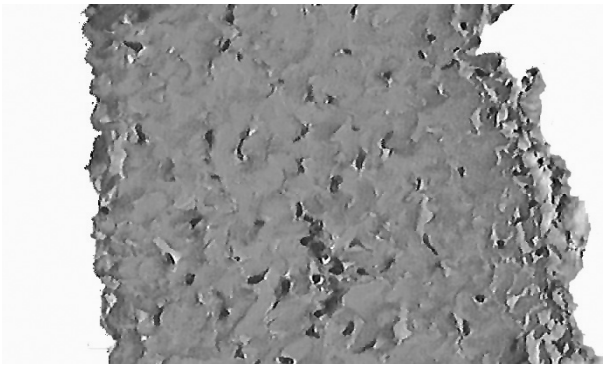

Figure 2: Native PP tissue (N) stained with hematoxylin and erythrosine (magnification 400×). Tight structure of the tissue. Delicate slits in extracellular matrix. Presence of fibroblasts.

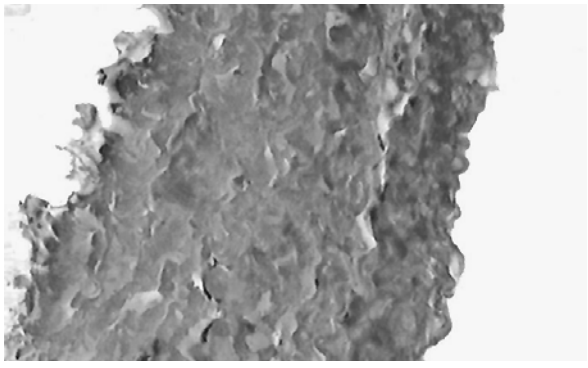

Figure 3: The PP tissue digested with pancreatin (N+PA), stained with hematoxylin and erythrosine (magnification 400×). Loosed structure of the tissue. Small cracks of extracellular matrix. Lack of fibroblasts.

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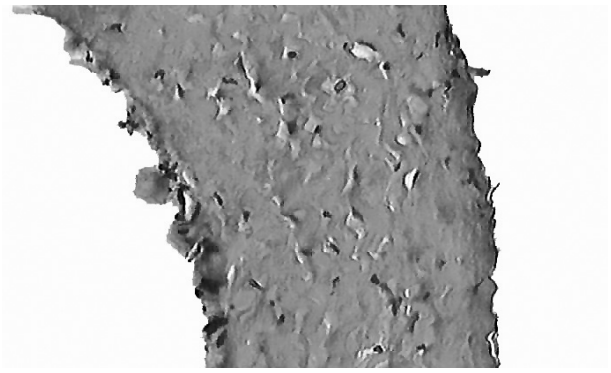

Figure 4: The PP tissue treated with penicillin (N+P), stained with hematoxylin and erythrosine (magnification 400×). Tight structure of the tissue. Delicate slits of extracellular matrix. Presence of fibroblasts.

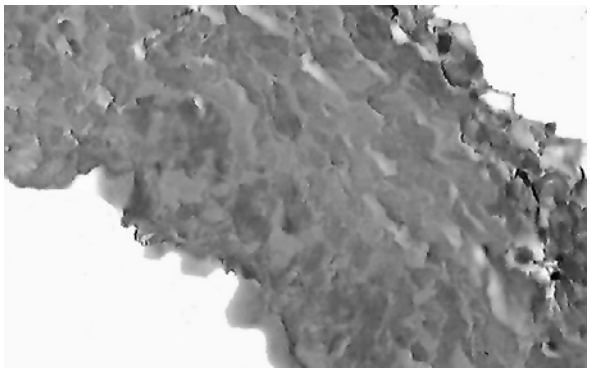

Figure 5: The PP tissue treated with penicillin and digested with pancreatin (N+P+PA), stained with hematoxylin and erythrosine (magnification 400×). Loosed structure of the tissue. Small cracks of extracellular matrix. Presence of fibroblasts.

Conclusions

1. DMS causes stabilization of the porcine pericardium structure; this effect is reflected by decrease in number of peptides extracted from the modified tissue as well as by its higher resistance to enzymatic digestion.
2. Electrophoretic studies are useful for reveal effects of the tissue stabilization.
3. Susceptibilities to the peptides extraction from the tissue were higher in case of the P- or the P- and DMS-treated tissues as compared with the native tissues. It may be stated that the tissue pre-treatment with penicillin (P) resulted in reduction of the DMS crosslinking action.
4. Differences between structure of native and DMS-treated tissues have been shown in histological studies.
5. The porcine pericardium tissue pre-treatment with penicillin did not cause significant changes in the tissue morphology.

Acknowledgements

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