fluorescent labeled Annexin V and CD61 antibodies. The samples were incubated for 10 minutes in the dark and next the labeled samples were processed in a BectonDickinson FACScan flow cytofluorymeter. Our preliminary study was performed for 12 hemodialyzed patients, 13 nondialyzed uremic patients and 12 controls. It was found that the blood platelet population of hemodialyzed patients exhibited significantly higher level of fluorescence intensity attributed to Annexin V. Furthermore, this intensity was comparable before and after hemodialysis and was independent on patient age. The results support the hypothesis that blood platelet contact with artificial surfaces during the process of hemodialysys may be partially responsible for triggering blood platelet apoptosis.

[Engineering of Biomaterials, 89-91, (2009), 29-30]

Acknowledgement

The work was supported by project No. N N401 227434.

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ENDOTHELIAL CELL PROTEOME CHANGED BY CONTACT WITH SURFACES OF BIOMATERIALS

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Abstract

Biomaterials used for medical implants or instruments production can cause numerous undesirable effects in human organism. They may affect cells being in a direct contact with them and can cause changes in genes expression, and as a consequence, also in protein profile of these cells. The aim of the present work was to examine an effect of medical steel 316L, poly-para-xylylene (Parylene) and nanocrystalline diamond (NCD) surfaces on protein expression in human endothelial cell line EA.hy 926. Cells were grown in Dulbecco's MEM (DMEM) supplemented with antibiotics (penicillin and streptomycin), glucose, 10% heat inactivated fetal bovine serum and HAT-supplement. After 48h of incubation cells were washed with PBS and treated with lysis buffer (7M urea, 2M thiourea, 4% CHAPS, 2 % IPG buffer pH 4-7, 1% DTT). Proteins were purified from cell lysates with 2-D CleanUp Kit, and concentration was assessed with 2D Quant Kit. After overnight rehydration of IEF strips (pH 4-7, 11cm), in the presence of purified proteins, isoelectric focusing procedure was performed until 40kVh. Then, stripes were equilibrated, and focused proteins were separated in 12,5% polyacrylamide gels (SDS PAGE). Silver stained gels were recorded with ImageScanner and analyzed with ImageMaster 2D Platinium 6.0 (GE Healthcare) software. Numerous changes in protein expression were detected in endothelial cells exposed to artificial surfaces of tested materials (see TABLE I).

[Engineering of Biomaterials, 89-92, (2009), 30]

Biomaterial	Total number of detected spots	Number of matched spots	Number of over-expressed spots	Number of suppressed spots
Medical steel 316	301	187	45	66
Parylene	283	164	59	54
NCD	423	224	38	75
None (control)	339	339	-	-

Acknowledgement

The project was supported by grant No. 05/WK/P01/0001/SPB-PSS/2008.

NANOSTRUCTURE OF BOVINE PERICARDIUM TREATED WITH TRYPSIN

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Abstract

Various methods of xenogeneic tissues stabilization have been proposed for the purpose of preparing many tissue-derived biomaterials. One of the most important treatments that may lead to obtaining the good-quality tissue biomaterials seems to be decellularization of such tissues. This process may contribute to the reduction of the most frequent failures resulting from the tissues stabilization. The aim of this work was to determine nanostructure of trypsin-treated bovine pericardium, using atomic force microscopy (AFM). The treatment of bovine pericardium with trypsin in EDTA solution resulted in non significant changes in tissue's morphology. Demonstrated AFM studies of these tissues revealed no failures on the fibers' surface in the nanoscale. Thus, our results confirm the expectation that decellularization may be considered as one of the most promising methods of the allogeneic and xenogeneic tissues stabilization.

Keywords: *nanostructure, bovine pericardium, collagen fibers, trypsin*

[Engineering of Biomaterials, 89-91, (2009), 30-32]

Introduction

Nowadays, various methods of xenogeneic tissues stabilization are proposed for the purpose of preparing many tissue-derived biomaterials [1-3]. One of commercial methods of tissues stabilization is the crosslinking of extracellular matrix by glutaraldehyde (GA) action [4,5]. However, GA is responsible for cytotoxic effect [6] and induction of calcification [7]. Moreover, a key role in the premature biodegradation of tissues stabilized in various ways is played by the cellular debris [7] and phospholipids [8]. Decellularization of xenogeneic tissues may contribute to reduction of these failures [9]. The tissue treatment with trypsin in EDTA solution belongs to the most often studied methods of the tissues decellularization [10-12]. However, some risk of connective fibers damage is possible. The aim of this work was to determine nanostructure of bovine pericardium after trypsin treatment, using atomic force microscopy (AFM).

Materials and methods

Bovine pericardium from hearts of 5–6 month old domestic cattle (Bos taurus) was obtained from the local abattoir and subsequently transported to the laboratory in buffered physiological saline solution (PBS; pH 6.5) at 4°C according to Simionescu and co-workers [13] procedure for the pericardium selection for bioprosthetic heart valves. Fatty tissue and sections with heavy vasculature were gently removed from prepared samples.

Tissue samples were modified by incubation under continuous shaking in the solution containing trypsin and EDTA (0.5% trypsin and 0.2% EDTA; Sigma), and PBS in the ratio 1:10, at 37°C for 48 hours. During this period, the digestion solution was changed twice [14].

The nanostructures of native and modified tissue samples were evaluated by AFM. AFM imaging was performed using MultiMode 3 (di-Veeco, CA) working in the tapping mode under atmospheric conditions. Two standard AFM signals were registered: the signal corresponding to the topography of the sample (Height) and the differential signal (Deflection), which is useful for direct observation. Before measurements, tissue samples were gently air-dried, at room temperature in the laminar flow box, until the excess of water had evaporated from the samples' surfaces [2]. All AFM images were processed using the software package WSxM (Nanotec Electronica, Spain) [15].

Results and discussions

FIGURE 1 shows the AFM Deflection (FIG.1A) and Height (FIG.1B) images of native structure of bovine pericardium. Generally, parallel arrangement of collagen fibers was revealed. However, some fibril groups formed by 2-3 fibers and more run in various directions (FIG.1A). Topography analysis of single fiber is presented in FIGURE 1C, where an axial profile taken along the marked line on the surface of collagen fiber in the Height image (FIG.1B) was shown. Collagen fibers in native bovine pericardium showed the regular D-banding pattern characteristic for collagen fibrils type I with the distance of 68-78nm.

The soaking of bovine pericardium in PBS solution with trypsin and EDTA resulted in non significant changes in tissues' morphology. FIGURE 2 shows the AFM Deflection (FIG.2A) and Height (FIG.2B) images of bovine pericardium treated in that manner. Generally, surfaces of trypsin-treated connective tissue samples were free of the extracellular matrix debris. It is important that the collagen fibers structure remains intact as it is clearly visible in FIG.2A. Topography of collagen fiber representing tissue treated with trypsin in EDTA-PBS solution, presented as an axial profile (FIG.2C) taken along the marked line on the fibril surface in the Height image (FIG.2B), reveals regular periodicity. The boundaries between bands are more visible (FIG.2C), which probably results from removal of low molecular proteins from the tissue structure. These data correspond to results of our earlier studies. We have shown that electrophoretic profile of proteins released from porcine pericardium treated in the same way revealed the lack of polypeptides with molecular weight below 24 kDa [12]. Demonstrated results of AFM studies of bovine pericardium treated with trypsin in EDTA-PBS solution do not reveal any failures on the fibers' surface in the nanoscale.

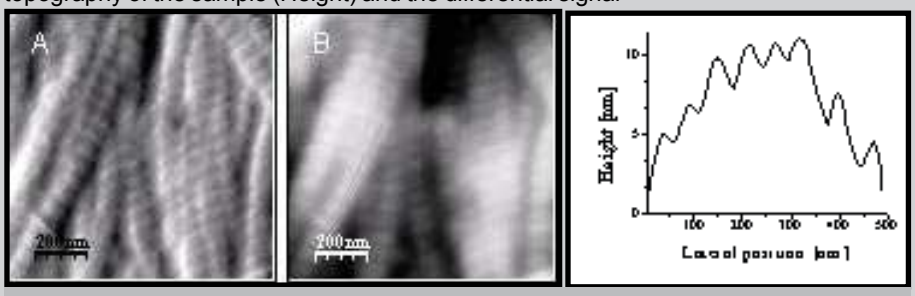

FIG.1. AFM Deflection (A) and Height (B) images (1.0×1.0μm) of the native bovine pericardium; (C) represents an axial profile of the collagen fibril taken along the marked line in the Height image (B).

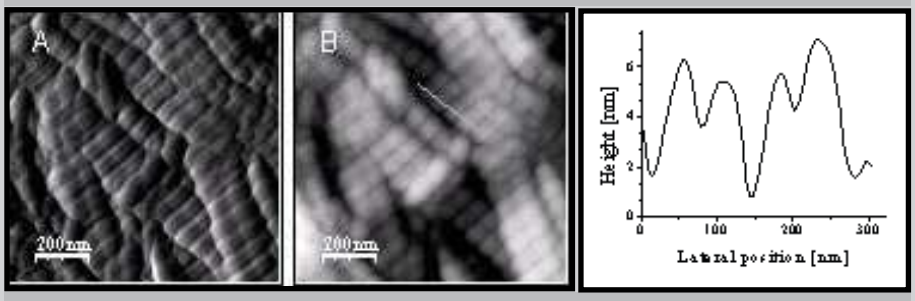

FIG.2. AFM Deflection (A) and Height (B) images (1.0×1.0μm) of the trypsin-treated bovine pericardium; (C) represents an axial profile of the collagen fibril taken along the marked line in the Height image (B).

Conclusions

Although the enzymatic decellularization belongs to the most promising methods of allogeneic and xenogeneic tissues stabilization, the biodegradation processes in modified tissues are complex and still not recognized. It has been found upon presented results that enzymatic "purification" of bovine pericardium surface may influence the reduction of biodegradation processes by elimination of cellular debris and immunogenic agents. The most important aspect of this finding is the lack of deterioration of collagen fibers in the tissue surface layer.

Acknowledgements

This work was financially supported by Silesian Medical University.

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MORPHOLOGICAL STUDIES OF TISSUES STABILIZED BY GLUTARALDEHYDE AND TANNIC ACID

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Abstract

Despite the disadvantages of glutaraldehyde (GA)-stabilization of tissues, it is the method most often used for xenogeneic tissues preparation. Nowadays, partial elimination of drawbacks of this method is achieved by using GA in the mixture with other crosslinking reagents, which completes the stabilization effects and acts synergistically. The aim of this work was to determine microstructure and nanostructure of porcine pericardium stabilized by GA and tannic acid (TA). The microstructure was examined by optical microscopy and the nanostructure by atomic force microscopy (AFM). Different results on the level of micro- and nanostructure were observed. No essential changes in the tissue morphology after crosslinking with GA and TA were observed under optical microscope, but significant morphological differences were revealed in AFM studies.

***Keywords**: glutaraldehyde, tannic acid, porcine pericardium, microstructure, nanostructure*

[Engineering of Biomaterials, 89-91, (2009), 32-34]

Introduction

Glutaraldehyde (GA) is the crosslinking agent most often used in stabilization of xenogeneic tissues [1]. However, GA is cytotoxic [2] and, moreover, the GA-stabilized tissues are susceptible to premature calcification [3]. Procedures of the tissue stabilization with GA are modified by use of GA low concentrations. Partial elimination of drawbacks in the GA-stabilized tissues may also be achieved by using GA in the mixture with other crosslinking reagents, which completes the stabilization effects and acts synergistically. Lately, the attention has been paid on tannic acid (TA) as stabilizing reagent [4-6]. According to these data, the tissue treatment with TA alone or with mixture of GA and TA is proposed as a new method of the connective tissues stabilization. TA-treatment leads to the decrease in the tissue calcification [7] and the increase of extracellular matrix integrity [4].

The aim of this work was to determine microstructure and nanostructure of porcine pericardium stabilized by GA and TA.

Materials and methods

Porcine pericardium from hearts of 5–6 month old domestic pig (Sus scrofa domestica) was obtained from the local