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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

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 [8] 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 crosslinked using solution containing 0.2% GA (Sigma) and 2% TA (Sigma), at the temperature 4°C, during 4h.

Changes on the level of microstructure were observed using the optical microscope Polyvar (Leica) under magnification 200x. Tissues were stained with hematoxylin and erythrosine. The preparation and documentation were performed using Quantament 500 Plus System.

The nanostructures of native and modified tissue samples were evaluated by atomic force microscopy (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 [5]. All AFM images were processed using the software package WSxM (Nanotec Electronica, Spain) [9].

Results and discussion

Histological studies of porcine pericardium stabilized with mixture of GA and TA did not reveal any significant changes in microstructure (FIG.1). Tight structure of collagen fiber-rich connective tissue with slits was observed. The crosslinking of porcine pericardium with the mixture of GA and TA (during 4h) influenced the preservation of fibers structure.

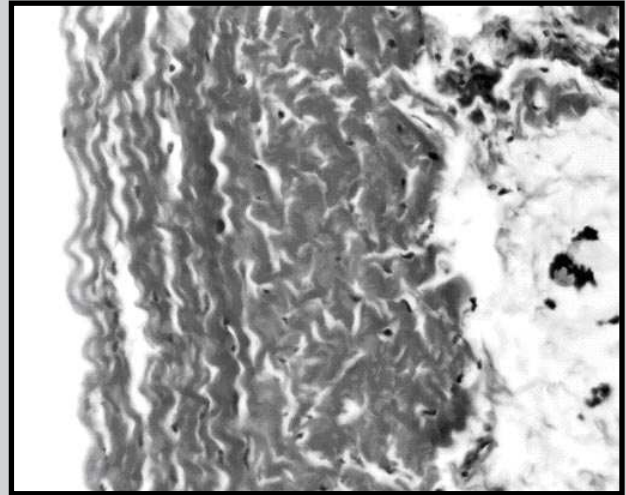


FIG.1. Histological image (magnification 200×) of porcine pericardium crosslinked with the mixture of glutaraldehyde (GA) and tannic acid (TA). Tissue stained with hematoxylin and erythrosine.

However, in the nanoscale significant changes in collagen fibers structure representing tissue modified with mixture of GA and TA were revealed by AFM study (FIG.2).

The crosslinking of porcine pericardium with mixture of GA and TA influences the broadening of collagen fibers (compare FIGs.2A and 2D), which results from the forming of additional crosslinks between tropocollagen chains [5,6]. The tissue crosslinking using mixture of GA and TA influences an axial profile (FIG.2C) taken along the marked line in the Height image (FIG.2B), which reveals irregular periodicity of collagen fiber.

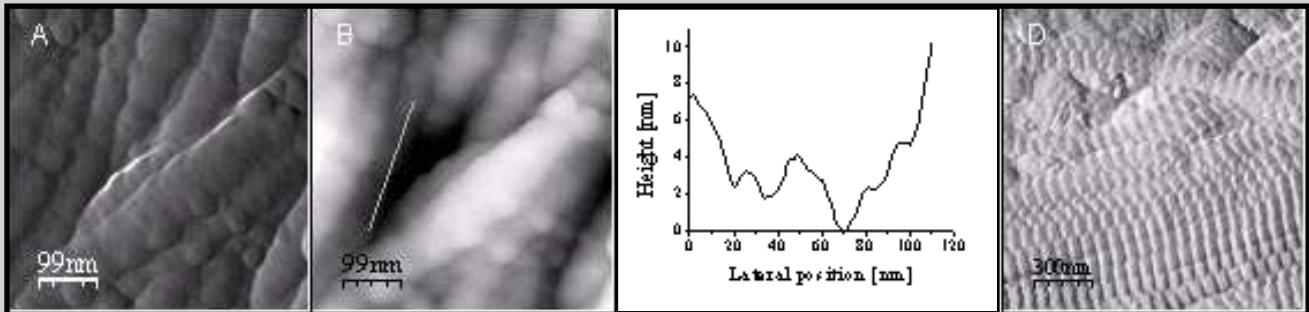


FIG.2. AFM Deflection (A) and Height (B) images (483.2nm×483.2nm) of the porcine pericardium crosslinked with the mixture of GA and TA; (C) represents an axial profile of the collagen fiber taken along the marked line in the Height image (B); (D) - Deflection image of the native pericardium.

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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. These methods of the tissues structure imaging are useful in the research of the tissues stabilization effects.

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HISTOLOGICAL EVALUATION OF THE SOFT TISSUE REACTION AFTER IMPLANTATION OF HERNIA POLYPROPYLENE MESHES

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Introduction

A new type of Dallop® M1) hernia mesh was designed using nonresorbable, monofilament polypropylene yarn by knitting. Resulted implant differs in surface fracture: smooth from one side and coarse on the another and shows significantly high macroporosity (macropore surface - 3,3mm²) and low surface weight.

The aim of the study was to evaluate local tissue reaction after designed medical device implantation. The commercial, polypropylene, monofilament hernia mesh (Duramesh™, CE-marked, Sukol Inc) was used as a control. The samples of the designed implant and control were implanted subcutaneously and into the back muscles of the rabbits (albino New Zealand breed), for 2, 4, 12, 24 and 52 weeks. Macroscopic evaluation indicated the healing process coursed without any complications. Symptoms of low irritation around the subcutaneously implanted samples were present up to 12 weeks due to the constant movements of the samples. In the long time after 26 and 52 weeks neither in case of tested mesh nor in control mesh maintaining inflammation state were macroscopically observed. The histological evaluation of designed hernia mesh Dallop® M presented slight irritation in subcutaneous tissue after 2 weeks that disappeared after 26 weeks of implantation. The process of implant integration caused the formation

of thin layer of connective tissue with fat infiltration around the mesh fibres both in subcutaneous and muscle tissue. After 26 week of implantation the connective tissue covering implants had double-layer structure: fibrous connected with the surrounding tissues and loose and rich-cell of the implant side. After 52 weeks, in direct vicinity of mesh fibres, especially in spaces between filaments, were still present small bands of loose rich-cell connective tissue including: fibroblasts, lymphocytes, single giant cells. Moreover, fat infiltration had different degree both around the tested as well as control implant. Reaction of subcutaneous and muscle tissues in term 2-52 weeks on implanted designed Dallop® M polypropylene hernia mesh allows to recognize it as biocompatible material.

1) DALLOP® M is a design code of commercial OP-TOMESH™ hernia mesh.

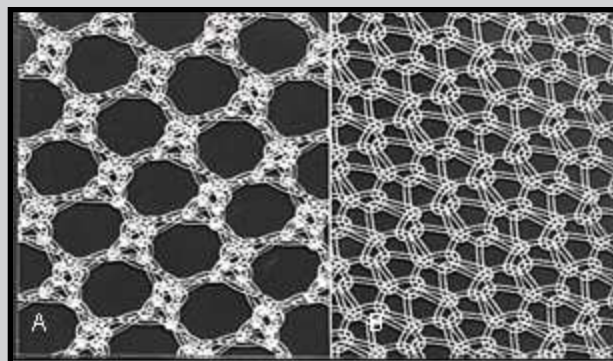


FIG.1. A. Designed Dallop® M hernia mesh, B. Commercial Duramesh™ hernia mesh.

Materials and methods

The following samples for testing were prepared:

- tested material namely polypropylene hernia mesh Dallop® M, in the form of discs at the diameter of 15mm, subject to ethylene oxide sterilization complies with the requirements as for chemical purity and physical properties stated in Initial Quality Release Conditions for the designed product Dallop® M.

- as a control material polypropylene mesh Duramesh™ (manufactured by Sukol Scientific Inc, Germany).

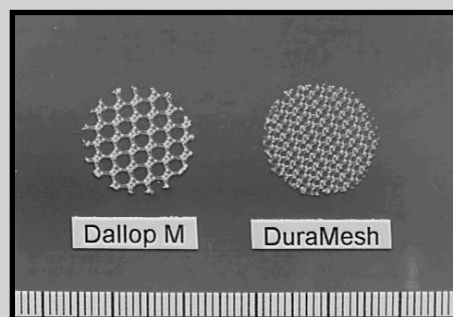


FIG. 2. Samples of tested medical devices.

Operations

In the opinion no. 28/05 dated 21.09.2005 1st Local Ethical Commission on Animal Experiments recognized all planned tests within this project as acceptable.

Tests were conducted on 12 rabbits of New Zealand breed of both sexes. In animal quarters animals were kept separately in cages under controlled humidity (28-37%) and temperature (16-20°C). Animals had unlimited access to water and were fed with standard granulated fodder for rabbits LSK. 24 hours before planned procedure rabbits were fasting with access to water. Hair on the back of the animals on the area of 15cm in length and 10cm in width was