Economical Technology of Creation of Cell-Free Matrix of Animal and Human Arterial Vessels

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We present a technology of creation of blood vessel connective tissue framework by 2-3-h vessel perfusion with detergents. The technology ensures effective removal of vascular cells without damaging collagen and elastic fibers. The connective tissue frameworks prepared by this method can the used for restoring blood flow in various vascular pathologies. The presented approach attenuates the damaging effect of treatment on the vascular framework due to maximum simplification and shortening of the duration of treatment and is universal for human and animal vessels.

Key Words: *connective tissue framework of blood vessel; decellularization*

Plastic surgical reconstruction of great vessels involved in pathological process is now performed in case of ineffective therapeutic treatment [2]. In these interventions, synthetic vascular prostheses are used. Correction of lesions in other organs and tissues often requires transplantation of donor materials.. Regenerative medicine, a new field of medicine aimed at restoration of tissue and organs via using SC, is now actively developing [1,4,7]. For successful and controlled use of SC for the formation of complex spatial structure, a special matrix imitating the shape of restored tissue is required [6]. It is known that vascular wall in humans and animals has a complex multilayer structure on a connective tissue framework presented by collagen fibers. Collagen structures are successfully used as a feeder for SC culturing [3]. However, creation of an extensive volume collagen construct copying the vessel structure is hardly possible. This circumstance stimulated studies aimed at the development of a technology of obtaining decellularized connective tissue matrixes of vessels and other organs preserving their spatial organization [6]. These matrixes can be used for SC application and formation of full-value biological transplants. The known methods of decellularizartion imply the use of multipompotent solutions containing proteolytic enzymes and bioactive compounds, long incubations, and mechanical treatment [8]. However, these factors increase the risk of matrix damage, which will make impossible its further use.

Here we developed a method of obtaining decellularized matrixes of arterial vessels excluding damage to their connective tissue framework.

MATERIALS AND METHODS

The study was performed on arterial vessels of muscular type: rat abdominal aorta and a fragment of human *a. mammaria*. The abdominal aortas were isolated from Wistar rats weighing 350-400 g; to this end, the thorax was opened under rausch-narcosis and the heart together with the abdominal aorta was isolated. The organs were placed in distilled water and the aorta was separated. The aorta cleansed from adjacent tissues was placed in a perfusion chamber and mounted on a cannula. Fragments of *a. mammaria* obtained during coronary bypass surgeries were also used. After isolation, the operation material was stored before treatment

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in antibiotic solution at 4-8°C (2-12 h). The vascular fragment was prepared and cleansed from the adjacent tissues in a dish with distilled water, transferred to a perfusion chamber, and mounted on a cannula.

In all cases, the cleansed vessel was washed from the blood by perfusion with water using a peristaltic pump (10-15 ml at a rate of 20 ml/min). Then, the vessel was washed with working solutions; every hour, a fragment was cut off, fixed in 10% neutral formalin, treated routinely with ascending alcohol concentrations, and embedded in paraffin blocks. For histological study, serial paraffin sections were stained with hematoxylin and eosin.

The working solutions (Table 1) were prepared using Sigma reagents.

RESULTS

In our previous studies, previously described schemes of obtaining decellularized matrix of biological tissues including long-term (16-48 h) treatment with multicomponent solutions [5,9-12] yielded extremely negative results. The effects of various working solutions prepared on the basis of published data on the state of the vessel after 2-h perfusion are summarized in Table 1. Analysis of these results showed that detergents sodium dodecyl sulfate (SDS) and Triton X-100 were the most effective agents for cell removal. Further efforts were aimed at the search of the most effective combination of agents and optimal time of treatment.

The following sequence of treatments was the most optimal. The vessel mounted on the cannula was washed from blood with distilled water for 10-15 min. Then the vessel was successively perfused using a peristaltic pump with 1% SDS and 1% TritonX 100 (1 h for each agent, 20 ml/min perfusion rate) and washed free from detergents with distilled water and physiological saline (10-15 min each). All manipulations were carried out at room temperature.

Preparation of the cell-free matrix could be supplemented by treatment with physiological saline containing DNase and RNase (20 μg/ml each) when needed. In our study this treatment was performed for 1 h at 37°C. Enzyme activity was stopped by 10-15-min treatment with cold physiological saline (4-8°C).

The described technology of decullarization was tested on 40 laboratory animals and 20 operation samples of human vessels.

The efficiency of the decellularization procedure was evaluated by comparing histological preparations of the same vessel from experimental animals (Fig. 1) and humans (Fig. 2) before and after treatment. Analysis of histological preparations of rat abdominal aorta showed that its wall (Fig. 1, *a*) initially has typical multilayer structure including cell elements and connective tissue components. After decellularization (Fig. 1, *b*) the multilayer structure of the vascular wall

TABLE 1. Effects of Working Solutions of Different Compositions on the State of Perfused Vessel

Solution	Result after 2-h perfusion
Trypsin — 0.05%, EDTA — 5 mM, Tris — 10 mM, pH 8.0	Cell count decreased by 67% compared to the control, equal distribution of cells in the media, no cells in the media and adventitia, loosening of vascular wall, and zones of pericellular loosening of the stroma were observed
Trypsin — 0.5%, EDTA — 0.05%, pH 8.0	Cell count decreased by 64%, but cells were unevenly removed from the vascular wall, solitary foci of elastic membrane damage, reduced content of acid and neutral mucopolysaccharides, zones of partial destruction of the vascular wall were noted
EDTA — 5 mM, tris — 10 mM, pH 8.0 (hypotonic solution)	Cell count decreased by 36% compared to the control, this decrease was uneven, adventitial and endothelial cells were partially preserved, uneven loosening of the vascular wall and decrease in the number of thin collagen fibers were observed, the content of acid mucopolysaccharides decreased throughout the vascular wall thickness, contours of elastic membranes looked interrupted
Triton X-100 — 1%, NaCl — 1 M, EDTA — 10 mM, tris — 50 mM, pH 8.8	Endothelial and adventitial cells were absent, irregular cell removal from the media (53-57%), vast zone of vascular wall damage
Triton X-100 — 1%, pH 9.0	Cell content in the media decreased by 49% compared to the control, the cells are unequally distributed on section, adventitial and endothelial cells are completely absent, vascular wall was little changed, solitary lacunes were seen
SDS — 1%, pH 9.0	The content of cell elements evenly decreased (to 47%), 100% decellularization of the adventitia, complete removal of endothelial cells, preserved structure of the vascular wall, partial destruction zones

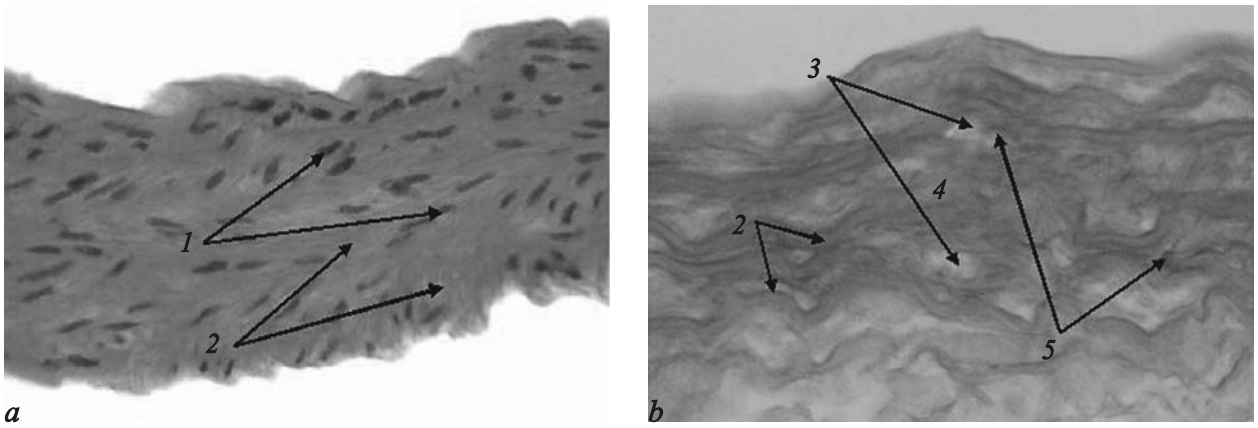

Fig. 1. Rat blood vessel before (*a*) and after treatment (*b*). Hematoxylin and eosin staining, ×400. *1*) cells; *2*) connective tissue; *3*) lacunes; *4*) zone of connective fiber homogenization; *5*) chromatin lumps.

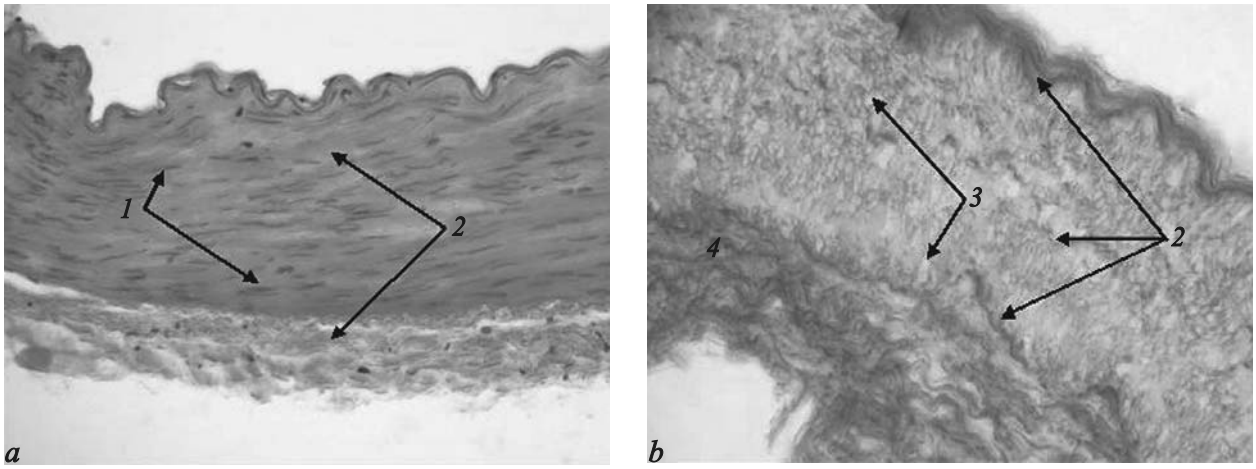

Fig. 2. Human blood vessel before (*a*) and after treatment (*b*). Hematoxylin and eosin staining, ×400. *1*) cells; *2*) connective tissue; *3*) lacunes; *4*) zone of connective fiber homogenization.

was preserved, but cell elements were completely absent. The areas previously occupied by cell elements looked like lacunes. In some cases, chromatin lumps were seen at the sites of destructed cells. The elements of the connective tissue framework are well preserved and have loosened structure. Examination of serial sections revealed solitary small foci of homogenization of connective tissue fibers.

Histological study of intact human *a. mammaria* showed that its wall also consists of the connective tissue framework and cells forming three main layers: endothelium, media, and adventitia (Fig. 2, *a*). After completion of the treatment cycle (Fig. 2, *b*), no cell elements were seen in these three layers, while the connective tissue framework looked like a reticulum. The thickness of the vascular wall was relatively even. In some preparations, swelling of solitary connective tissue elements in the media was observed.

Thus, we chose conditions ensuring removal of cell elements from the arterial wall without damaging its connective tissue framework. The total duration of treatment did not exceed 4 h and the proposed protocol can be used for the treatment of both animal and human vessels.

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