

THE EFFICIENCY OF DECELLULARIZATION OF BOVINE PERICARDIUM BY DIFFERENT CONCENTRATIONS OF SODIUM DODECYL SULFATE

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Background. In modern cardiovascular surgery, it is a promising method to use xenotissues, which in their properties are close to human tissues, in order to restore the integrity of the heart chambers, its walls or valves. Decellularization of extracellular matrix (ECM) is applied in the process of creation of such bioprotheses. In ECM, the elastin and collagen components are preserved, and antigenic molecules are eliminated resulting in reduction the risk of rejection. The study is devoted to assessment of the histological, molecular-genetic and cytotoxic properties of decellularized bovine pericardium, processed with various concentrations of trypsin enzyme.

Objective. The aim of the work is evaluation of efficiency of bovine pericardial decellularization, based on the use of trypsin enzyme with 1% Sodium Dodecyl Sulfate (SDS) and 0.1% SDS.

Methods. Bovine pericardium was used as a biomaterial for decellularization. Decellularization protocol 1 envisages processing of samples with 0.25% Trypsin solution at 24 °C with constant shaking (200 rpm) along with processing with 1% ionic SDS detergent. The samples, prepared according to protocol 2, were processed with a low concentration of 0.1% SDS. Histological and morphological properties along with detection of nucleic acids concentration in the samples were studied. Matrix samples were cultured in a human fibroblasts cell culture suspension in order to determine cytotoxicity.

Results. Histological examination has not revealed any presence of cells in tissues, decellularized in accordance with both protocols. More than 99% of the nucleic acids was removed from decellularized bovine matrix. During our study, we have not observed cytotoxic effect *in vitro* for protocol 2 matrix samples, decellularized with only 0.1% SDS. Focal destruction of fibroblasts was observed in conditions of long-term cultivation in protocol 1 samples (Trypsin + 1% SDS). Cells formed abnormal morphological aggregates. Samples of this group have also demonstrated structural changes in collagen and elastin fibers.

Conclusions. Studies have shown that pericardial matrix tissue, decellularized with low-concentration of 0.1% SDS, has the same biological properties as the native pericardium. Decellularization of bovine pericardium, using trypsin enzyme with 1% SDS, has a cytotoxic effect on human cells.

Keywords: pericardium; decellularization; sodium dodecyl sulfate; tissue engineering.

Introduction

Decellularized extracellular matrix (dECM), extracted from pericardium, has been extensively investigated as a natural scaffold for cardiac tissue engineering applications [1]. dECM is used in cardiac surgery for congenital and acquired heart pathologies to renew heart chambers walls or heart valves integrity [2, 3]. The bioimplant should be as similar as possible to natural composition of myocardial biopolymers (e.g. collagen, elastin and glycosaminoglycans), mechanically integral and support cardiac cells *in vitro*. Nowadays dECM of bovine pericardium scaffold is of interest for tissue reconstruction purposes, arising upon implantation of damaged heart [4]. However, there are several

issues that need to be solved prior to clinical application of cardiac dECM which included developing of optimal decellularization method, preservation of vasculature and ECM composition, recellularization strategies providing proper reintroduction of cells into specific compartment of the scaffold and prevascularization of thick cardiac dECM [5]. In Center for pediatric cardiology and cardiac surgery we conduct the study aimed at determining the safety and efficacy of different methods of bovine pericardium decellularization. Basing on the scientific investigation results, the most appropriate method of cardiac implant manufacturing was chosen. This method envisages the use of low-concentration Sodium Dodecyl Sulphate (SDS) and Trypsin enzyme. The process has demonstrated the removal