Adherence and Growth of Endothelial Cells in Treated Bovine Pericardium

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Introduction

Bovine pericardium (BP) is one of the most used biomaterial to manufacture living tissue implant as heart valves. Among a variety crosslinlers for BP, glutaraldehyde has been found to yield a stable product although calcification occurs in about 40% of patients by 10 years. By the other hand, there is a considerable interest in promoting rapid and firm adhesion of endothelial cells for a number of clinical applications, including seeding of vascular tissue engineering. In order to obtain a BP for heart valves with improved properties for endothelial cell seeding, the tissue was crosslinked with phenethylamine diepoxide [1] and lyophilization [2] was used to decrease the cytotoxicity of the samples. The aim of the present study was to compare the proliferation of human umbilical vein endothelial cells (HUVEC) on treated lyophilized membranes of BP.

Materials and Methods

Bovine pericardium (BP): Samples of pericardium were collected at slaughthouse, cleaned, washed, and stored in glycerol solution for 3 months. Before use, pericardium was rehydreted with saline solution (NaCl 0,9%) (1) used as control and in the others treatments, (2) treated with glutaraldehyde, washed with saline solution and lyophilized; (3) lyophilized and immersed in phenethyl amine-diepoxide and lyophilized again.

Cell culturing: Human umbilical vein endothelial cells (HUVEC) from ATCC (CRL 1730) were maintained in F12 medium supplemented with antibiotic and antimicotic solution (final concentration: 100 units/mL penicillin, 100 µg/mL streptomycin and 0.025 µg/mL amphotericin), 2mM glutamine, 20µg/mL endothelial cell growth supplement, 90µg/mL heparin and 10% bovine fetal serum, at 37° C in a humidified 5% CO_2 atmosphere until they reached confluence. For subculturing and for experiments, cells were harvested using 0.05% trypsin and 0.02% EDTA in phosphate-buffered saline, pH 7.4.

Cell seeding onto BP: The PB were placed in a 6 multiwell plate and a seeding ring was accommodated on it with culture medium for HUVEC for 24 hours. After this time, the culture medium was changed for a cell suspension with $10x10^3$ cells/mL. The plate was left in the incubator with medium changing each 3 days. After 21 days the membranes were reacted with antibody mouse anti – human factor VIII and FICT anti – mouse for cytoplasm identification and ethidium bromide for nucleus identification and analyzed at the confocal microscopy for cells identification. The same samples were analyzed in a scan electron microscopy (SEM) for BP structure observation.

Results and discussion

The micrographics images (frontal and transversal) in Figure 1 were captured with a confocal laser scanning microscope and SEM, of BP membranes treated with glycerol (control), glutaraldehyde and phenethylamine diepoxide.

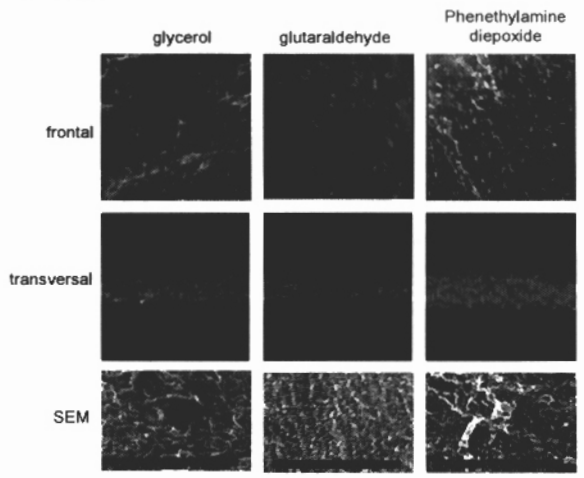

Figure 1. Bovine pericardium seeded with HUVEC.

The comparison of SEM micrographics pointed out porosity on the surface of the third BP sample. That topography justifies the adhesion and the greater spreading of the cells. Using immunocytochemical marker for HUVEC, the photos clearly showed that the glutaraldehyde treated BP promoted a smaller cell population growth, due probably on its more compact structure. These results also demonstrate a promising use of phenethylamine diepoxide as crosslinker for BP membranes.

Conclusion

Treated and lyophilized bovine pericardium with reduced cytotoxicity showed a high degree of biofunctionality when seeded with endothelial cells (HUVEC) from ATCC. The treatment of BP with phenethylamine diepoxide as crosslinker provided a porous structure in the membrane suitable for cell growth.

References:

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