Abstract

Currently used xenogeneic biological heart valves have several limitations in clinical practice. The main problem is the development of degenerative changes leading to valve failure. Re-surgery is required in approximately 65% of patients at 15 years after implantation. The challenge of heart valve tissue engineering is to create a new type of autologous biological heart valve prosthesis for clinical use with living cells capable of valve tissue remodeling. Several approaches are used with different types of scaffolds, and with a variety of cells and laboratory protocols. Most of them have not proven themselves, due to limitations such as scaffold immune system incompatibility, non-optimal mechanical properties, scaffold shrinkage, poor cell penetration, low extracellular matrix production and poor biomechanical properties and no remodeling potential after implantation *in vivo*.

In the first part of the research, the objective is to compare the cellular matrix, the extracellular matrix structure and the mechanical properties of human pericardium as a potential scaffold for autologous heart valve tissue engineering with the structure of the normal human aortic heart valve.

The second part of the research deals with the preparation of a dynamic culture system (a bioreactor) for *in vivo* human pericardial tissue three-dimensional (3D) conditioning with pulse and regulated flow of the culture medium.

In the third part of the research, a pilot study with the use of a new method for preparing a three-cusp heart valve construct from human pericardium for potential use as a heart valve replacement based on 3D pericardial tissue conditioning (mechanotransduction). Human pericardium samples were harvested during cardiac surgery and were cultured under dynamic conditions *in vitro* in the shape of the three cusp aortic heart valve for up to four weeks. After this time, the conditioned pericardial samples were compared with the control unconditioned pericardial samples from the same patient, and with the normal aortic heart valve obtained during heart transplantation.

Human pericardium consists of vimentin-positive pericardial interstitial cells (PICs), which have similar properties to those of human valve interstitial cells (VICs). These cells are able to respond to mechanical stresses through a process called 3D mechanotransduction, by proliferating and differentiating into an active phenotype capable of producing new extracellular matrix (ECM). This was shown by a statistically significant increase in vimentin and alpha smooth muscle actin (α-SMA) positive cells after conditioning, and also by increased production of collagen I, elastin and glycosaminoglycans (GAGs). The histological

structure of the conditioned pericardium is very similar to that of the normal aortic heart valve, and 3D dynamic conditioning was proven to be important for PIC activation and tissue remodeling.

Based on the results of this study, autologous human pericardium may be a promising tissue from which to construct a living heart valve substitute. A heart valve replacement of this type may possess optimal biomechanical and hemodynamic properties and may be free of negative immune response after implantation *in vivo*.