

***In vitro* development and preconditioning and *in vivo* testing of biological heart valve substitutes derived from the extracellular matrix**

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The premises of the study:

Based on the proportion of heart valve diseases in both pediatric and adult patients groups with a growing life expectancy and the well-known disadvantages of current valve replacements - the need for permanent anticoagulation for mechanical prostheses and the large number of biological valve replacements due to degeneration processes, it is necessary to design the next generation of valve replacements. Using the fundamentals of regenerative medicine and tissue engineering technologies, our study is based on a translational scenario of generating, preconditioning and testing a new type of heart valve prosthesis using temporary supports - scaffolds (decellularized heart valves), which have been repopulated with heart valves specific cells (differentiated from autologous stem cells derived from adipose tissue), and the newly obtained structures were preconditioned *in vitro* (in a bioreactor dedicated to the heart valves) and finally implanted in ovine experimental animals, subsequently followed by cardiac ultrasound for six months after implantation.

Objectives:

Study #1: aimed to obtain temporary cellular supports, scaffolds derived from the extracellular matrix. Two procedures of cells removal (decellularization) from porcine pulmonary valves were used and compared. The initial used procedure and protocol showed unsatisfactory results with nucleic material residues in the matrix when assessed histologically. The new protocol sought to improve the results by adding a pressure gradient. The two protocols were analyzed in terms of quality control - sterility, histology and DNA extraction.

Study #2: was dedicated to stem cells derived from adipose tissue of ovine origin, trying to identify a solution to preserve them and expand their usage over the time. The stem cells underwent differentiation protocols towards specific heart valves cell lines (endothelial cells and fibroblasts).

Study #3: presents the activity of cell repopulation of scaffolds *in vitro* at the endoluminal, interstitial and adventitial level with their subsequent preconditioning in a heart valve dedicated bioreactor. The newly obtained valve was implanted by open heart surgery in extracorporeal circulation in the pulmonary orthotopic position in ovine model experience animal and tested *in vivo* for a period of six months. The functionality and performance of the valves were monitored and documented by transesophageal, epicardial

and transthoracic echocardiography and the explants were analyzed by classical histology and immunohistochemistry.

Study #4: represents a case presentation of a patient with a mechanical prosthesis in aortic position that associated a hematological pathology more than 20 years after the implant. This association with the anticoagulant status of the patient conferred the premises of a neurosurgical condition culminating in fatal end.

Conclusions:

Study #1: usage of physical agents in decellularization procedures - the pressure gradient results in an improved removal of the cells, ensuring an acellular extracellular matrix. The quality control revealed absence of cell nuclei at the histological analysis of the valve components, respectively undetectable DNA levels in the extraction procedures for the perfusion decellularized valves group.

Study #2: ovine adipose tissue derived stem cells are an important and feasible source of stem cells in scientific research. Their preservation for long time periods can be achieved with 10% DMSO solution. Their differentiation towards the fibroblastic line is acquired using growth factors respectively towards the endothelial using chemical and physical stimuli (shear stress). Exposure to mechanical stimuli is performed in the cells incubator, using a manufactured rotation system that has no impact towards the incubator environment.

Study #3: cell seeding procedures on scaffolds revealed viable cells but repopulation was performed on limited areas, microscopy also revealing presence of acellular areas. The use of the bioreactor highlighted functional valves and its use did not have a negative impact on valves integrity. Surgical implantation procedures in the animal model were successful but the echocardiographic follow-up was difficult due to suboptimal windows. The analysis of the explanted valves revealed cells with homogeneous distribution, present especially at the level of the cusps.

Study #4: although they provide valvular patients with a much-improved quality of life and increased life expectancy, mechanical heart valve prostheses, require anticoagulant therapy that exposes the patient to a number of risks such as thrombosis or bleeding. When the risks overlapped with other pathological conditions such as hematologic disease, their lethal potential increases, clinicians being placed in front of life saving decision-making situations that are difficult to balance.