Univerzita Karlova v Praze

2. lékařská fakulta

Studijní program: Fyziologie a patofyziologie člověka

MUDr. František Straka

Využití mechanismů tkáňové modifikace k přípravě autologní perikardiální chlopenní náhrady

The use of tissue modification mechanisms for preparing an autologous pericardial heart valve replacement

Dizertační práce

Školitel: prof. MUDr. Jan Pirk, DrSc.

Praha, 2016

Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracoval samostatně a že jsem řádně uvedl a citoval všechny použité prameny a literaturu. Současně prohlašuji, že práce nebyla využita k získání jiného nebo stejného titulu.

Souhlasím s trvalým uložením elektronické verze mé práce v databázi systému meziuniverzitního projektu Theses.cz za účelem soustavné kontroly podobnosti kvalifikačních prací.

V Praze dne 6.6. 2016

František Straka

Identifikační záznam:

STRAKA, František. Využití mechanismů tkáňové modifikace k přípravě autologní perikardiální chlopenní náhrady *[*The use of tissue modification mechanisms for preparing an autologous pericardial heart valve replacement*]*. Praha, 2016. Počet stran 99, počet příloh 7. Dizertační práce (Ph.D.). Univerzita Karlova v Praze, 2. lékařská fakulta, Institut klinické a experimentální mediciny v Praze. Školitel prof. MUDr. Jan Pirk, DrSc.

Abstrakt

Xenogenní biologické srdeční chlopenní náhrady používané v současnosti v klinické praxi mají celou řadu limitací. Hlavním problémem je rozvoj degenerativních změn, který vede k dysfunkci chlopně. Reoperace je nutná až u 65% pacientů za 15 let po implantaci těchto chlopní. Cílem tkáňového inženýrství je vytvořit nový typ autologní biologické náhrady srdečních chlopní pro klinickou praxi, která bude obsahovat živé autologní buňky a bude mít zachovánu schopnost remodelace. Za tímto účelem se používají různé typy scafoldů, buněk a různé laboratorní postupy. Většina z nich se však neosvědčila pro různé limitace, jako je nekompatibilita s imunitním systémem, špatná penetrace buněk do scafoldů, nízká produkce mezibuněčné hmoty, špatné biomechanické vlastnosti a nezachovaná schopnost remodelace po implantaci *in vivo*.

V první části výzkumu bylo cílem porovnat buněčné složení a strukturu mezibuněčné hmoty a mechanické vlastnosti lidského perikardu jako potenciálního scafoldu pro tkáňové inženýrství autologní srdeční chlopennní náhrady se strukturou normální lidské aortální chlopně.

Druhá část výzkumu byla soustředěna na přípravu dynamického kultivačního systému (bioreaktoru) pro trojrozměrné (3D) dynamické kondicionování tkáně lidského perikardu regulovaným pulsním průtokem kultivačního media.

Ve třetí části výzkumu byla provedena pilotní studie s užitím nové metody přípravy trojcípé srdeční chlopenní náhrady založené na 3D kondicionování tkáně lidského perikardu (mechanotransdukci). Vzorky lidského perikardu byly odebírány během kardiochirurgických výkonů a kultivovány 4 týdny v dynamických podmínkách *in vitro* ve tvaru trojcípé aortální chlopně. Poté byly vzorky kondicionované tkáně perikardu srovnány s tkání nekondicionovaného perikardu od stejného pacienta a s normální aortální chlopní odebranou při srdečních transplantacích.

Lidský perikard obsahuje vimentin pozitivní perikardiální intersticiální buňky (PICs), které mají podobné vlastnosti jako chlopenní intersticiální buňky (VICs). Tyto buňky jsou schopné reagovat na mechanické zatěžování v procesu nazývaném 3D mechanotransdukce proliferací a diferenciací do aktivního fenotypu a produkcí nové mezibuněčné hmoty (ECM).

To bylo dokumentováno statisticky významným vzestupem vimentin a α-SMA pozitivních PICs buněk, a také zvýšenou produkcí kolagenu I, elastinu a glykosaminoglykanů (GAGs). Histologická struktura lidského perikardu po kondicionování je velmi podobná normální

aortální chlopni a bylo prokázáno, že 3D mechanické kondicionování je důležité pro aktivaci PICs buněk a remodelaci tkáně.

Na základě výsledků této studie lze konstatovat, že autologní lidský perikard může být slibnou tkání k připravě srdeční chlopenní náhrady se živými buňkami. Náhrada srdeční chlopně tohoto typu může mít optimální biomechanické a hemodynamické vlastnosti a nebude vyvolávat nežádoucí imunitní reakci po implantaci *in vivo*.

Klíčová slova: autologní lidský perikard, perikardiální intersticiální buňky, intersticiální buňky aortální chlopně, mezibuněčná hmota, 3D zatěžování, mechanotransdukce, bioreactor, sečný modul pružnosti, tkáňové inženýrství.

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*.

Keywords: autologous human pericardium, pericardial interstitial cells, aortic heart valve, valvular interstitial cells, extracellular matrix, 3D conditioning, mechanotransduction, bioreactor. secant elastic modulus, tissue engineering.

Poděkování:

Chtěl bych velmi poděkovat především panu profesoru Janu Pirkovi, Dr.Sc., přednostovi Kardiocentra IKEM, který mi umožnil věnovat se vědecké práci i při mé klinické praxi v IKEM, za jeho odborné a profesionální vedení, mimořádně lidský přístup, pochopení, trpělivost a podporu nejen při řešení problémů každodenní klinické práce v IKEM, ale rovněž při zavádění inovativních metod a řešení otázek souvisejících s touto vědeckou prací. Můj dík patří i mnoha dalším spolupracovníkům a kolegům z IKEM, kteří mi pomáhali realizovat tento výzkum.

Z dalších spolupracovníků chci poděkovat doc. MUDr. Lucii Bačákové, CSc., a kolektivu Oddělení tkáňového inženýrství Akademie věd ČR, se kterými jsem na vědecké práci spolupracoval, za jejich odbornou pomoc při realizaci tohoto projektu.

Další mé poděkování patří kolegům z ČVUT v Praze, zejména Ing. Hynku Chlupovi a jeho kolegům, kteří se podíleli především na hodnocení biomechanických vlastností tkání.

Současně bych chtěl poděkovat i všem mnoha dalším spolupracovníkům s technickým či biologickým zaměřením za spolupráci při řešení různých problémů, které se vyskytly v rámci této vědecké práce.

V neposlední řádě mé poděkování patří mým rodinným příslušníkům a příbuzným za podporu a pochopení během mé vědecké práce.

Acknowledgements:

I would particularly like to acknowledge Professor Jan Pirk, M.D., Dr.Sc., head of the Cardiology Centre and Cardiovascular Surgery Department of the Institute for Clinical and Experimental Medicine (IKEM). He enabled me to focus on my research work during my clinical practice at IKEM, and I would like to thank him for his expert and professional leadership, his exceptionally human approach, understanding, patience and support in solving problems in daily clinical practice at IKEM, introducing innovative methods and resolving questions regarding this scientific work. I would also like to thank many other collaborators and colleagues from IKEM, who have helped me execute this research.

Among other colleagues I would like to thank Associate Professor Lucie Bačáková, M.D., Ph.D, and the team of the Department of Biomaterials and Tissue Engineering at the Academy of Science of the Czech Republic, with whom I collaborated, for their professional help in executing this project.

My further thanks go to colleagues from the Czech Technical University in Prague, particularly Hynek Chlup, M.Sc. and his team, who specifically contributed to evaluating the biomechanical characteristics of tissues.

I also appreciate the help of many colleagues with technical or biological specializations for collaborating on a solving various problems that occurred over the course of this scientific work.

Last but not least, my thanks go to my family members for their support and understanding during my research.

1. Table of contents

2. Abbreviations

α-SMA alpha - Smooth Muscle Actin

CAD computer-aided drafting

CD31 Cluster Differentiation 31, platelet-endothelial cell adhesion molecule, also referred to as PECAM-1

CMFDA 5-chloromethylflorescein diacetate

CW Continuous-Wave Doppler

DMEM Dulbecco's Modified Eagle's Medium

ECM ExtraCellular Matrix

EOA Effective Orifice Area

Es Elastic secant modulus

ESEM Environmental Scanning Electron Microscope

FBS Fetal Bovine Serum

GAGs GlycosAminoGlycans

H&E Hematoxylin-eosin and elastica staining

HPF High-Power Fields - the maximum magnification power of the objective

HP unconditioned Human Pericardium

Ki-67 nuclear marker for cell proliferation

LCA common LeukoCyte Antigen

NAV Native (normal) Aortic Valve

PICs Pericardial Interstitial Cells

PW Pulsed-Wave Doppler

SEM Scanning Electron Microscopy

SHG Second Harmonic Generation microscopy

STL format technology called STereoLithography) for 3D printing

SV Stroke Volume

VICs Valve Interstitial Cells

3D 3 Dimensional

TEV Tissue-Engineered Valves

TPEM two-photon excitation microscopy

2D 2 Dimensional

VTI Velocity Time Integral

3. Introduction

Heart valves are very important for normal blood flow in the heart. Valve defects are either stenoses, meaning narrowing, or regurgitations caused by incompetence of the valves. The prevalence of heart valve diseases increases with age. It is estimated that around 300,000 valve replacements are implanted worldwide each year and this number will likely rise manifold in the upcoming decades (Yacoub M.H., Takkenberg J.J., 2005).

The valve replacements currently used (for examples, see Fig. 1) have a number of limitations and risks for the patient. Mechanical valve replacements are made from pyrolytic carbon or titanium components and, upon implanting, require permanent anti-coagulation treatment. They are subject to a risk of dysfunction of the valve replacement due to the structural defects of mechanical valves, paravalvular regurgitation, hemolysis, and thrombosis, fibrous tissue of the pannus or infectious endocarditis on their surfaces that is not natural for the body. The incidence of mechanical valve dysfunction is reported in 2-4% of patients each year and the mortality caused by this dysfunction is 1-2% of patients per year (Vojáček J., Kettner J. et al., 2009; Vahanian A. et al., 2012).

Exogenous bovine valves fixated with glutaraldehyde, valves produced from bovine pericardium, and cryopreserved allografts (homografts) from human donors (see Fig. 1) are widely used biological valve types at the present time. Pericardial valves, crosslinked and fixated by glutaraldehyde, have greater resistance against degradation by collagenases. They further prevent infiltration by cells and the natural remodeling of the valve, and have a tendency toward more frequent occurrence of undesirable degenerative changes and calcification, which are described in 2-4% of patients per year (Popelová J. et al., 2007). In the case of biological valve replacements, the necessity of reoperation due to structural changes (see Fig. 2 A,B,C) occurs in up to 65% of patients under 60 years of age within 15 years of implantation. For this reason, they are primarily used in patients over 65 years of age (Popelová J. et al., 2007; Vahanian A. et al., 2012; Cheung D.Y. et al., 2015, Welke K.F. et al., 2011). Compared with native heart valves, they have more problematic biomechanical characteristics and their surfaces are not covered with endothelium (Vojáček J., Kettner J. et al., 2009; Vahanian A. et al., 2012).

Ross' operation (a diseased aortic valve is replaced with the patient's pulmonary valve, which then replaced either by a mechanical valve or by a biological valve) is surgically difficult, and is linked with up to 10% higher early mortality. It has unconvincing long-term results, and it is necessary to reoperate in up to 20% of patients within 10 years. In a long-

term study, the main complication is stenosis of the homograft, for which replacement of the pulmonary valve is required (Kallio M. et al., 2015).

Therefore, there is currently no optimal valve replacement available in clinical cardiology and in cardio surgery that would be "custom-made for each patient", be natural for the body and contain live autologous cells capable of remodeling the extracellular matrix (ECM), have a surface covered with endothelium, and not cause an undesirable immune response leading to an earlier development of degenerative changes (Fig. 2A, B, C) or the need to re-operate.

Fig. 1. Some types of present-day valve replacements – a double-disc mechanical valve and a biological valve replacement for surgical and catheter implantation.

Fig. 2A. An explanted biological valve replacement with significant degenerative changes, thickening, calcification, and rigidity of the cusps.

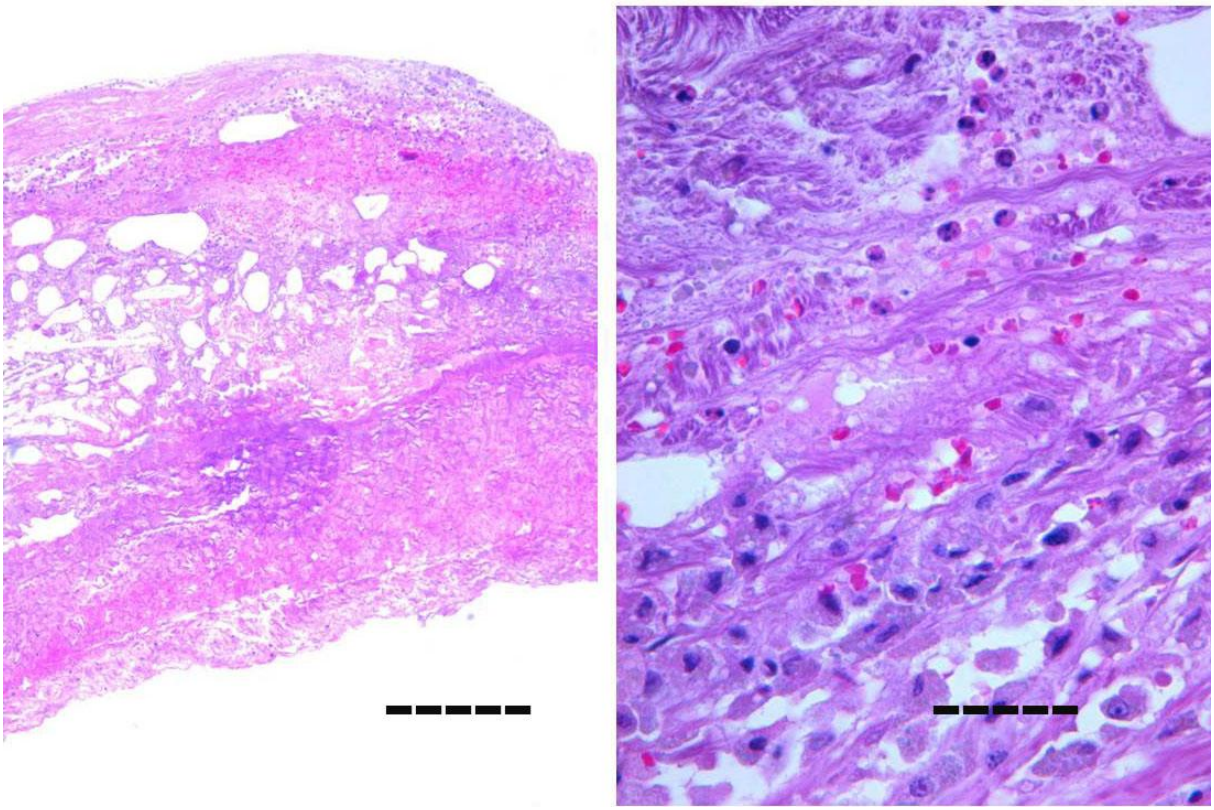


Fig. 2B. Biological valve replacement with degenerative changes and infiltration of immune cells - H&E stain (magnification 50x, 400x). Scale bar = 200 µm,100 µm.

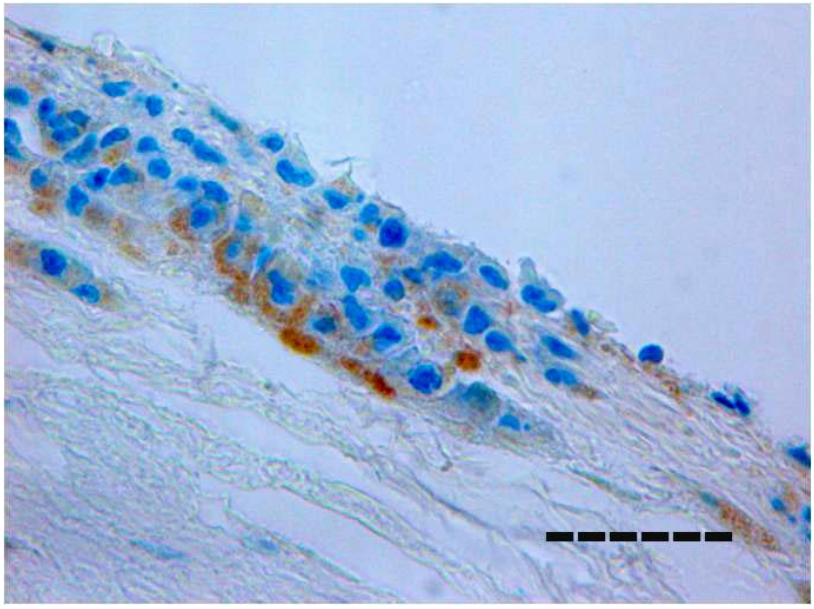


Fig. 2C. Plasmatic positivities CD68 (shown in brown) were focally found in an explanted bovine aortic valve replacement, identifying the presence of macrophages in the surface part of the valve in the context of the immune response to the xenogeneic valve (magnification 400x). Scale bar = 100 µm.

A normal aortic valve has a specific shape (Fig. 3) and is composed of three histological layers. Lamina ventricularis (C) is found on the chamber-side of the valve and mostly contains elastic fibers. Lamina fibrosa (A) is on the opposite side of the valve, facing the ascending aorta, and is composed of thickly-arranged collagen fibers. This layer is responsible for most of the mechanical characteristics of the aortic valve. The central part of the valve is created by lamina spongiosa (B), composed primarily of glycosaminoglycans and proteoglycans (GAGs). The surface of the valve is covered by endothelial cells (Fig. 4).

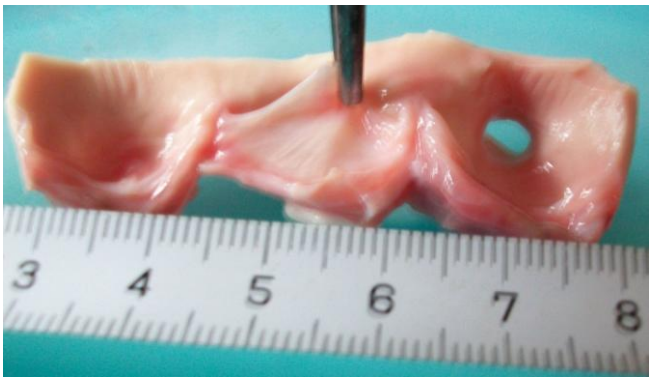


Fig. 3. A tricuspid aortic valve with visible sinus of Valsalva and connection of coronary arteries.

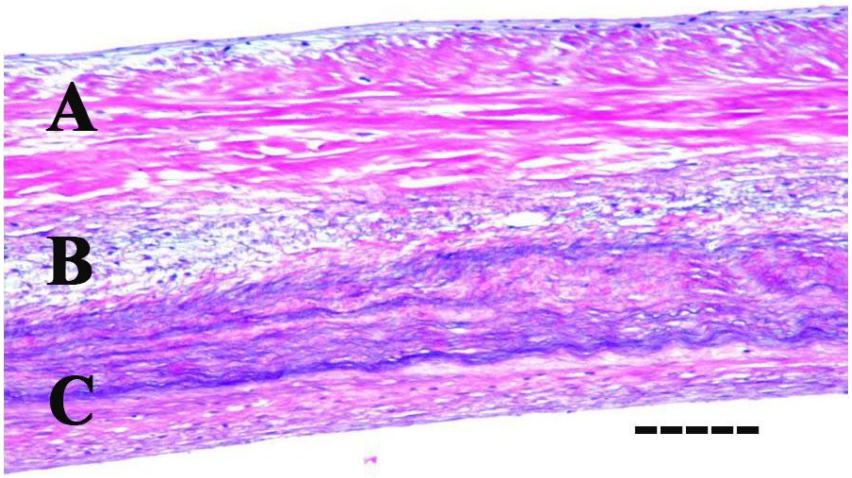


Fig. 4. Normal aortic valve. H&E stain (magnification 100x). Scale bar = 200 μm. Collagen fibers are apparent in red, particularly lamina fibrosa (A), elastic fibers in lamina ventricularis (C) in purple and the central part is lamina spongiosa (B).

The main cellular type of aortic valve is valve interstitial cells (VICs). These are cells of mesenchymal type that occur in multiple phenotypes. The ratio of the individual phenotypes present in the aortic valve changes over the course of a lifetime from the embryonic period to adulthood (Schoen F.J., 2008). VICs are highly dynamic and ductile cells, able to respond to stimuli from the extracellular environment. They are closely attached to the valvular ECM that they both produce and remodel. These cells are also mutually closely attached, using cytoplasmic processes and junction connections and, as such, they create a complex three-dimensional structure within the ECM (Plant A.L. et al., 2009). This structure is apparent in examinations using confocal microscopy (Fig. 5).

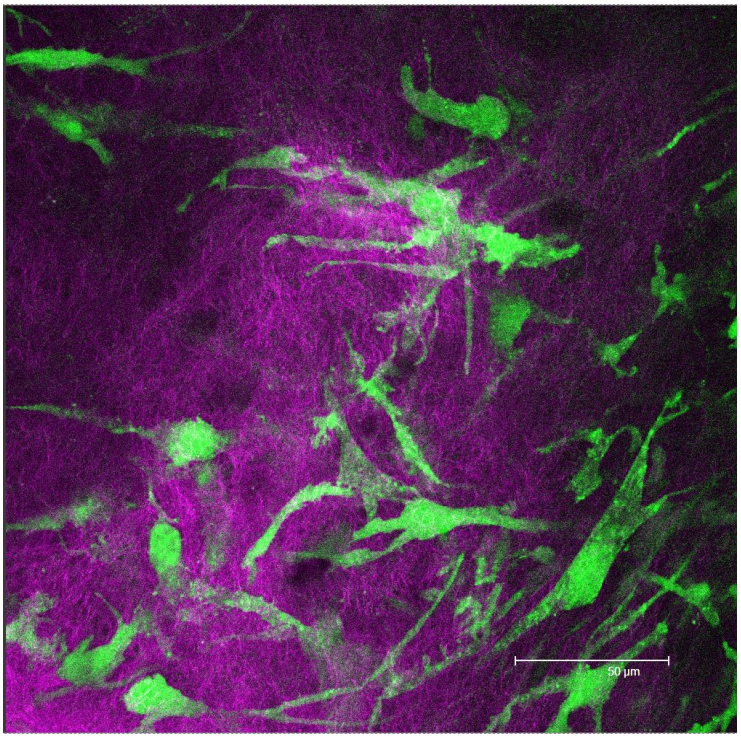


Fig. 5. Aortic heart valve, lamina fibrosa. Second harmonic generation (SHG) and two-photon excitation (TPEM) microscopy imaging: bright green fluorescence - 5-chloromethylfluorescein diacetate (CMFDA) staining of living cells (VICs), autofluorescence of elastin, magenta - SHG signal of collagen.

The changes in the composition of ECM and mechanical straining are perceived by the VICs, using receptors for growth factors and communication of the cells with ECM. The external impulses are transformed into intracellular signals that govern the genetic behavior of the cell and can therefore also participate in the change of its phenotype. It is assumed that

signaling uses growth-factor functions in synergy with direct mechanical strain (mechanotransduction), and leads to the proliferation and differentiation of these cells (Schoen, F.J., 2008; Plant, A.L. et al., 2009).

The signal paths lead to the creation of a specific structure of the tissue and, affected by the uneven straining of the surface of the valve, they also form the basis for anisotropy of its structure and mechanical characteristics. The amount and the quality of newly-created or remodeled ECM (including the orientation of the ECM fibers in the 3D structure of the tissue), the type of collagen, elastin and its crosslinking, as well as the content of GAGs, determine the mechanical characteristics of the valve tissue, including its pliancy, strength in traction and anisotropy (Engelmayr Jr. G.C., Sacks M.S., 2008).

In adults, VICs usually have a resting phenotype called quiescent fibroblast-like phenotype (identified as qVICs), which characteristically expresses low levels of cellular marker for smooth-muscle alfa actin (α-SMA). Their role is similar to that of chondrocytes in the cartilage or osteocytes in bones; that is, to sustain an even level of production and degradation of ECM, thus maintaining the adequate structure and function of the heart valve. VICs may be activated into a myofibroblast-like phenotype (identified as aVICs), i.e. into active cells that express α-SMA and mediate the process of remodeling the valve tissue. This change into an active phenotype occurs as a reaction to chemical or mechanical signals from the cellular environment (Ku C. et al., 2006; Colazzo F. et al., 2011).

As a whole, VICs behave in ways to create and remodel the ECM, also based on sensing damaged ECM or from sensing pressure changes in their own surroundings. This is a very effective and sophisticated method of perceiving defects in the ECM structure, which they attempt to repair this way in order to sustain the normal structure and function of the tissue. (Ku C. et al., 2006; Colazzo F. et al., 2011).

Although tissue engineering of heart valves has made significant progress, when it comes to understanding the structure and the function of these valves and their clinical applications, there remain certain limitations within these proceedings in order to create a live heart valve that is capable of remodeling and, in the case of child populations, that is also capable of growth and biological integration. A heart valve replacement created by tissue engineering (TEHV) should optimally cause no undesirable immune reactions against the valve tissue after implanting. Further, it should be non-thrombogenic; that is, ideally the surface should be covered with endothelium. It would also be valuable for the valve to have an appropriate anatomical shape, mechanical characteristics, proper function and for it to be easily implantable in clinical practice.

Tissue engineering has various methods for producing a biological valve replacement. There are various types of scaffolds and scaffold processing, as well as various types of cells for seeding scaffolds. In this case, natural biomaterials, such as collagen and fibrin, appear suitable for creating conditions for cell proliferation and differentiation. For example, collagen was used in the form of a hydrogel to create cusps of a valve and an entire conduit (Neidert M.R., Tranquillo R.T., 2006). Flanagan et al. created a completely autologous valve replacement in the form of a mold from fibrin, using sheep cells from their carotid artery that were implanted into the stem of the pulmonary artery of a sheep (Flanagan T.C. et al., 2007).

Generally, the main problem with these TEHV (made from biological materials without a firm structure of EMC created in vitro in hydrogel conditions) is that these materials are not stable in shape upon straining. They may retract, thus causing the loss of coaptation of the valve (Van Loosdregt I.A. et al., 2014). Synthetic polymers have the advantage of easier processing into the required shape of the valve, and better mechanical characteristics. Among such polymers typically used are polyglycolic acid (PGA), polylactic acid (PLA), polycaprolactone (PCL), poly(4-hydroxybutyrate) (P4HB) (Filova E. et al., 2009), or combined scaffolds such as PGA/P4HB TEHV scaffold (Dijkman P.E. et al., 2012).

The scaffolds are seeded with cells and cultivated and dynamically strained in vitro. A problem may occur with excessively fast biodegradation of the scaffold, before it is strengthened by the new production of ECM, or in retraction of the cusps of the valve. This may lead to structural changes and valve incompetence. However, slow degradation of the synthetic scaffold can cause a chronic inflammatory reaction, leading to fibrosis and dysfunction of the valve (Ghanbari H. et al., 2009; Claiborne T.E. et al., 2012).

A problem also remains in the penetration of cells into deeper layers of the scaffolds, depending on the size of the pores or the character of the scaffold. The interactions of cells with matrixes are highly specific, arranged by mediators and sometimes difficult to simulate under laboratory conditions (Plant A.L. et al., 2009).

New technologies such as electrospinning have enabled the development of TEHV on the basis of scaffolds in a controlled nanofiber structure that simulates the arrangement of ECM fibers, anisotropy of normal heart valve tissue, and enables the growth and differentiation of cells (Fallahiarezoudar E. et al., 2015). Such TEHVs have been created, for example, from polyurethane (Fan R. et al., 2013), or from poly(glycerol sebacate) (PGS) (Masoumi N. et al., 2014), with VIC cells that produced new ECM and remodeled the scaffold (Sant S. et al.,2013).

Rapid prototyping (3D bioprinting) is used in the preparation of valves that correspond to anatomical shapes according to CT or MR examinations. They also attempt to respect the individual layers of the tissue, that is, the character of the ECM and cell content. For example, valves from P4HB (Sodian R. et al., 2002) or from poly(ethylene glycol) – diacrylate supplemented by alginate (Hockaday L.A. et al., 2012) were created in this way. Migration and proliferation of VICs and their ability to produce new ECM (collagen, GAGs) and remodel the valve tissue was documented in the case of 3D valves created from a hydrogel of methacrylated hyaluronic acid and gelatin from human VICs by cells from the aortic valve (Duan B. et al., 2013).

Another way to prepare valve replacements in tissue engineering is to remove cells from suitable tissue through physical methods (freezing and thawing), through chemical methods (hypertonic or hypotonic buffers, detergents) or through biological methods (chelators, enzymes) and thus to decellularize it while sustaining its ECM structure.

For example, DNases/RNases were used for decellularization (Paniagua Gutierrez J.R. et al., 2015), Triton X (Akhyari P. et al., 2010), and also sodium deoxycholate (Zhou J. et al., 2010), sodium dodecyl sulfate (Flameng W. et al., 2014) and ethylenediaminetetraacetic acid (Takagi K. et al., 2006). Decellularized valve tissue should not contain any nuclear material and only minimal amounts of dsDNA, less than 50 ng/mg of dry weight of ECM (Crapo P.M. et al., 2011). Allogeneic or xenogeneic tissue or a valve can be used for this purpose. Given the preservation of the ECM structure, decellularized valves have similar hemodynamic characteristics to biological valves (Jiao T. et al., 2012). However, unlike them, they are not crosslinked with glutaraldehyde, thus enabling cellularization by autologous cells after implantation (Neumann A. et al., 2014).

Until now, glutaraldehyde has been used to crosslink biological valve replacements. New procedures are also being developed, for example the use of carbodiimide, neomycin trisulphate and pentagalloyl glucose (TRI) (Tam H. et al., 2015), or substances such as quercin (Somers P. et al., 2015). However, it is apparent from the studies and from clinical experience with the use of decellularized scaffolds that these allogeneic or xenogeneic tissues can cause undesirable immune reactions against foreign tissues in vivo. These can negatively impact the "survival" of the valve after implantation, its remodeling, etc. (Zhou J. et al., 2010).

Chemical crosslinking and decellularizion processes have been used with varied success to decrease undesirable immune responses to the xenogeneic tissue, but in general, it is apparent that further studies are necessary to improve our understanding of this immune reaction.

Ligands and ECM structure can be changed by crosslinking and decellularization processes, thus impairing the natural molecular mechanisms necessary for the normal function of interstitial cells (Knight R.L. et al., 2008). It has been demonstrated that these processes are not sufficiently reliable in removing cells and achieving immune tolerance of the valve tissue, and also because they can even influence the early and long-term survival of the heart valve after implantation (Kasimir M.T. et al., 2006).

For example, xenogeneic decellularized valve replacements Matrix P® and Matrix P plus® were used in clinical practice to replace the pulmonary valve. A surgical or transcatheter exchange of this valve replacement had to be performed in 52% of the patients within 19 months, due to its failure caused by an immune reaction against the valve cusps. This caused an inflammation leading to tissue fibrosis, as documented by histology and magnetic resonance examinations (Voges I. et al., 2013).

The goal of tissue engineering efforts is to create an autologous valve replacement, in order to prevent undesirable immune reactions against the valve tissue in vivo. To achieve this, some authors use molds from biocompatible materials in the shape of a heart valve, which they temporarily transplant under the skin of animals, so-called *'in-body tissue architecture'* technology. Skin fibroblasts grow into the mold and create a new ECM. The result is an autologous valve that can be used for implantation (Kishimoto S. et al., 2015). This approach appears to be promising. However, we are waiting for further results of studies after implanting these valves to aortal position, in which the valve is exposed to different hemodynamic conditions and greater strain than for the position of the pulmonary valve replacement.

An alternative method for creating autologous heart valve replacement that we will examine in our work is the use of human tissue of a similar histological composition as the aortic valve, and its modification in vitro. Human pericardium (Fig. 6) is a tissue of this type. It is composed of two layers, fibrous (A) and serous (B), and contains pericardial interstitial cells (PICs) of mesenchymal type, similar to VICs in heart valves. The fibrous part is composed of a dense network of collagen fibers, while the serous layer (closer to the pericardial cavity) contains (in comparison with the fibrous layer) fewer collagen fibers and is covered by mesothelial cells that have a secretory function.

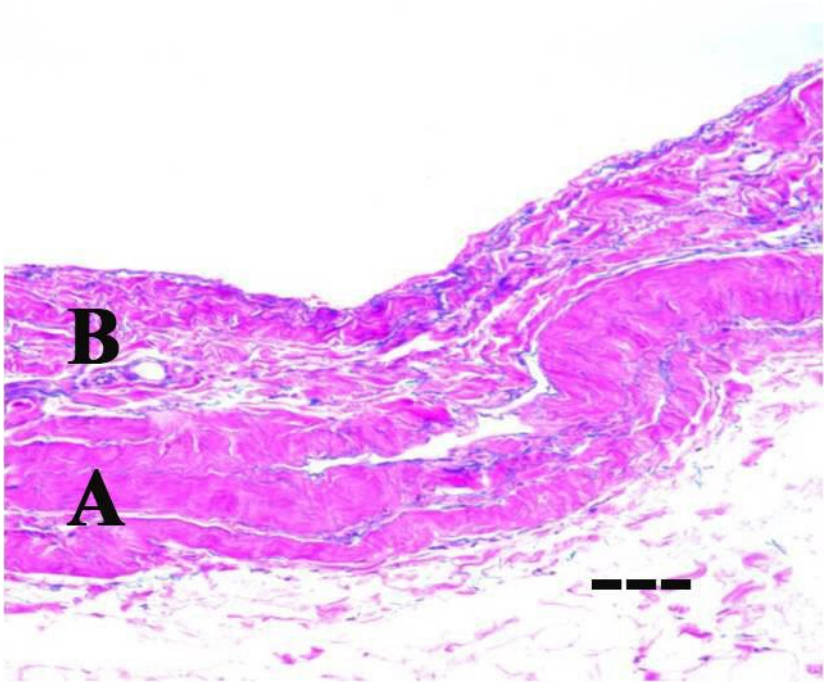


Fig. 6.

Human pericardium. H&E stain (magnification 100x).). Scale bar = 200 μm. Dense collagen fibers in the fibrous layer are apparent in red.

Modification of the human pericardium in vitro can be a new approach to creating autologous heart valve replacements with live cells capable of remodeling in vivo. The harvesting of pericardium can already be performed with minimal invasiveness in clinical practice today. A valve of the necessary size and dimensions (a stent) can be prepared for the patient based on MR, CT or 3D echocardiography examination with the use of CAD (computer-assisted design) technology and 3D printing (rapid prototyping). Using these methods, it is possible to create an anatomically shaped stent for a specific patient. It will be possible to evaluate the function of the valve replacement in a dynamic cultivation system under simulated physiological conditions prior to implantation. Using an echocardiographic examination, the evaluation includes an assessment of the opening of the valve, coaptation of the cusps, glow gradients and effective orifice area (EOA), as well as valve regurgitation.

In our estimates, dynamic conditioning in the bioreactor may lead to positive remodeling of the human pericardium tissue by PICs prior to actual implantation, provided that the PICs behave similarly to VICs (Berry J.L. et al., 2010). We can evaluate the quality of pericardial tissue, the structure and viability of PICs prior to implantation using immunohistochemical techniques or confocal microscopy.

Besides sustaining their regeneration function after implantation, an advantage of autologous valves from human pericardium with endothelium-covered surfaces may also be their non-thrombogenic surface. In these valves we can expect a decreased risk of infectious endocarditis. We can also expect that long-term anticoagulation treatment will no longer be necessary. The potential growth of these valves after implantation in child patients is also promising.

Human pericardium is a prosmising tissue for the preparation of autologous heart valve replacements. It is a complex tissue with a sustained ECM structure and a network of live PICs that can proliferate, differentiate and modify the pericardial tissue through the production of new EMC under the influence of a specific mechanical strain (3D mechanotransduction), similarly to the proliferation of VICs (Colazzo F. et al., 2011). It is reasonable to assume that the cells will retain their remodeling capability even after implantation in vivo in the blood circulation.

The mechanical characteristics of the human pericardial tissue may be similar to the tissue of a normal aortic valve, which likely also relates to the similarity of the histologic structure of both these tissues (particularly the composition of their fibrous layer). The anisotropy of the tissue of a normal aortic valve is given by the arrangement of collagen fibers, primarily in the circumferential direction (Martin C., Sun W., 2012).

4. Objectives

1. Compare the cellular and extracellular matrix (ECM) structure and 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.

2. Construct the dynamic culture system with pulse flow of the culture medium for three-dimensional (3D) conditioning of human pericardial tissue.

3. Perform a pilot study with the use of a new method for preparing a three-cusp heart valve construct from autologous human pericardium for potential use as a heart valve replacement based on 3D conditioning (mechanotransduction) of human pericardial tissue.

5. Hypothesis:

The cellular matrix and the extracellular matrix structure and mechanical properties of human pericardium as a potential scaffold for autologous heart valve tissue engineering are similar to the structure and the mechanical properties of the normal human aortic heart valve.

A dynamic culture system will be constructed with pulse flow of the culture medium for three-dimensional (3D) conditioning of human pericardial tissue.

In vitro modification of the autologous human pericardial tissue structure by 3D dynamic conditioning will prove that pericardial interstitial cell (PIC) behavior is similar to that of valve interstitial cells (VICs) of normal aortic heart valve. These cells are able to respond to mechanical stresses (mechanotransduction) by proliferating and differentiating into an activated myofibroblast-like phenotype capable of producing a new ECM and remodeling the histological structure of the tissue.

We assume that the biomechanical and hemodynamic properties human pericardial tissue will be comparable to the normal aortic heart valve.

6. Materials and methods

6.1 Histological and immuhistochemical analysis

For the histological evaluation, the samples were fixed in 10% formalin, embedded in paraffin, cut at 4 μm and stained with hematoxylin-eosin (H&E) and elastica Weigert - van Gieson. All tissue samples were also evaluated for several cell markers, such as cytoskeletal proteins vimentin (a type III intermediate filament protein) and desmin (a marker of striated muscles), alpha smooth muscle actin (α-SMA), CD31 (platelet-endothelial cell adhesion molecule, also referred to as PECAM-1), LCA (leukocyte common antigen) and β-catenin (a cell adhesion protein associated with cadherin junctions linking cadherins to the actin cytoskeleton). Alcian blue staining was used for staining acidic glycosaminoglycans (GAGs; mucopolysaccharides).

Immunohistochemical detection of collagen I, III and elastin was performed on paraffin sections 4 μm in thickness, using a two-step indirect method. The slides were deparaffinized in xylene and rehydrated in graded ethanol. After deparaffinization and rehydration, endogenous peroxidase was blocked by 0.3% H_2O_2 in 70% methanol for 30 minutes. A primary antibody was applied for 30 minutes at RT, and antibody detection was performed using Histofine Simple Stain MAX PO (MULTI) Universal Immuno-peroxidase Polymer,

anti-Mouse and anti-Rabbit (Histofine; Nichirei, Japan). Immunohistochemical detection of vimentin, desmin, α-SMA, CD31, LCA and β-catenin was performed on sections of paraffin-embedded tissues 4 μm in thickness, using the Ventana Benchmark Ultra system (Tuscon, AZ, USA) with the ultraView Universal DAB Detection Kit. The primary antibodies were: anti-vimentin (1:500; V9, Dako, Glostrup, Denmark), anti-α-SMA (1:1000; 1A4, Dako, Glostrup, Denmark), anti-CD 31(1:100; JC7OA, Dako, Glostrup, Denmark), anti-LCA (1:100; 2B11+PD7/26, Dako, Glostrup, Denmark), anti-β catenin (1:100; 17C2, Leica Biosystems, Germany), anti-desmin (1:100; D33, Dako, Glostrup, Denmark), anti-collagen I (1:50; polyclonal, CosmoBio Co., Japan), anti-collagen III (1:100; FH-7A, Abcam, Cambridge, UK) and anti-elastin (1:200; polyclonal, Abcam, Cambridge, UK). Finally, the specimens were stained with Dako Liquid DAB (3.3 diaminobenzidine) + Substrate-Chromogen System (Dako, Glostrup, Denmark) for 5 min, and were then counterstained with Harris's hematoxilin and were mounted.

The stained specimens were evaluated by light microscopy (Leica DMLB 100S) and microphotographs were taken (software Leica image manager). The average number of cells was calculated from 10 high-power fields (HPF - the maximum magnification power of the objective) in each group of evaluated tissue samples, using an Olympus BX 40 microscope, 400x magnification level (microscopic field ≈ 440 x 440 μm).

6.2 Histomorphometric analysis

A histomorphometric evaluation of the presence of collagen I, elastin and GAG was performed using the ImageJ analysis program (Image Processing and Analysis in Java; National Institute of Health, USA). Ten image files were scanned from each immunohistochemically (collagen I, elastin) stained sample or Alcian blue (GAGs) stained sample, (100x magnification), giving a total of 300 images of unconditioned pericardium, pericardium after dynamic conditioning, and normal aortic valve. Ten lines of bar profiles were subsequently evaluated for each image for an investigation of the structure of the tissue. We evaluated this with the use of so-called "integrated density" - the total amount of collagen I, elastin and mucopolysaccharides in the examined tissue was determined, with the exclusion of non-compacted tissue structures, which are artifacts resulting from the processing of histological samples. The content of the investigated ECM proteins (collagen I, elastin) and GAGs in the tissue was calculated as the integral of the density of the pixel values (color intensity) along the lines of the bar profiles, which is like the tissue thickness of the stained ECM component multiplied by its color intensity (Schneider et al., 2012).

6.3 Scanning electron microscopy (SEM)

An FEI Quanta 200 ESEM microscope (Institute of Botany, Academy of Sciences of the Czech Republic) was used to examine the surface of the tissue by SEM (scanning electron microscopy). This microscope has outstanding non-high-vacuum operating modes: Low Vacuum and ESEM (Environmental Scanning Electron Microscope) additionally improved by the Peltier cooling stage for the samples (equipped with an external water chiller).

The ESEM technology makes it possible to investigate specimens in their natural state, under conditions of a water vapour atmosphere at pressures up to 2600 Pa (=26 mbar =20 Torr). Gas (water vapour) ionisation inhibits accumulation of the charge on the surface of non-conductive specimens and amplifies the secondary electron signal. There is no need for time-consuming conventional preparation techniques (such as coating), which may produce unwanted artefacts in the sample.

In addition, the cooling system makes it possible to explore easily-damageable living specimens by freezing them to temperatures from 0°C to –20°C with accuracy of 0.1°C. Cooling is effective for reducing the danger of over-drying and wizening of the sample and the danger that burns or swellings can be made by an intensive electron beam.

Suitable usage of these abilities, together with high resolution (up to 3.5 nm at 30 kV) electron optics and the sensitive Gaseous Secondary Electron Detector, makes it possible to observe natural state biological specimens with useful magnifications from 6x up to ordinary 20,000x - 50,000x, depending on the quality of the sample material (the microscope is built to maximum magnification >1,000,000x).

Our specimens were scanned using the ESEM mode in their natural state under water vapour atmosphere at pressures around 300 Pa and at temperatures slightly below -10°C. The electron beam accelerating voltage was 20 kV and the magnification was up to 1000x.

6.4 Second harmonic generation (SHG) and two-photon excitation (TPEM) microscopy

All images were acquired by a Leica TCS SP2 acoustooptical beamsplitter (AOBS) multiphoton confocal laser scanning microscope based on the Leica DM IRE2 inverted microscope and equipped with the following light sources: Ar laser (458 nm/5 mW, 476 nm/5 mW, 488 nm/20 mW, 514 nm/20 mW), HeNe lasers (543 nm/1.2 mW, 633 nm/10 mW) for one-photon excitation, and a mode-locked Titanium(ti) - Sapphire Chameleon Ultra laser (Coherent Inc., Santa Clara, *CA, USA*), tuneable from 690 to 1040 nm for TPEM.

The light emitted from collagen was detected by NDD - RLD photomultipliers installed directly under the lens of a scanning confocal microscope head. Signal collagen using SHG

was recorded as λex (wavelength of the excited photons) 860 nm and λem (wavelength of the emitted photons) 430 nm.

Live cells were labelled by CellTracker™ Green CMFDA (5-chloromethylflorescein diacetate, Molecular Probes, Invitrogen Detection Technologies). Tissue samples were immersed in CMFDA dye solution (at a concentration of 10.0 μM/L in serum-free medium) for 30 minutes at 37 °C in an incubator, according to the manufacturer's instructions. CMFDA is a colorless dye that releases a brightly fluorescent product after being cleaved by the cell's cytosolic esterases, a reaction that also involves glutathione-SH. The time management between tissue dissection and image acquisition was no longer than 45 min.

Two-photon excitation microscopy (TPEM) was used to assess the cell morphology, distribution, viability, cell-cell and cell-matrix interactions and elastin fiber architecture (Imanishi Y. et al., 2007). SHG imaging was performed to assess the architecture and the spatial distribution of the collagen fiber (Campagnola P.J. et al., 2002). Both surfaces of aortic heart valve cusps (lamina fibrosa and ventricularis) and unconditioned pericardium (inner and outer part) were assessed to the maximal depth of penetration (around 80-100 um). In our study, temperature had the greatest impact on cell viability. A heated chamber and a heated objective were applied to optimize the temperature (37°C) during image acquisition.

6.5 3D conditioning (mechanotransduction) in a bioreactor

Human pericardium was sutured onto a valve holder in the shape of a tricuspid heart valve, using 4-0 monofilament polypropylene (Surgipro™ II) sutures under sterile conditions. The pericardium heart valve constructs (annulus diameter 20 mm) were placed into a cylindrical conditioning chamber. Within this chamber we conditioned the pericardial heart valve construct for 4 weeks. A human pericardial heart valve construct was conditioned in a bioreactor by a pulse flow of culture medium containing DMEM, 10% fetal bovine serum (FBS; Sebak GmbH, Aidenbach, Germany) and 2% w/v ABAM (antibiotic/antimycotic solution containing 10,000 I.U./ml Penicillin G, 10,000 μg/ml Streptomycin and 25 μg/ml Amphotericin B, Cat. No. 30-004-CI, Mediatech, USA).

The human pericardial heart valve constructs were conditioned in the incubator by gradually increasing the pulse flow of the culture medium at weekly intervals from 300 to 600 ml/min, in a humidified atmosphere of 5% CO_2 in air, temperature 37°C and pH 7.4. The culture medium was replaced weekly.

During conditioning of the pericardial heart valve constructs, the pericardial heart valve leaflets were subjected to a shear stress of approximately 5-15 dynes/cm² due to the flow of

medium when the valve was opened, to a flexural stress due to cyclical opening and closure of the leaflets, and to a tensile stress when the valve was closed (Choon Hwai Yap *et al.*, 2012). The average maximal systolic pressure in the system was 100-120 mmHg, the mid diastolic pressure at valve level during valve closure was 60-80 mmHg, and the end diastolic pressure in the chamber for conditioning was 0-5 mmHg. Pressures were measured invasively by pressure transducer technology (B. Braun Combitrans double Monitoring kit ref No. 5200011), GE Carescape 8850 monitoring system. After 4 weeks, the conditioned pericardium valve cusp samples were sent for histological and immunohistochemical analysis, and for biomechanical testing. The results were compared with the unconditioned pericardium samples obtained from the same patient, and with the native aortic heart valve cusp tissue.

6.6 Assessing the biomechanical properties

The specimens were transported to the Laboratory of Biomechanics at the Faculty of Mechanical Engineering of the Czech Technical University in Prague, and were documented and processed as soon as possible (the average time interval after tissue harvesting during cardiac surgery or after dynamic conditioning was 4 hours). The dimensions of the samples were contactlessly measured by laser-profiled scanCONTROL 2800 (Micro – Epsilon, Ortenburg, Germany – Fig. 7). Strips typically 0.2 ÷ 0.5 mm in thickness, 3 ÷ 5 mm in width and 10 ÷ 15 mm in length were prepared for mechanical testing.

Mechanical experiments were carried out on a tensile testing machine for soft tissues (Messphysik Materials Testing GmbH, Fürstenfeld, Austria) equipped with a 25 N load cell (Fig. 8). Deformations were assessed *via* image analysis of video records conducted by a built-in videoextensometer. Monotonic uniaxial loading was applied at a loading rate of 1 mm/s, which corresponds to a strain rate of $d\varepsilon/dt \approx 0.1\ s^{-1}$. The results were obtained after five cycles of preconditioning (loading/unloading) in order to attain repeatable behavior of a specimen. $\boldsymbol{\varepsilon_{max}}$ was defined as the maximum strain at which the stress increases monotonically (after this point, the slope to an $\boldsymbol{\sigma}$–$\boldsymbol{\varepsilon}$ graph starts to decline, which indicates the initiation of a tissue failure process). Mechanical properties were evaluated from this last loading cycle. The evaluation of the mechanical properties was based on the secant elastic modulus $\boldsymbol{E_s}$ defined in {1}:

$$E_s = \frac{\sigma_{max}}{\varepsilon_{max}} \qquad \{1\}$$

Here, σ_{max} is the maximum Cauchy stress achieved within the monotonic response of the material. It was computed as $F \cdot (1+\varepsilon)/S$; where F, S and ε denote the loading force, the reference cross-section area and the engineering strain, respectively.

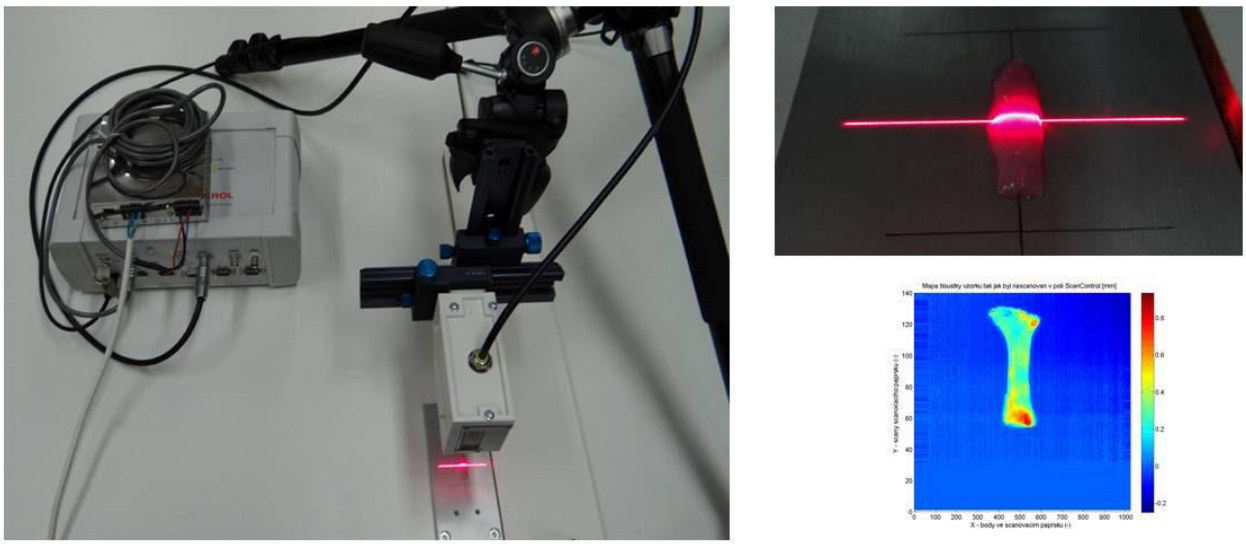


Figure 7. Laser profile sensor scanCONTROL 2800 (Micro-Epsilon, Ortenburg, Germany) reading a sample profile (thickness and width), a detail of scanning and processed data.

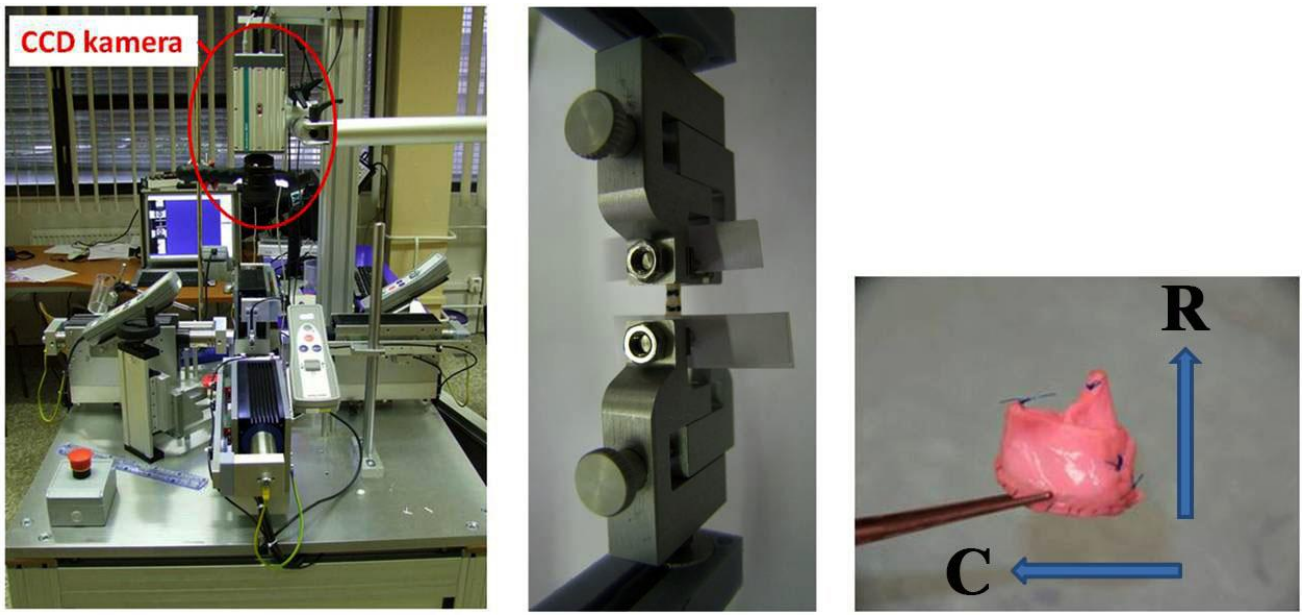


Figure 8. Left – biaxial testing machine for soft tissues Zwick/Roell. Right – a detail of a sample stretched in the clamps of the machine. The sample is identified by marks in between which the longitudinal deformation is evaluated. TEV samples were tested in radial (R) and circumferential (C) direction.

6.7 Assessing echocardiographic and hemodynamic properties

The hemodynamic performance of TEV was assessed visually and by echocardiography at systemic pressures (120/80 mmHg) using a pulse flow mechanical circulatory support device for simulating the physiological condition of human circulation in vitro. The pericardial valve function was estimated in a chamber for valve construct conditioning. The pressure gradients and the effective orifice area (EOA) of the valves was measured using a Vivid *i* GE Healthcare echocardiography machine and a GE 3S-RS cardiac sector array ultrasound probe with a 1.5 – 3.6 MHz broadband multi-frequency range for 2D, color and Doppler imaging. The pressure gradients obtained by the Bernoulli equation correlate well with the hemodynamically measured gradients. EOA represents the minimal cross-sectional area of the flow jet downstream of the valve. Doppler EOA was determined using pulsed-wave (PW) and continuous-wave (CW) Doppler and the continuity equation EOA = SV/VTI_{vc}, where SV is stroke volume and VTI_{vc} is the velocity time integral at the level of the vena contracta measured by CW Doppler (Garcia et al., 2004; Garcia and Kadem, 2006). In the Doppler measurements, the ultrasonic beam was oriented parallel to the flow across the human pericardial valve in the chamber for conditioning, guided by 2D imaging and assisted by color flow imaging.

6.8 Statistical analysis

All parameters are expressed as mean values ± standard deviation and as a median with a range. A two-sample t-test or an unpaired Wilcoxon test for non-Gaussian distributed variables was used. The Bonferroni correction for multiple group comparison was applied. All tests were two-sided, with $p < 0.05$ considered statistically significant. The statistical analyses were performed using MedCalc software.

The histomorphometric data were evaluated using ANOVA and Student-Newman-Keuls multiple comparisons. The data were evaluated in the SigmaStat program.

7. Results

7.1 Comparison of the cellular and extracellular matrix structure of human pericardium and normal aortic heart valve

The objective of this part of study was to evaluate and compare the cellular and ECM structure and the mechanical properties of human pericardium with the properties of the normal human aortic heart valve, and to assess whether this tissue may serve as a promising scaffold for autologous heart valve tissue engineering. We have compared human pericardial tissues and normal aortic heart valves harvested during cardiovascular surgery and heart transplantation, using histological, immunohistochemical analysis, scanning electron microscopy (SEM) and confocal microscopy (SHG/TPEM) evaluation. The properties of biomechanical tissues were assessed and compared on the basis of the secant elastic modulus E_s.

7.1.1 Study group

The study group consists of 16 patients (donors). All patients signed an informed consent form prior to enrolment in the study. The project was approved by the Ethical Committee of the Institute for Clinical and Experimental Medicine in Prague, Czech Republic. Native human pericardium samples, approximately 4 x 10 cm, were harvested at the time of heart surgery from 11 patients (undergoing coronary artery bypass grafting or valve surgery, 2 women, 9 men, average age 68 ± 8 years). Normal aortic heart valve samples were obtained from 5 other patients (5 men with dilated cardiomyopathy, average age 51 ± 15 years) during heart transplantation. The tissue samples were obtained at different times, depending on the scheduled surgery (Fig. 9), and were transported to the laboratory in a sealed container containing DMEM (Dulbecco's Modified Eagle's Medium; Sigma, St. Louis, MO, U.S.A; Cat. No. D5648) at body temperature (37^{o}C).

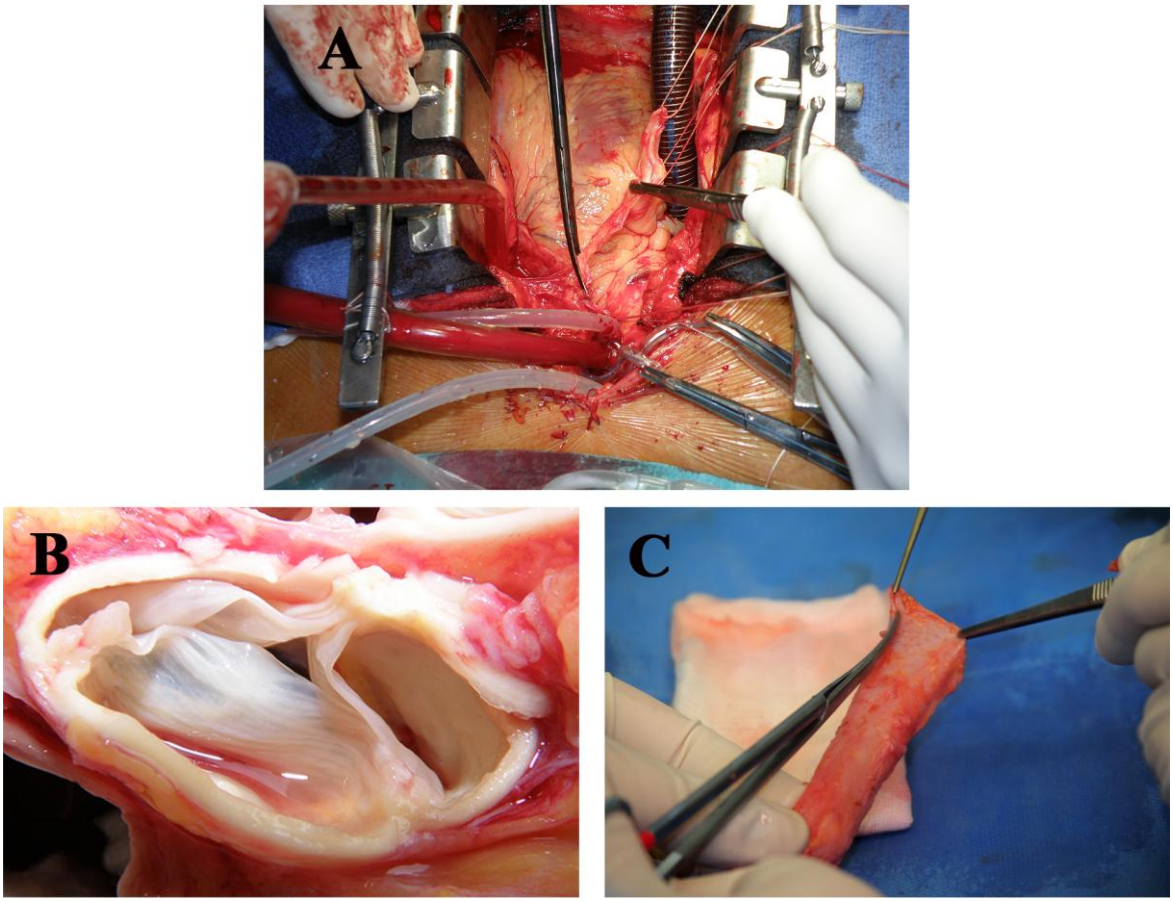


Figure 9. Human pericardium (C) and normal aortic heart valve (B) were harvested during open heart surgery (A).

7.1.2 The cellular and ECM structure of the normal aortic heart valve

H&E staining of the normal aortic heart valve (Fig. 10), consists of three specific layers. Lamina fibrosa is the part facing the outflow surface, the middle is lamina spongiosa, and the lamina ventricularis is the part facing the inflow surface. Human aortic heart valve cusp ECM is composed primarily of collagen type I, III, elastin and GAGs.

On the surface of the heart valve there is endothelium, which is CD31 and β-catenin positive. The ECM is produced by VICs that exists in several phenotypes. VICs of spindle-shaped and polygonal morphology are present mostly in the lamina fibrosa in connection with stress-bearing collagen fibres. A smaller amount of VICs is present in the lamina spongiosa and in the lamina ventricularis. Most of them are positive for vimentin staining, i.e. a marker of cells of mesenchymal origin, and represent a quiescent state of VICs (i.e., qVICs). Aortic heart valve cusp tissue also contains a small amount of α-SMA positive cells, which represent activated myofibroblast-like VICs (aVICs). These cells are also found predominantly in the

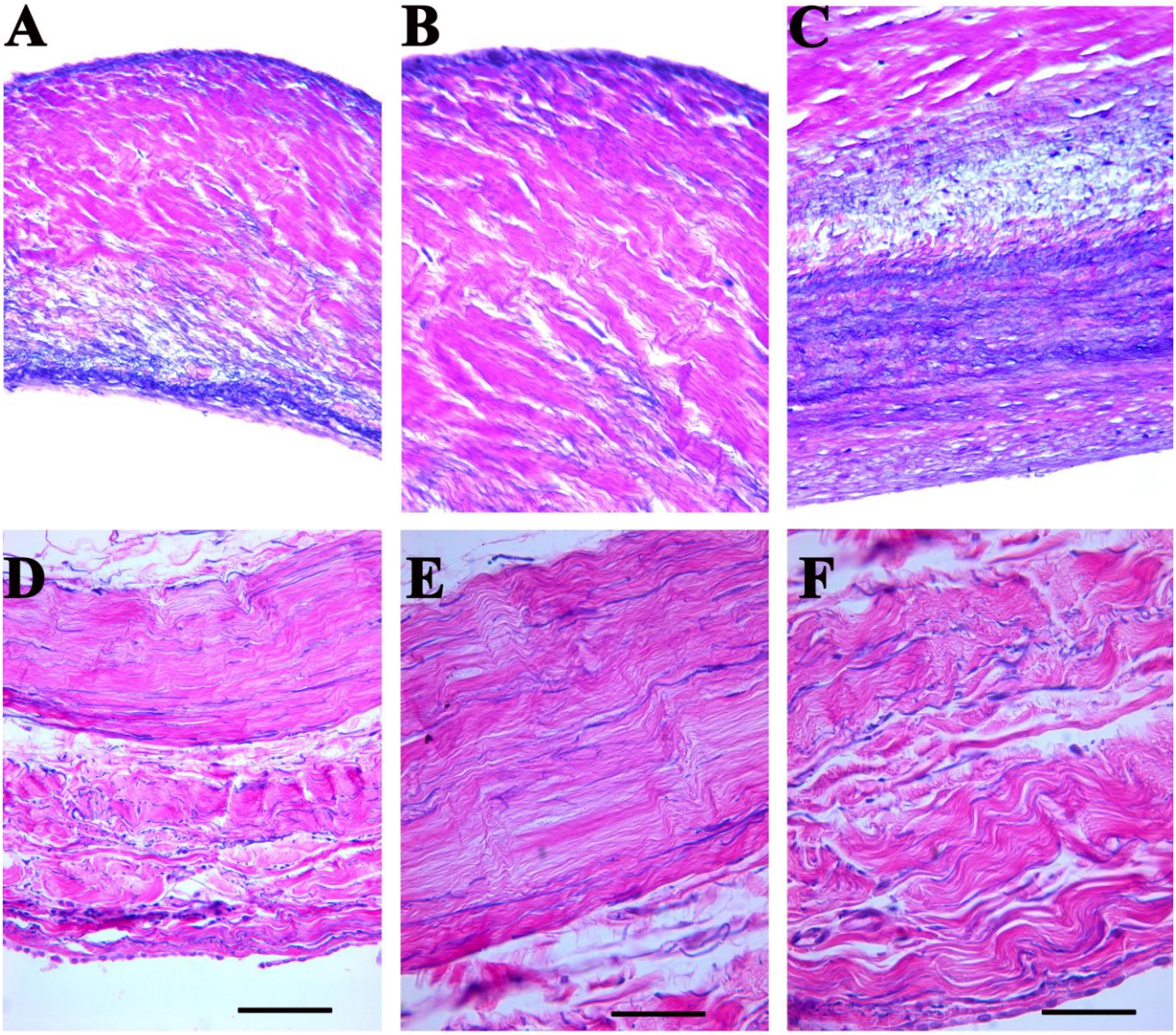


Figure 10. Hematoxylin-eosin and elastica staining (H&E) of a normal (native) aortic heart valve (A,B,C) and human pericardium (D, E, F), magnification 200x. Scale bar = 200 μm. Lamina fibrosa (B), lamina spongiosa and lamina ventricularis (C) of the normal aortic heart valve, magnification 400x. Human pericardium, fibrous part (E) and serous part (F), magnification 400x. Scale bar = 100 μm. The nuclei of the cells are blue, the collagen fibres are red, the elastin fibres are blue-purple.

lamina fibrosa, where the valvular tissue is under the influence of the greatest shear stress and strain forces. Activated VICs (aVICs) represent approximately 15-17% of all VICs (Fig. 11).

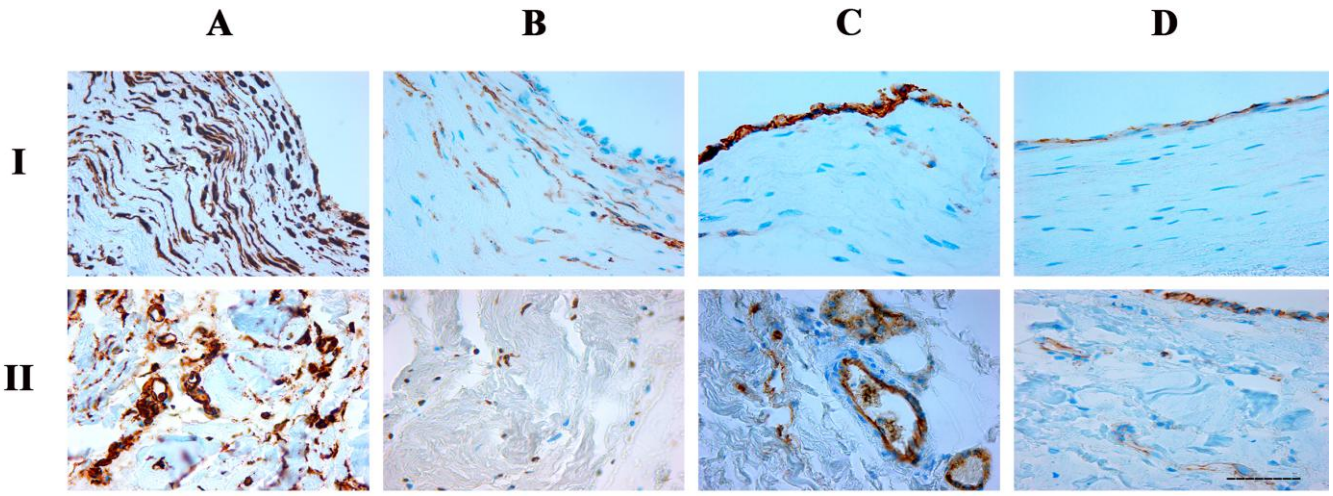


Figure 11. Cell characterization in a normal (native) aortic heart valve (I), - upper part of images. Human pericardium (II), - lower part of images. Immunohistochemistry staining for vimentin (A), α-SMA (B), CD31 (C), and β-catenin (D), magnification 400x, scale bar = 100 μm. Immunopositivity of the targeted proteins in the cells is depicted in intense brown color, nuclei are in blue.

Lamina fibrosa consists of rich collagen type I and III fibers with parallel orientation and crimping, red colored by H&E. This is confirmed by immunohistochemical staining for collagen I and III. In lamina fibrosa, collagen fibres are aligned preferentially in the circumferential direction. These fibres are the main stress-bearing structure in the aortic heart valve. There are elastin fibres in small amounts in the lamina fibrosa between the collagen fibres, and their wavy formation network provides connections between collagen fibres and other valve layers (Fig. 10, 12).

Collagen I and III fibres are present in smaller amounts in the lamina spongiosa. They are loosely packed and are thinner. The elastin fibres in the spongiosa layer continue from the fibrosa layer to the lamina ventricularis, and are in smaller amounts than in the lamina

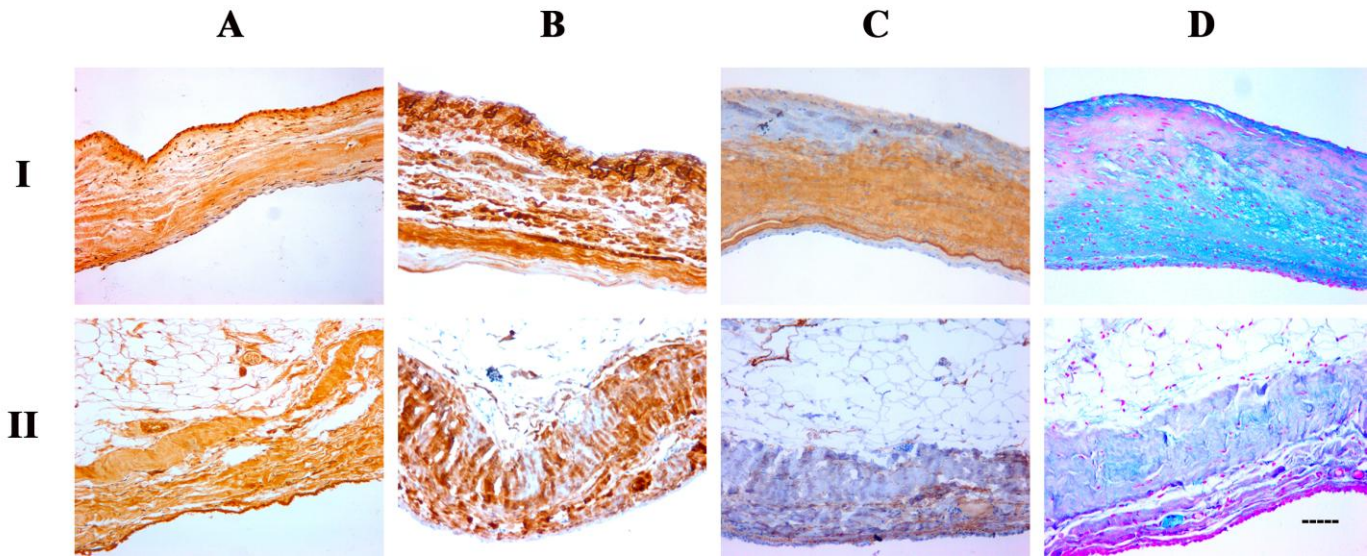


Figure 12. ECM characterization in a normal (native) aortic heart valve (I), - upper part of images. Human pericardium with surrounding fatty tissue after harvesting (II), - lower part of images. Immunohistochemical staining for collagen type I (A), for collagen type III (B) and for elastin (C); the samples are counterstained with Harris's hematoxylin. Detected proteins are positive in brown color, nuclei are in blue. D: Alcian blue staining for GAGs, positivity is shown in blue or dark blue, nuclei are in red. Magnification 100x, scale bar = 200 μm.

ventricularis. The lamina spongiosa consists predominantly of a well-developed GAG layer (glycosaminoglycans and proteoglycans). This layer is very important for absorbing energy during valve compression in the cardiac cycle (valve closure and opening). A small amount of GASs are also distributed in other all histological layers of the aortic heart valve cusp (Fig. 12).

In the lamina ventricularis, predominantly well-developed rich wavy arranged and radially aligned elastin fibers (blue-purple colored by H&E) are seen, together with small amounts of collagen I and III, which have a thinner fibre appearance than in the lamina fibrosa. Elastin fibres are responsible for restoring the wavy and crimped structure of the collagen during aortic valve unloading. GASs are also present, but in a smaller amount than in the lamina spongiosa (Fig. 10, 12).

7.1.3 The cellular and ECM structure of the normal human pericardium

Normal human pericardium consists of two specific layers, i.e. the serous part facing the heart, and the fibrous part on the opposite side, which merges into the fatty tissue surrounding the pericardium on the outer side. Mesothelial cells cover the inner pericardial surface facing the heart (the serous part) (Fig. 10). These cells are positive for vimentin, α-SMA positive staining and negative for CD31 staining. Beta-catenin-positive staining is also detected between mesothelial cells (Fig. 11). In the human pericardial tissue there are pericardial interstitial cells (PICs) that are of mesenchymal origin. They are found predominantly in the fibrous part of pericardial tissue, and have a spindle-shaped and polygonal morphology. PICs are stained by H&E, and also display vimentin positivity, similar to VICs in the normal aortic heart valve, and represent a quiescent state (qPICs). Only a small proportion of these cells are stained for α-SMA and represent an activated cell form of PICs (aPICs), similar to aVICs in the aortic heart valve. Immuhistochemical cell staining is well depicted by a plasma-intensive brown color. PICs and also VICs in the normal aortic heart valve are desmin-negative and LCA-negative. No staining for β-catenin was evident between PICs. In the outer layer of the pericardium, which is in transition with surrounding adipose tissue, small capillaries can be seen with endothelial cells in the lumen staining positively for CD31 and beta-catenin (Fig. 11).

The fibrous part of the native human pericardium is very similar to the fibrosa layer of the normal aortic heart valve. It consists of densely-packed collagen fiber bundles that have a crimped appearance on H&E (Fig. 10). They consist of collagen type I and III, as was confirmed by immunohistochemical staining (Fig. 12). In this part of the pericardial tissue PICs also have a more spindle-shaped morphology, probably due to higher collagen density in the surrounding ECM, similar to the situation observed in the fibrosa layer of the aortic heart valve. In the serous part of the pericardium, collagen I and III bundles can be seen that are more loosely-packed or more tightly-packed. PICs have a more spread-out (polygonal) morphology in the serous part of the pericardium, corresponding to the ECM character that surrounds these cells. Again, elastin is well developed throughout the pericardium, forming a scaffold interspersed with the fibrous and serous part of collagen**.** Elastin fibers are particularly well developed in the inner pericardial side facing the heart (the serous part), but in smaller amounts throughout the tissue structure, and the amounts are smaller than in the normal human aortic heart valve. GAGs are present in both layers of human pericardial tissue, but in greater amounts in the serous part (Fig. 10, 11, 12).

7.1.4 A comparison of the cellular and the ECM structure of normal human aortic heart valve and pericardium

The histological structure of aortic heart valve and pericardium tissues is very similar. The surface of the aortic heart valve is covered by endothelial cells. Mesothelial cells are observed on the inner pericardial surface facing the heart (the serous part). The fibrous part on the opposite side is connected with fatty tissue surrounding the pericardium on the outer side. When this adipose tissue is removed from human pericardium, we can see a typical surface pattern in SEM (Fig. 13). SEM of the human pericardium and of the aortic heart valve cusp tissue revealed close similarities in surface morphology. The outer surface of pericardium (the fibrous layer) and lamina fibrosa of the aortic heart valve display microscopic grooves and ridges formed by well-developed thick collagen bundles of ECM. This specific surface pattern with corrugation is more profound in the normal aortic heart valve. These tissue layers possess the highest amounts of collagen I and III and therefore impart the greatest strength and resilience to the tissue.

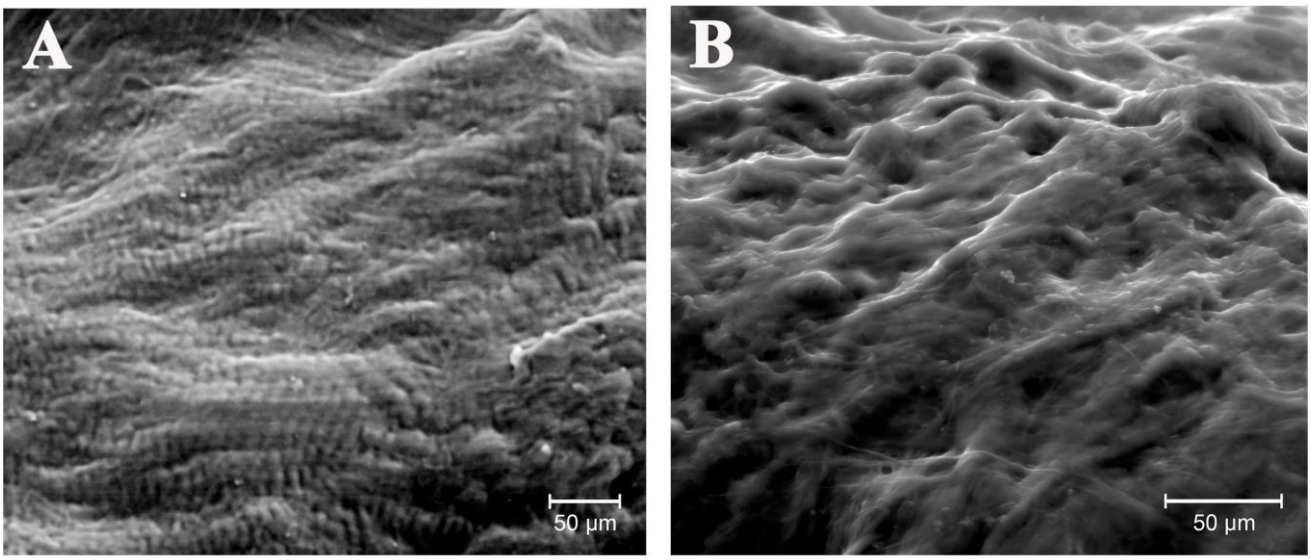


Figure 13. Scanning electron microscopy (SEM) of the human pericardium and of the normal aortic heart valve cusp. The lamina fibrosa of the aortic heart valve (A), and the outer surface of pericardium, fibrous layer (B) display microscopic grooves and ridges that are formed by well-developed thick collagen bundles of ECM. Scale bar = 50 µm.

The surface of the heart valve is covered by endothelium, which is CD31 and β-catenin positive (Fig. 11). Mesothelial cells are present on the inner pericardial surface facing the heart (the serous part), visualized by H&E (Fig. 10). These cells are vimentin- and α-SMA positive, and CD31 negative (Fig. 11).

Both normal human aortic heart valve tissues and human pericardium tissues contain cells of mesenchymal origin - VICs, PICs, which are specifically arranged in the ECM structure. Both of these cells exist in quiescent and activated (fibroblast-like) phenotype and are responsible for tissue remodeling, ECM production and degradation.

The lamina fibrosa of the aortic heart valve and the fibrous part of human pericardium contain thick bundles of collagen I and III. The middle part of the aortic heart valve contains mainly GAGs and sparsely distributed collagen I and III fibers. In the middle part of human pericardium we observe less densely arranged collagen fibers that mimic the structure of the lamina spongiosa of the normal aortic heart valve. GAGs are apparent relatively uniformly in all layers of human pericardial tissue, but in smaller amounts than in the normal aortic heart valve. Elastin fibers are apparent between the wavily arranged collagen fibers in all layers of the pericardial tissue, similarly as in the normal aortic heart valve, but in the pericardial tissue they are not arranged in densely corrugated structures, as they are in the lamina ventricularis of the normal aortic heart valve. The serous part of human pericardial tissue that corresponds to the lamina ventricularis of the normal aortic heart valve has a greater presence of collagen I and III fibers than lamina ventricularis of the normal aortic valve (Fig. 10, 12).

The surface layers of both tissues, the arrangement of the cells and the ECM are better shown by confocal and TPEM microscopy (Fig. 14A). Aortic heart valve cusps show a well-developed collagen network in the examined tissue, with the density of the crimped, densely-packed fiber being highest on the side of the lamina fibrosa. VICs in this densely packed collagen layer of the lamina fibrosa display predominantly a spindle-shaped cell morphology. The high density of thicker collagen bundles in this layer constrains these cells. They display a morphology that accommodates this architecture with their shape and cellular processes aligned in parallel to the collagen bundles that they produce and remodel. Similar huge and uniformly oriented bundles of collagen fibers are seen in the fibrous part of human pericardial tissue, and the PICs in this part have a more spindle-shaped morphology. In lamina vetricularis of the aortic heart valve and in the serous part of human pericardial tissue, the collagen fibers are more loosely packed with a non-uniform direction, and the VICs and PICs in both tissues display a more spread-out cytoplasmic morphology. The elastin fibers are much thinner than the collagen fibers, with branches around the collagen fibres. They are well

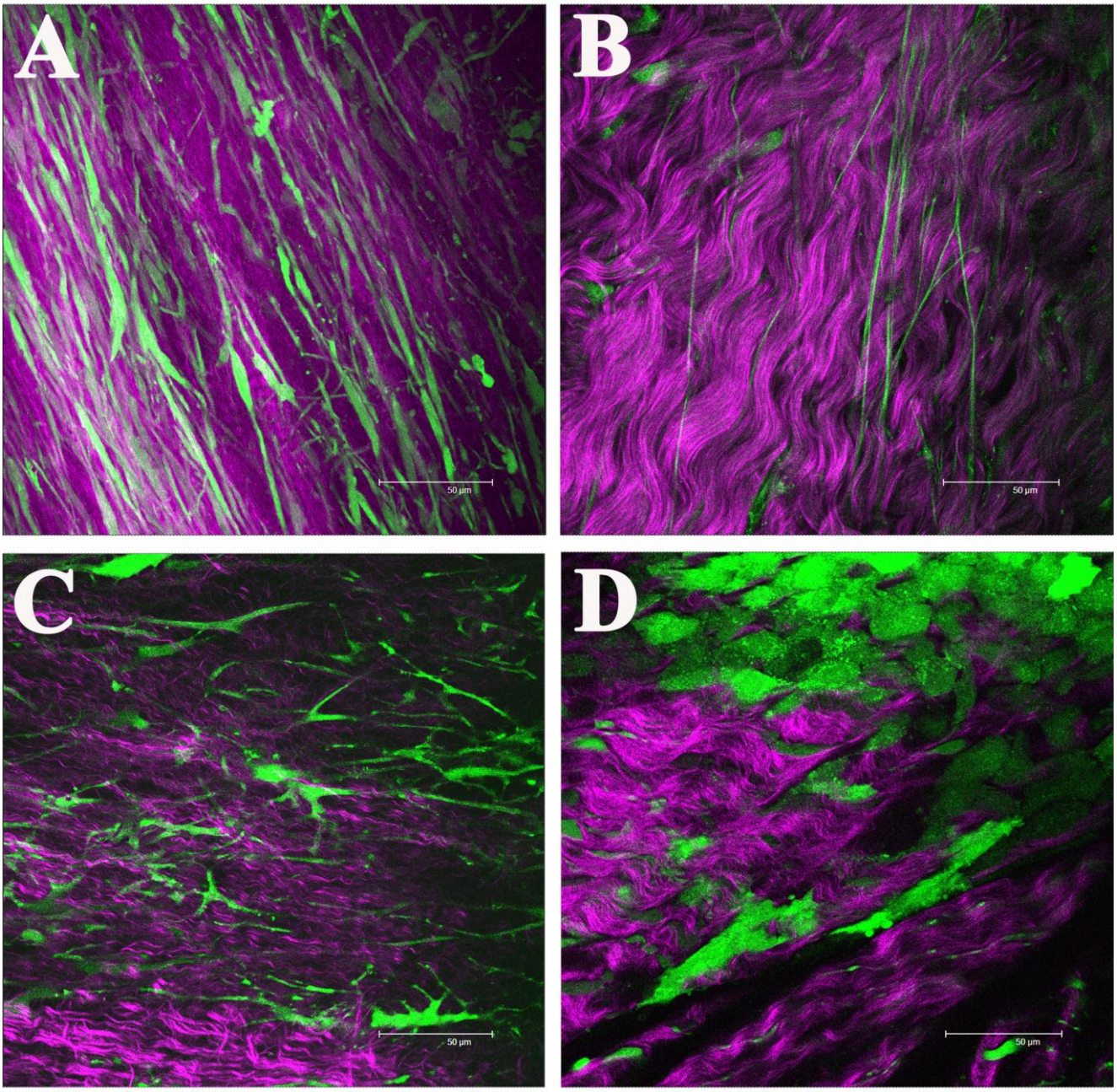


Figure 14A. Second harmonic generation (SHG) and two-photon excitation (TPEM) microscopy imaging: bright green fluorescence - 5-chloromethylfluorescein diacetate (CMFDA) staining of living cells (VICs, PICs, endothelial and mesothelial cells), autofluorescence of elastin, magenta - SHG signal of collagen in the lamina fibrosa (A) and lamina ventricularis (C) of the normal aortic heart valve and the fibrous (B) and serous part (D) of human pericardium. The collagen bundles are thicker and more densely arranged in the lamina fibrosa of normal aortic heart valve (A) and in the fibrous part of human pericardium (B) in comparison with lamina ventricularis of normal aortic heart valve (C) and the serous

part of human pericardium (D). VICs and PICs in these parts of the tissue have a more spindle-shaped morphology. In the lamina ventricularis of normal aortic heart valve and in the serous part of human pericardium, the collagen bundles are loosely arranged and VICs and PICs have a more spead-out cytoplasmatic morphology. Green elastin bundles can be seen between the collagen fibers and the cells. Scale bar = 50 μm.

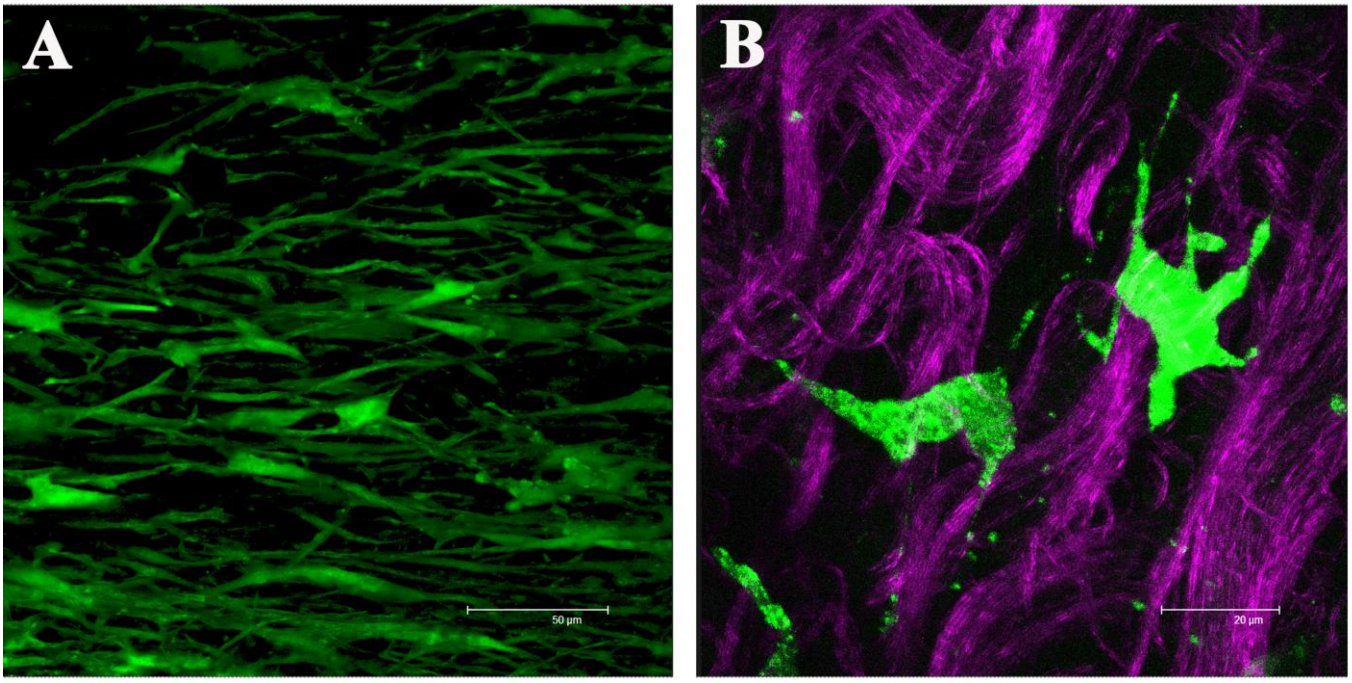


Figure 14B. Second harmonic generation (SHG) and two-photon excitation (TPEM) microscopy imaging: bright green fluorescence - 5-chloromethylfluorescein diacetate (CMFDA) staining of living cells (VICs, PICs, endothelial and mesothelial cells), autofluorescence of elastin, magenta – a SHG signal of collagen. Multiple VIC connections and inteactions are seen in the lamina fibrosa of normal aortic heart valve (A). Cell-to-cell and points of cellular attachment of PICs to collagen bundles in the ECM are present in human pericardial tissue (B). scale bar = 50, 20 μm.

developed both in the lamina fibrosa and in the lamina ventricularis, but predominantly in lamina ventricularis of the normal aortic heart valve, forming a scaffold interspersed with elastin fibres**.** Elastin fibers occur relatively rarely in human pericardial tissue. They are particularly well developed in the serous part, but are generally observed in smaller amounts

than in the normal human aortic heart valve. In both tissues, live VICs and PICs exhibited an intensely green florescence and well developed cytoplasmic processes when stained with CMFDA. PIC cytoplasmic extensions are shown in both pericardial layers, with cytoplasmic connections evident between the cells. Points of cellular attachment of PICs to collagen bundles are also visible, and this documents integrity of the cells with ECM in this tissue (Fig. 14B).

7.1.5 Biomechanical properties of the normal human aortic heart valve and native human pericardium

This part of the study compares the mechanical properties of normal human aortic valve and human pericardium in order to reveal whether pericardium-based valves are suitable for replacement purposes. In order to answer this question, uniaxial tensile tests were carried out with samples of human pericardium (HP) and normal aortic valve (NAV), and the results were compared. The total number of tested samples was: 22 for HP from 11 patients (donors) and 10 for NAV from 5 patients (donors).

NAV strips were cut in the circumferential direction of the valve. Samples of HP excised in longitudinal direction and in transversal direction showed comparable mechanical properties, which suggests isotropic behavior of pericardium. Therefore only samples cut in longitudinal direction (respecting the orientation of the heart) were included in our study.

Mechanical experiments provided secant elastic moduli for the tested specimens as follows: HP – E_s = 38.7 ± 32.9, and NAV – E_s = 31.9 ± 16.4 MPa (mean ± sample standard deviation). The results are displayed in Table 1. A two-sample t-test revealed non-significant differences between the groups (*p-value* = 0.44).

Table 1.

tissue	donors	samples	Es [MPa]	h [mm]	w [mm]
HP	11	22	38.7 ± 32.9	0.51 ± 0.26	5.22 ± 1.92
NAV	5	10	31.9 ± 16.4	0.50 ± 0.14	4.16 ± 0.84

Number of donors (patients) and samples for the tested tissues. HP - human pericardium; NAV - native aortic valve; *Es* - secant elastic modulus; h – thickness of samples; w - width of samples. For the thickness of the samples, the result of the t-test is: *p*(NAV–HP) = 0.87.

The results presented here were obtained after five cycles of preconditioning applied in order to attain repeatable behavior of a specimen. $\varepsilon_{\max}$ was defined as the maximum strain at which the stress increases monotonically (after this point, the slope to a σ–ε graph starts to decline, which indicates the initiation of a failure process). The samples attained mean ε_{max} in the range 0.09 - 0.15, and the mean corresponding maximum stress was 1.4 - 2.5 MPa.

7.2 Construction of the dynamic culture system for pericardial tissue 3D conditioning

We constructed a bioreactor capable of generating a pulse flow of culture medium. This high flow dynamic culture system consists of a pulsatile pump (Stőckert SIII, Sorin Group Deutschland GmbH) for driving the flow of the culture medium (thereby perfusing and conditioning the human pericardial heart valve), a reservoir for regulating the fluid capacitance and the function of the pericardial heart valve, a chamber for placing the pericardial heart valve (80 mm in internal length and 42 mm to 30 mm in inner circular diameter, corresponding to the normal size of the left ventricle and the ascending aorta in adults), containing a hollow cylindrical holder for the pericardial heart valve, and a gas exchanger for perfusing the culture medium with air mixed with 5% CO_2. The individual components of the bioreactor are connected by tubing (3/8 inch in inner diameter) and during conditioning the entire system is placed in a cell incubator. All components of the bioreactor are constructed from biocompatible materials (PVC, silicone, plexiglas) and were sterilized before use.

The components and function of each part of the dynamic culture system are described in detail in the patent:
Inventors/Applicants: Straka František, Mašín Jaroslav, Schorník David, Pirk Jan:

Title: A method of constructing a pericardial heart valve replacement, a pericardial heart valve replacement constructed according to this method, and a device for the in vitro conditioning and modification of tissue of autologous pericardium for the construction of a pericardial heart valve replacement. International Publication Number: WO 2013/182171 A1.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 December 2013 (12.12.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/182171 A1

(51) International Patent Classification:
A61L 27/36 (2006.01) *A61F 2/24* (2006.01)

(21) International Application Number:
PCT/CZ2012/000083

(22) International Filing Date:
17 August 2012 (17.08.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PV 2012-376 5 June 2012 (05.06.2012) CZ

(71) Applicant *(for all designated States except US)*: **INSTITUT KLINICKÉ A EXPERIMENTÁLNÍ MEDICÍNY** [CZ/CZ]; Vídeňská 1958/9, 140 21 Praha 4 (CZ).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **STRAKA, František** [CZ/CZ]; Třída 5. května 1042/34, 140 00 Praha 4 (CZ). **MAŠÍN, Jaroslav** [CZ/CZ]; Pitkovická 227, 251 01 Čestlice (CZ). **SCHORNÍK, David** [CZ/CZ]; Frýdlantská 23, 182 00 Praha 8 (CZ). **PIRK, Jan**; V domově 28, 130 00 Praha 3 (CZ).

(74) Agent: **PATENTCENTRUM SEDLÁK & PARTNERS S.R.O.**; Husova 5, P.O. Box 1, 370 01 České Budějovice (CZ).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *with amended claims (Art. 19(1))*

(54) Title: A METHOD OF CONSTRUCTING A PERICARDIAL HEART VALVE REPLACEMENT, A PERICARDIAL HEART VALVE REPLACEMENT CONSTRUCTED ACCORDING TO THIS METHOD, AND A DEVICE FOR THE IN VITRO CONDITIONING AND MODIFICATION OF TISSUE OF AUTOLOGOUS PERICARDIUM FOR THE CONSTRUCTION OF A PERICARDIAL HEART VALVE REPLACEMENT

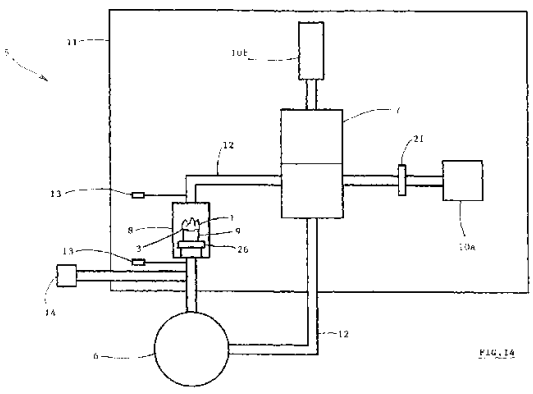


FIG. 14

(57) Abstract: The pericardial heart valve replacement (1) is constructed from living tissue of autologous pericardium containing living pericardial interstitial cells (PICs) and a living extracellular matrix (ECM) in the following way: in the first step the size and shape of the patient's native pathological heart valve is determined using real-time three-dimensional transesophageal echocardiography (TEE) or computer tomography (CT angiography) or magnetic resonance imaging (MRI); in the next step a stented (2) or a stentless pericardial heart valve replacement (1) is constructed from a single sheet of living autologous pericardium obtained from the patient; the size and shape of the pericardial heart valve replacement (1) corresponds to the size and shape of the patient's native heart valve and aortic or pulmonary root. The subject matter of the invention also includes the development of a device (5) for the conditioning and modification of the living autologous pericardial heart valve replacement (1) consisting of a ipulsatile pump (6) driving the flow of culture medium in the device (5) to perfuse the cells

[Continued on next page]

WO 2013/182171 A1

7.3 Modification of human pericardium by tissue 3D conditioning (mechanotransduction)

A pilot study was performed with the use of a new method for preparing a three-cusp heart valve construct from autologous human pericardium for potential use as a heart valve replacement. This method is described in detail in the patent (Straka F. et al., 2013). Our approach is based on harvesting living human pericardium, which is then subjected to three-dimensional (3D) modification of its histological structure using a specially-designed bioreactor for high pulse dynamic flow culture and conditioning. Like human aortic heart valve interstitial cells (VICs), pericardial interstitial cells (PICs) are activated by mechanical forces (3D mechanotransduction) to an active myofibroblast-like phenotype (aPICs) capable of producing and remodeling new ECM. We evaluated the pericardial heart valve construct for cell phenotype, ECM composition, mechanical properties and hemodynamic performance. We compared unconditioned human pericardial tissue with 4-week conditioned human pericardial tissue from the same patient, and with a native (normal) human aortic heart valve harvested during heart transplantation, using histological, immunohistochemical and biomechanical analysis and echocardiography assessment.

7.3.1 Study group

Our study group consisted of 8 patients. All patients signed an informed consent form prior to enrolment in the study. The project was approved by the Ethical Committee of the Institute for Clinical and Experimental Medicine in Prague, Czech Republic. Human pericardium samples were harvested at the time of heart surgery from 3 patients (from 2 men undergoing coronary artery bypass grafting and aortic valve replacement, and from 1 woman undergoing mitral valve replacement, average age 76 ± 3 years). The tissue samples were obtained at different times, depending on the scheduled surgery (Fig.15A). A pericardium sample of approximately 4 x 10 cm was obtained from each patient and was transported to the laboratory for heart valve construction in a sealed container containing DMEM (Dulbecco's Modified Eagle's Medium; Sigma, St. Louis, MO, U.S.A; Cat. No. D5648) at body temperature (37^{o}C). The human pericardial heart valve constructs were conditioned in the incubator by gradually increasing the pulse flow of the culture medium (Fig. 15). After conditioning in a bioreactor, these samples formed the conditioned pericardium group. Other pericardium samples obtained from the same patients were sent directly for histological and immunohistochemical evaluation. They formed the unconditioned pericardium group. Normal

(native) aortic heart valve samples were obtained from 5 other patients (5 men with dilated cardiomyopathy, average age 52 ± 16 years) during heart transplantation.

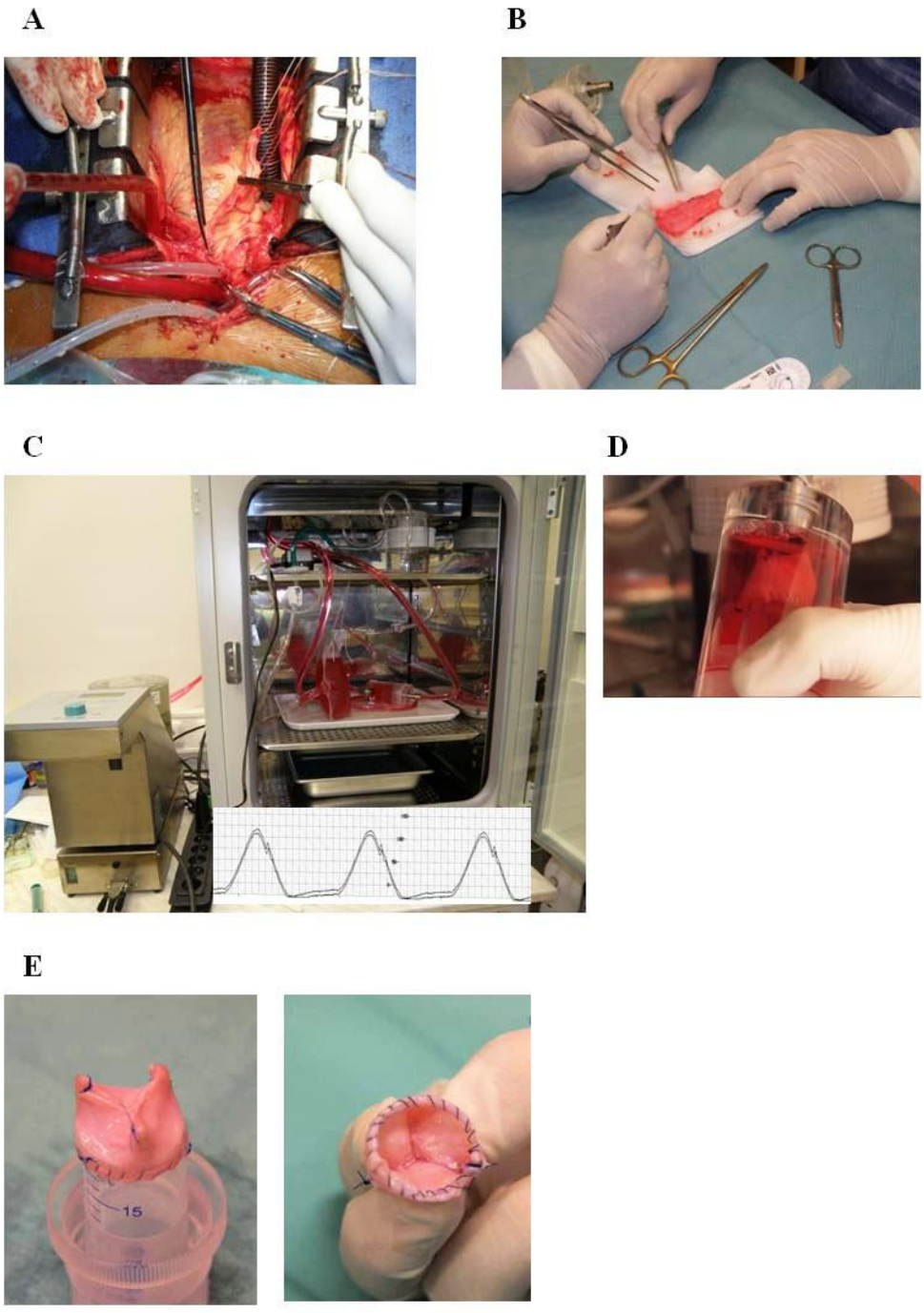


Figure 15. Human pericardium was harvested during open heart surgery (A) and was sutured to an anatomically-shaped stent (holding system) in the shape of a tricuspid aortic heart valve (B). The pericardial valve construct was conditioned in a bioreactor (C) in the chamber for conditioning with culture medium flow (D). E - tricuspid tissue-engineered human pericardial valve (TEV, conditioned human pericardium) after 4 weeks of 3D conditioning.

7.3.2 The cellular and ECM structure of human pericardium after 3D conditioning (mechanotransduction)

Dynamic conditioning of human pericardium in a bioreactor significantly influences PIC proliferation, differentiation and ECM production. A significant increase in the total PIC number is observed on H&E stained sections, together with a significant increase in the content of collagen, which also displays a crimped architecture (Fig. 16).

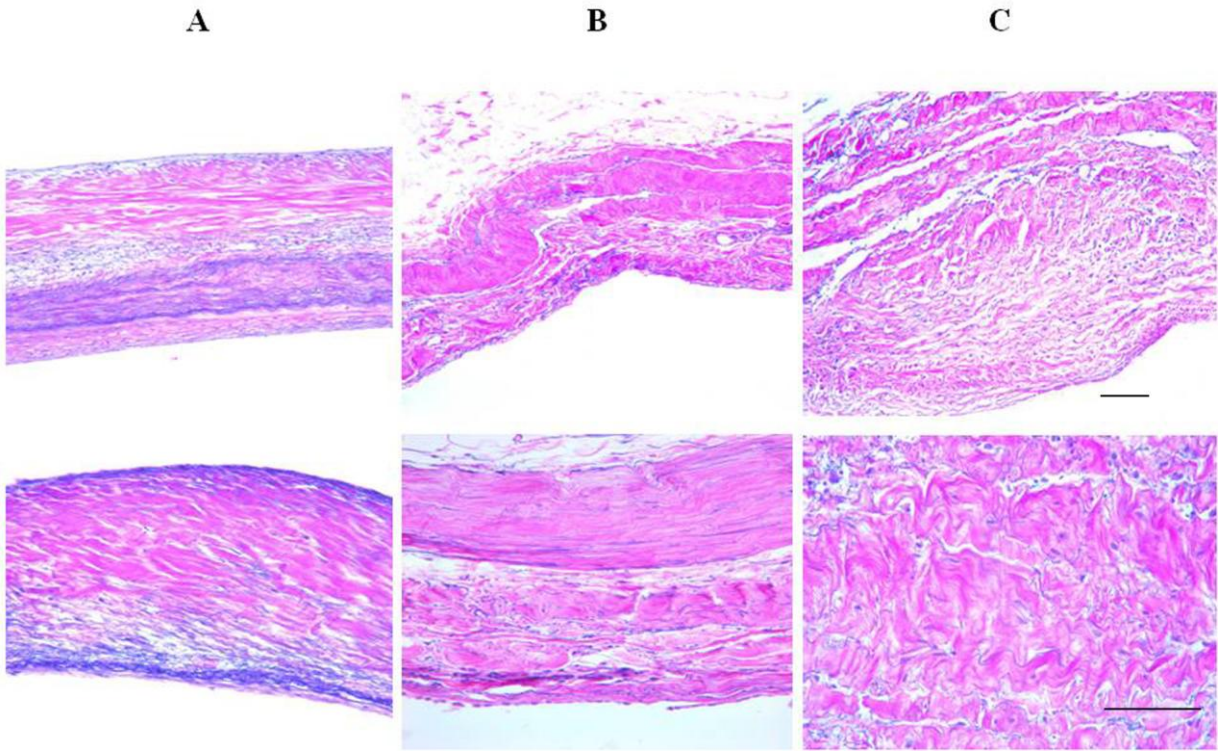


Figure 16. Hematoxylin-eosin and elastica staining of a normal (native) aortic heart valve (A), unconditioned human pericardium (B) and human pericardium after 3D conditioning (C), magnification 100x - upper part of the images, 200x - lower part of the images, scale bar = 200 μm. The nuclei of the cells are blue, the collagen fibres are red, the elastin fibres are blue-purple. There is a significant increase in the number of PICs and in the ECM content, which displays a collagen crimped architecture, and elastin fibres are also seen in human pericardium after conditioning.

Conditioned human pericardium shows a threefold increase in the number of vimentin-positive quiescent qPICs (Fig. 17) in comparison with unconditioned pericardium: unconditioned pericardium 7.5 ± 3.3, conditioned pericardium 23 ± 16.1, aortic heart valve

vimentin positive VICs 67.8 ± 21.5 average number of cells per one microscopic field in HPF magnification, 400x. The proliferation and differentiation of PICs in the tissue samples of pericardium was relatively uniform, and was most apparent in the fibrous layer. These differences are statistically significant between all three groups ($p \leq 0.001$).

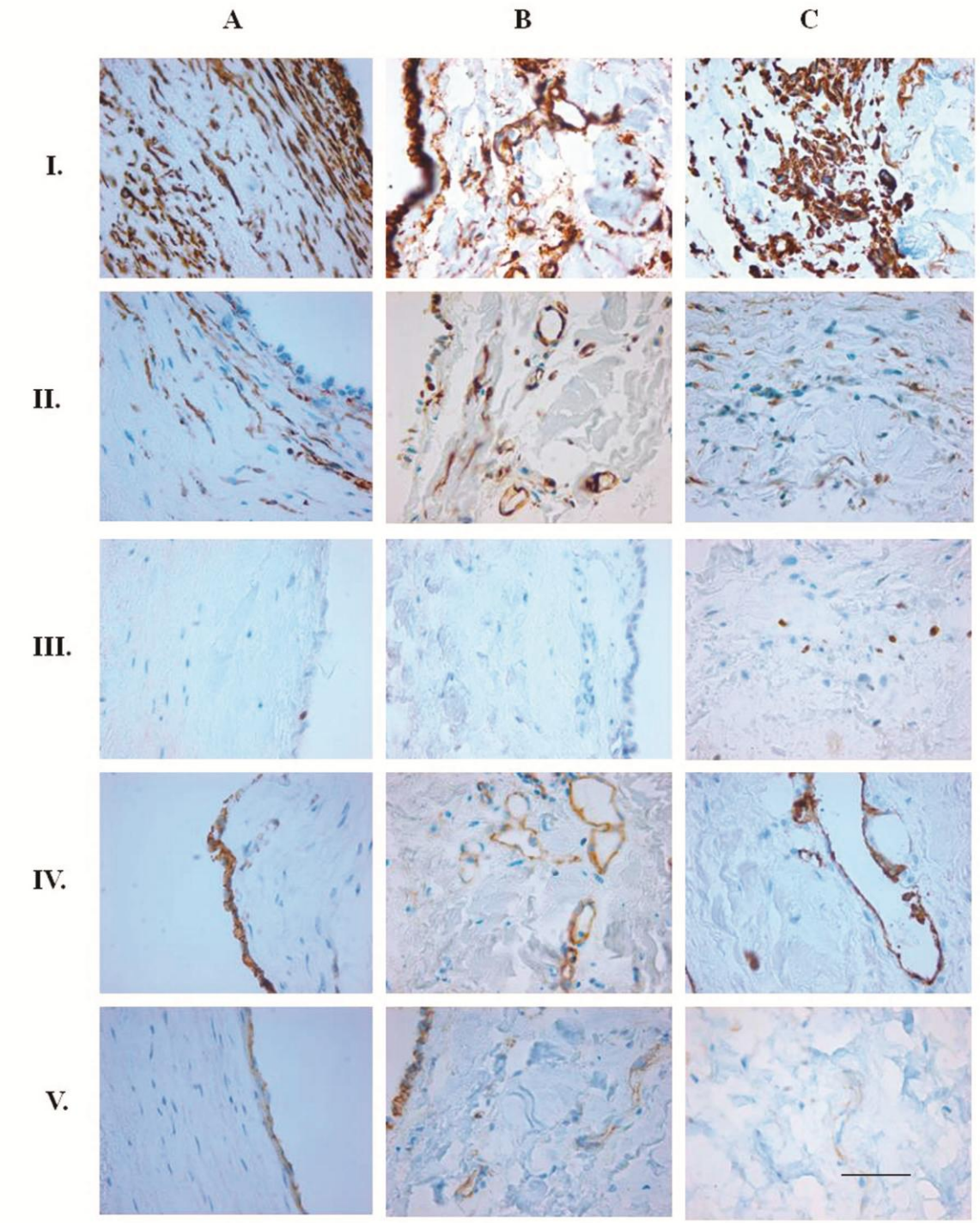


Figure 17. Cell characterization in a normal (native) aortic heart valve (A), in unconditioned human pericardium (B), and in human pericardium after 3D conditioning (C).

Immunohistochemistry staining for vimentin (I.), α-SMA (II.), Ki-67 (III.), CD31 (IV.), and β-catenin (V.), magnification 400x, scale bar = 100 μm. Immunopositivity of the targeted proteins in the cells is depicted in intense brown color, nuclei are in blue. Significant proliferation and differentiation of PICs (an increase in the number of vimentin and α-SMA positive cells) was observed in human pericardial tissue after conditioning.

The active ECM-producing phenotype of pericardial interstitial cells (aPICs) is confirmed by α-SMA positive staining of cytoplasm (brown color). We found a twofold increase in the number of α-SMA positive cells after dynamic conditioning in comparison with unconditioned pericardium (Fig. 17): unconditioned pericardium 3.3 ± 2.3, conditioned pericardium 8.9 ± 6.0, aortic heart valve α-SMA positive aVICs 11.7 ± 6.4, average number of cells per one microscopic field in HPF magnification, 400x. These differences in the number of α-SMA positive cells are statistically significant between unconditioned pericardium and conditioned pericardium ($p \leq 0.01$), and between unconditioned pericardium and aortic heart valve ($p \leq 0.001$), but are not significant between aortic heart valve and conditioned pericardium.

Ki-67, a specific nuclear marker of cellular proliferation, was also positively stained in a greater number of cells after dynamic conditioning: unconditioned pericardium expressed 0.17 ± 0.37, conditioned pericardium expressed 1.8 ± 2.0, and normal aortic heart valve expressed 0.33 ± 0.6 as the average number of cells per one microscopic field in HPF magnification, 400x. Although the number of Ki-67 positive cells is relatively small (Fig. 17), the differences between conditioned pericardium and unconditioned pericardium or normal aortic heart valve are statistically significant ($p \leq 0.001$). PICs proliferated and differentiated to an active aPICs phenotype in response to 3D dynamic loading (mechanotransduction) in a similar way as VICs are activated to aVIC phenotype in the aortic heart valve. PICs proliferated and differentiated preferentially in the superficial fibrous part of the conditioned human pericardial heart valve construct, which was the area of highest sheer stress and strain.

Beta-catenin staining positivity is lost from the mesothelial pericardial surface, most probably due to loss of these cells after conditioning, but it remains positive in the walls of a few capillaries, similar to CD31 positive staining (Fig.17).

Histological and immunohistochemical staining show a substantial increase in the amount of ECM in the conditioned pericardium tissue in comparison with unconditioned pericardium (Fig. 16, 18). The content of collagen I, (highest in the fibrosa layer), GAGs and elastin was

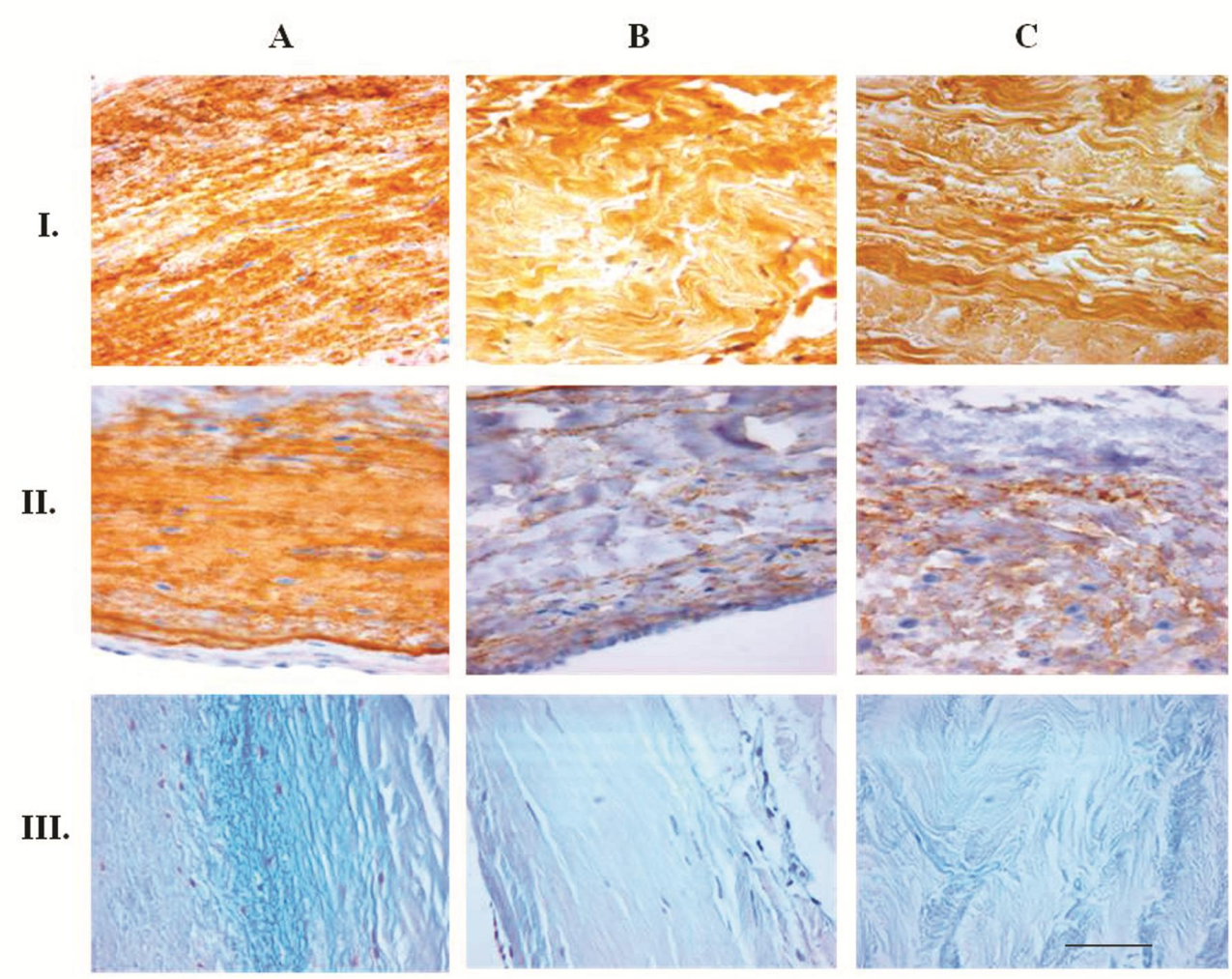


Figure 18. ECM characterization in a normal (native) aortic heart valve (A), in unconditioned human pericardium with surrounding fatty tissue (B), and in human pericardium after 3D conditioning (C). Immunohistochemical staining for collagen type I (I.) and for elastin (II.); the samples are counterstained with Harris's hematoxilin. Detected proteins are positive in brown color, nuclei are in blue. III: Alcian blue staining for GAGs, positivity is shown in blue or dark blue, nuclei are in red. Magnification 400x, scale bar = 100 μm. After conditioning, an increase was observed in the content of investigated proteins and GAGs in human pericardial tissue in comparison with unconditioned pericardium.

higher throughout the thickness of the conditioned pericardium, the width of which is also increased as a result of aPIC proliferation, differentiation and new ECM production induced by 3D mechanotransduction. These results were confirmed by histomorphometric analysis. Conditioned pericardium contained a greater amount of collagen I, elastin and GAGs than unconditioned pericardium, as measured by the integrated pixel values (color density), corresponding to the total amount of proteins and GAGs in the tissue: unconditioned pericardium – collagen I 40.3 ± 13.6, elastin 5.7 ± 2.7, GAGs 15.9 ± 8.4; conditioned pericardium - collagen I 58.6. ± 15.4, elastin 10.4 ± 3.6, GAGs 39.0 ± 14.7. We estimate that the human pericardium ECM content increased by about 20-30% after dynamic loading. There are also very important structural changes in the collagen fibers, which became thicker after conditioning, stronger and more densely arranged predominantly in the fibrous part of the pericardium. The amount of ECM components was higher in conditioned pericardium than in the aortic heart valve (the integrated pixel values: collagen I 25.9 ± 4.2, elastin 20.9 ± 4.5, GAGs 32.8. ± 13.4) with the exception of elastin. These differences are statistically significant between all three groups (unconditioned, conditioned pericardium and aortic heart valve) in the collagen I and elastin content, and in the GAG content between unconditioned and conditioned pericardium ($p \leq 0.001$), but they are not significant between aortic heart valve and conditioned pericardium in GAG content.

7.3.3 Biomechanical properties

Mechanical integrity and durability are desirable properties of bioprosthetic valves. Although pericardium-based valves have proved to be suitable for replacement purposes, their mechanical properties could be improved by a culturing procedure. In order to assess this effect, uniaxial tensile tests were carried out with pericardium after dynamic conditioning (referred to as tissue-engineered valves - TEV). Samples of unconditioned human pericardium (HP) and native aortic valve (NAV) were prepared and also underwent tensile testing, and the results were compared. The total number of tested samples from patients (donors) is listed in Table 2. NAV strips were cut in the circumferential direction of the valve; TEV strips were tested in radial and circumferential direction; HP strips were cut transversely and longitudinally with respect to the orientation of the heart.

Table 2.

tissue	donors number	samples number	Es [MPa]	h [mm]	w [mm]
HP	3	20	18.1 ± 10.6	0.72 ± 0.26	6.26 ± 1.42
NAV	5	11	32.0 ± 15.6	0.52 ± 0.15	4.27 ± 0.88
TEV	3	11	21.0 ± 15.3	0.67 ± 0.17	3.83 ± 0.88
HP_L	3	8	17.1 ± 11.4	0.69 ± 0.36	5.58 ± 1.38
HP_T	3	12	19.0 ± 10.5	0.75 ± 0.14	6.62 ± 1.45
TEV_C	2	6	22.6 ± 14.8	0.63 ± 0.11	3.73 ± 0.95
TEV_R	2	5	19.6 ± 17.6	0.72 ± 0.23	3.96 ± 0.88

The number of patients (donors) and samples of the tested tissues: unconditioned human pericardium (HP); conditioned human pericardium (tissue engineered heart valve - TEV); normal (native) aortic heart valve (NAV); HP_L – human pericardium in longitudinal direction; HP_T – human pericardium in transversal direction; TEV_C – tissue-engineered valve in circumferential direction and TEV_R – tissue-engineered valve in radial direction; Es – secant elastic modulus; h – thickness of the samples; w – width of the samples.

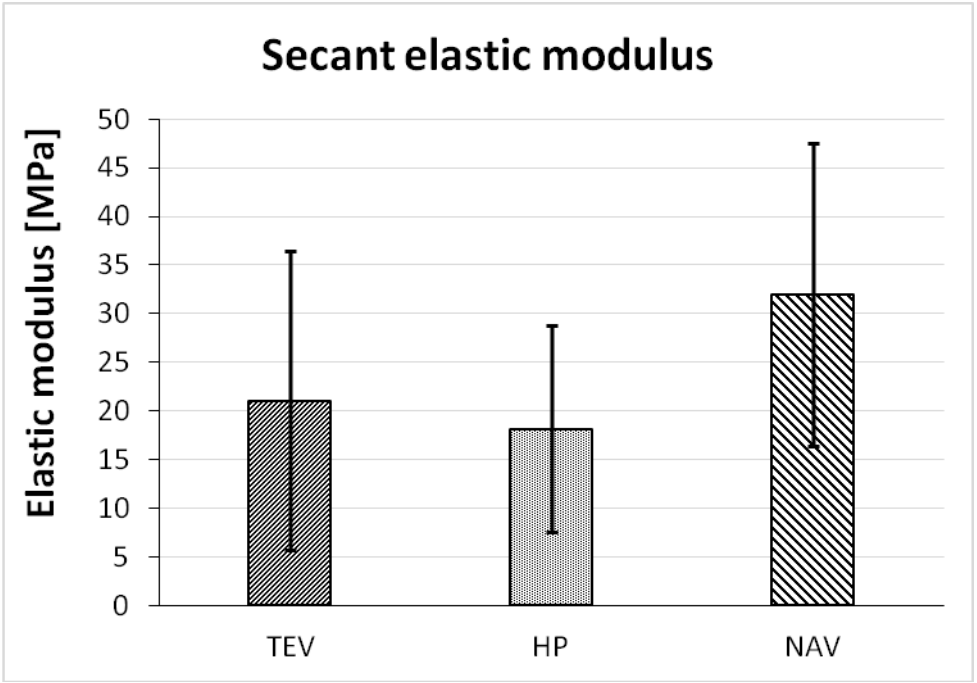


Figure 19. Averaged secant elastic moduli (***Es***) in each group: normal (native) aortic heart valve (NAV), unconditioned human pericardium (HP) and conditioned human pericardium (tissue engineered heart valve - TEV). Bars indicate standard deviation of the sample. A two-sample t-test revealed significant differences in ***Es*** between NAV – HP: p(NAV–HP) = 0.02.

The differences between other groups are statistically non-significant: p(TEV–HP) = 0.60, p(TEV–NAV) = 0.11.

Mechanical experiments gave the secant elastic moduli for the tested specimens as follows: TEV – E_s = 21.0 ± 15.3 MPa, HP – E_s = 18.1 ± 10.6, and NAV – E_s = 32.0 ± 15.6 MPa (mean ± sample standard deviation). The results are displayed in Fig. 19. The influence of sample orientation for TEV is displayed in Fig. 20, and for HP in Fig. 21.

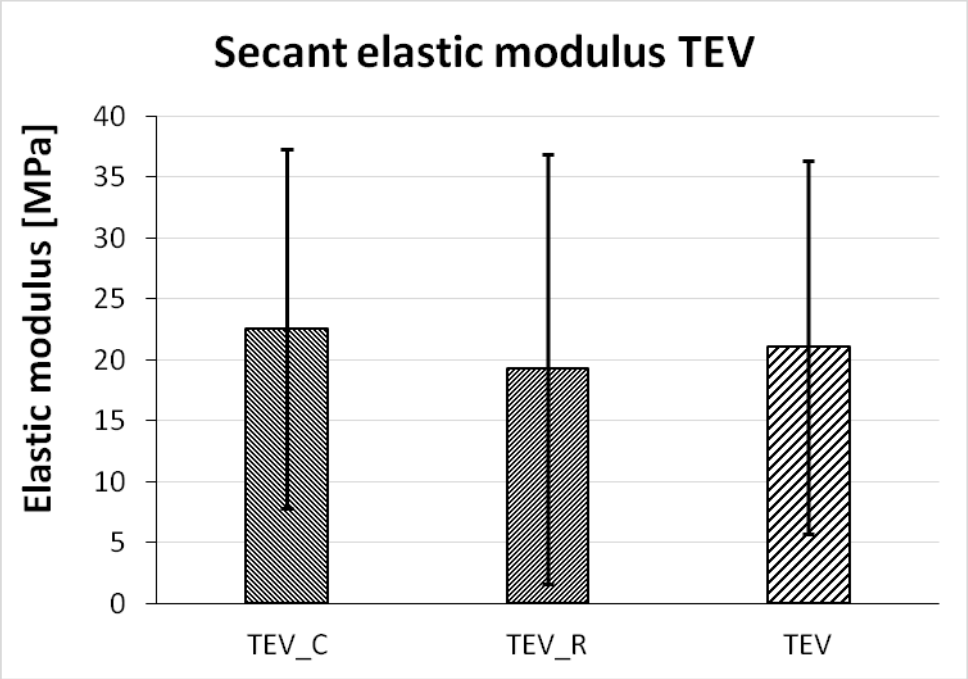


Figure 20. Averaged secant elastic moduli (***Es***) for TEV cut in radial direction (TEV_R) and in circumferential direction (TEV_C). Bars indicate the standard deviation of the sample. A two-sample t-test revealed non-significant differences between radial and circumferential direction; p(TEV_R - TEV_C) = 0.75.

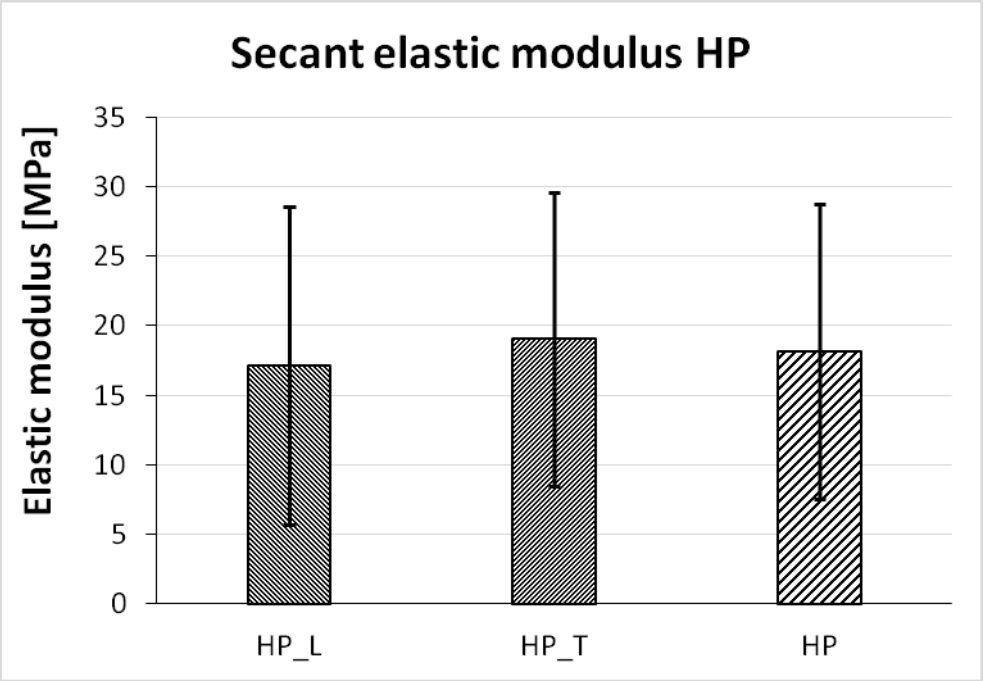


Figure 21. Averaged secant elastic moduli (***Es***) for HP cut in longitudinal direction (HP_L) and in transversal direction (HP_T). Bars indicate standard deviation of the sample. A two-sample t-test revealed non-significant differences between the directions; *p*(HP_L – HP_T) = 0.74.

Table 3.

compared groups	***Es*** ***p-value***	***h*** ***p-value***
HP-NAV	0.02*	0.02*
TEV-NAV	0.11	0.29
HP-TEV	0.60	0.55
HP-HP_L	0.85	0.84
HP-HP_T	0.85	0.74
HP_L-NAV	0.03*	0.27
TEV-TEV_C	0.85	0.57
TEV-TEV_R	0.85	0.69
HP_L-HP_T	0.74	0.70
TEV_C-TEV_R	0.75	0.46
HP_L-TEV_C	0.48	0.69

Results of a two-sample t-test and corresponding *p-values* for secant elastic modulus (Es) and thickness of the samples (h) in the compared groups: unconditioned human pericardium (HP); conditioned human pericardium (tissue engineered heart valve - TEV);

normal (native) aortic heart valve (NAV); HP_L – human pericardium in longitudinal direction; HP_T – human pericardium in transversal direction; TEV_C – tissue-engineered valve in circumferential direction and TEV_R – tissue-engineered valve in radial direction. An asterisk indicates a group with significant differences (*p-value* $\leq$ 0.05).

The following secant moduli were obtained for the anisotropy: TEV_C (in circumferential direction) – E_s = 22.6 ± 14.8 MPa, TEV_R (in radial direction) – E_s = 19.6 ± 17.6 MPa, HP_L (in longitudinal direction) – E_s = 17.1 ± 11.4 MPa, HP_T (in transversal direction) – E_s = 19.0 ± 10.5 MPa. The results of the statistical analyses (*p-values*) of the differences in the secant modulus and in the thickness of the samples (h) between each of the groups are listed in Table 3. The samples attained mean ε_{max} in the range 0.09 - 0.15, and the mean corresponding maximum stress was 1.4 - 2.5 MPa.

Fig. 19 shows that the secant modulus of HP is approximately two times lower than for NAV (p = 0.02). However, during the cultivation process, the modulus was increased and the two sample t-test revealed no significant differences between NAV and tissue engineered (TEV) valve. This suggests that the mechanical properties of the conditioned human pericardium were positively changed by the cultivation procedure and are comparable with the properties of NAV.

A comparison between HP samples excised in longitudinal direction and in transversal direction revealed isotropic behavior of pericardium, Fig. 21. The averaged secant elastic moduli (***Es***) for TEV cut in circumferential direction (TEV_C) were higher than in radial direction (TEV_R), but the differences were statistically non-significant; p(TEV_R - TEV_C) = 0.75, Fig. 20.

7.3.4 Echocardiographic and hemodynamic properties of a human pericardial valve in vitro

After 3D mechanotransduction and modification of its histological structure, the TEV exhibited valve cusp motion similar to that of a normal aortic heart valve with normal opening and closing of the valve leaflets and no regurgitation. The TEV with annulus size of 20 mm at a physiological intravalvular Doppler flow rate of 1.1-1.2 m s^{-1} has a maximal transvalvular gradient of 4-7 mmHg, a mean gradient of 2-3 mmHg, and an effective orifice area of 2.6-2.7

cm². The gradients and the cusp motion did not deteriorate during the course of TEV conditioning (Fig.22).

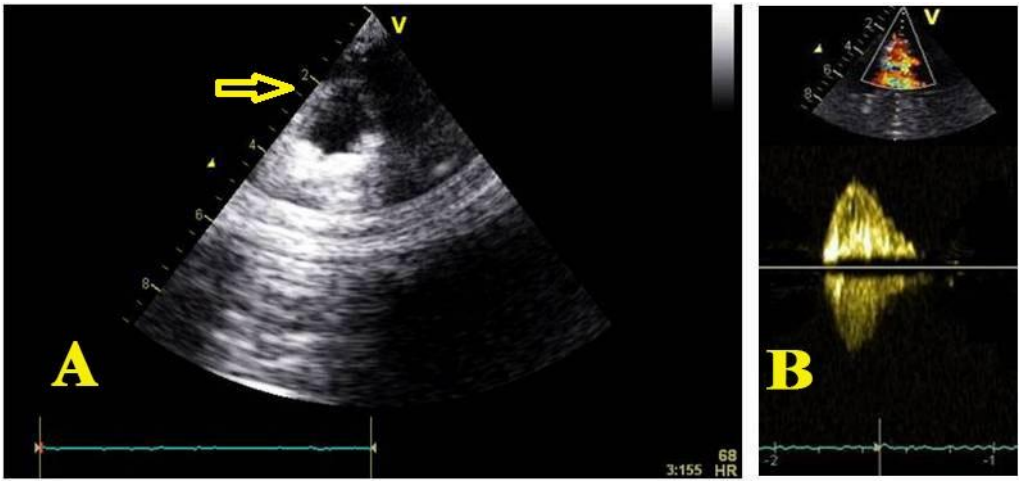


Fig 22. Echocardiographic evaluation. TEV is seen in its opened position in the chamber for conditioning (real-time 2D imaging), Doppler gradient 4-7 mmHg.

8. Discussion

Human autologous pericardial tissue may provide an appropriate scaffold for tissue engineering of the aortic valve, because it closely resembles the histological structure, the cell phenotype and the mechanical properties of the native aortic heart valve. VICs are the main cell type of normal aortic heart valve. The proportion of the phenotypes present in the aortic heart valve changes through the embryonic period, childhood and adult life. In adults, VICs are mostly of a quiescent fibroblast-like phenotype (qVICs), and express low levels of the cellular marker α-SMA) (Liu A.C. et al, 2007; Taylor P.M. et al., 2000). These cells not only interact with the ECM but also interact one with another through cytoplasmatic extensions and cellular junctions. They form a complex three-dimensional network within the ECM, which is well visible in SHG/TPEM imaging. Several molecules are involved in these communications, e.g. N-cadherin (cadherens junction), desmoglein (desmosomal junction), connexins 26 and 45 (gap junction), several integrin molecules, i.e. α1, α2, α3, α4, α5, α6 and β1 (Latif N., et al., 2006). This three-dimensional network and these cell associations are important for sensing strains and stresses in valve tissue and for reacting to them appropriately. Forces exerted on the ECM by the flow of blood *in vivo* are sensed by the VICs, and probably through cell-ECM adhesions. These signals are transformed

intracellularly by signal transduction pathways, which influence the function and the genetic behavior of the cells (Schoen F.J., 2008). VICs continuously respond to their environment by proliferating and differentiating to an activated myofibroblast-like phenotype called aVICs, which produce, remodel and crosslink the ECM through the production of several enzymes, including lysyl oxidase (LOX), lysyl hydroxylase, matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) (Chester A.H., Taylor P.M., 2007). Natural collagen crosslinking within the ECM is also influenced by the biological environment and by the specific steric organization of the collagen (Reiser K. et al., 1992). This unique biological behavior of VICs permits heart valve tissue growth, repair and adaptation to circulatory forces, which is very important for maintaining long-term heart valve function. The amount and the quality of the ECM that is produced, including the collagen fiber orientation, the collagen type and the degree of crosslinking, determine the mechanical properties of the tissue, such as compliance, elastic modulus and tensile strength. This is important for connective tissue that serves as a structural scaffold for load bearing, e.g. the heart valve (Schoen F.J., 2008).

PICs are of similar mesenchymal original as VICs (Taylor P.M. et al., 2000). In human pericardial tissue PICs exist, similarly as VICs in the aortic heart valve, in a quiescent and activated (fibroblast-like) phenotype, and they are probably responsible for tissue remodeling. As was confirmed in our study by the results of SHG/TPEM imaging, the shape of the VICs and PICs corresponds to the surrounding ECM structure, mainly in the nature and the arrangement of the collagen fibres. In parts of the tissue with a loosely-packed arrangement of the collagen fibers, such as the lamina ventricularis of the aortic valve or the serous part of the pericardium, the cells have a more spread-out cytoplasmic morphology. However, the cells have a rather spindle-like morphology between the densely packed collagen bundles in the lamina fibrosa aortic valve or in the fibrous part of human pericardial tissue. Cell-to-cell and cell-to-ECM communication are well visible in SHG imaging.

Mesothelial cells form a monolayer of flattened squamous-like epithelial cells resting on a thin basement membrane supported by dense connective tissue. The main purpose of these cells is to produce a lubricating fluid that is released between pericardial layers. These cells do not have the structure or function of valvular endothelial cells. When using human pericardium as a scaffold to prepare heart valve replacements, these cells should be removed from the surface of the pericardium.

SEM of the human pericardium and aortic heart valve cusp tissue revealed close similarities in surface morphology (corrugations, grooves and ridges) based on the

arrangement of huge collagen I and III bundles. Human pericardial tissue has a very similar ECM structure to the normal aortic heart valve.

Aortic valve leaflets are composed primarily of collagen (60% of total ECM dry weight) (Kunzelman K.S. et al., 1993). The main collagen types in the aortic heart valve are collagen I (74% of the total collagen) and collagen III (24% of the total collagen). Elastin represents 13%, and is responsible for the elastic properties of the valve leaflet and for restoring the crimped (corrugated) state of collagen fibers during unloading (Ku C.H. et al., 2006). Collagen bundles, which are the main load-bearing component, have a specific architecture that endows heart valve tissue with the ability to withstand the circulatory forces (which are highest in the circumferential direction) over the course of a lifetime. Its fiber orientation in the direction of the largest tensile stress, density and cell associations are very important for this purpose (Schoen F.J., 2008). Collagen I and collagen III fibres are also found in the tissue of human pericardium, predominantly in the fibrous part, where they form thick bundles. In addition, collagen fibers are present in the serous part of pericardial tissue, which corresponds to the lamina ventricularis of a normal aortic heart valve. In this part of human pericardial tissue, collagen I and collagen III fibers are more frequent and thicker than the lamina ventricularis of a normal aortic valve.

Elastin occurs in the aortic heart valve cusp tissue as a crosslink formed by lysyl oxidase from tropoelastin monomer, and it is surrounded by a fine mesh of microfibrils, predominantly fibrillin-1 and fibrillin-2 (Wagenseil J.E., Mecham R.P., 2007). These fibrillins can bind integrins, proteoglycans and growth factors, and can play a role in cell signaling (Bax D.V. et al., 2007). Dense wavy elastin fibers are observed in the lamina ventricularis in the aortic heart valve, and sparsely-distributed elastin fibers are also present in the lamina spongiosa. There are also thin layers in the lamina fibrosa that surrounds and connects the collagen fibers. This arrangement forms the characteristic corrugated structure of the fibrosa layer. It is assumed that the elastic fibers present in the lamina spongiosa connect the elastic fibers in the lamina ventricularis with collagen fibers in the lamina fibrosa. This enables the mechanical properties of these two layers to combine when the aortic heart valve cusps open and close during the cardiac cycle (Stella J.A., Sacks M.S., 2007). Elastin fibers can be observed between the wavily arranged collagen I and III fibers in all layers of the pericardial tissue, but they are not arranged in a densely corrugated structure as they are in the lamina ventricularis of a normal aortic heart valve.

Glycosaminoglycans (hyaluronan, heparin sulfate, chondroitin sulfate, dermatan sulfate, keratin sulfate) and proteoglycans (i.e. decorin, biglycan, versican) are comprised

preferentially in the lamina spongiosa of the aortic heart valve leaflets, where collagen I and collagen III fibers are also sparsely distributed. GAGs dampen vibrations and allow compressibility of the leaflets and changes in the arrangement of collagen and elastic fibers in the aortic heart valve leaflet during the cardiac cycle (Tseng H., Grande-Allen K.J., 2011; Lincoln J. et al., 2006). GAGs are also seen relatively uniformly in all layers of human pericardial tissue, and in smaller amounts than in the normal aortic heart valve. In the middle part of human pericardium we also observe less densely arranged collagen fibers. This part of the pericardial tissue between the fibrous and serous part mimic the lamina spongiosa layer of a normal aortic heart valve, but it is not so clearly expressed in the histological structure.

Human pericardium ECM contains well-developed and crimped collagen fibres interspersed with fibres of elastin and GAGs. Like in the aortic heart valve, interactions between these components of the ECM are responsible for the mechanical properties of the tissue. Confocal microscopy and TPEM of human pericardium confirmed a complex three-dimensional PICs and ECM network very similar to that of the native aortic heart valve, with intimate associations present between cells and between cells and collagen fibers. It is very difficult to achieve such a complex cellular and ECM histological structure for artificially generated scaffolds from several biocompatible materials for heart valve tissue engineering.

The significant increase in the total number of PICs, aPICs and α-SMA expression in human pericardial tissue during dynamic conditioning shows that PICs can proliferate and differentiate, and can be activated to an active pericardial interstitial cell phenotype similar to aVICs. Ki-67 staining proved increased proliferation of aPICs, which is mostly apparent in the superficial layer (the fibrous part) of the pericardial heart valve construct exposed to the highest shear stress and strain. The increase in collagen, elastin and GAGs in human pericardial tissue after *in vitro* conditioning means that PICs remain metabolically active and are capable of responding to mechanical stress and strain in their environment through the production of new ECM and tissue remodeling. PICs survived *in vitro* for 4 weeks of conditioning, suggesting that the nutrient diffusion is sufficient to sustain pericardial living tissue in the bioreactor.

3D mechanotransduction can be used in tissue engineering as a method for *in vitro* tissue modification to improve its histological structure. The main forces that may cause cell activation and tissue remodeling are shear stress, hydrostatic pressure, compressive and tensile forces. It was confirmed *in vivo* that the cellular response to mechanical stimulation can be influenced by the direction of the applied forces, by the extracellular matrix architecture, and by cell morphology and orientation (Kurpinski K. et al., 2006). Forces

exerted on the ECM by the flow of blood *in vivo*, or by the flow of the culture medium in vitro, stretch the heart valve leaflets and are sensed by VICs, and probably also by PICs, through cell-ECM adhesions. These signals are transformed intracellularly by signal transduction pathways, which influence the function and the genetic behavior of the cells. Growth factor signaling is thought to act cooperatively, leading to directed cell proliferation and differentiation. Together, these signaling pathways lead to specialized tissue formation and remodeling (Schoen F.J., 2008). Several cellular and subcellular mechanisms are thought to be involved in the 3D mechanotransduction process. A very important factor is tissue stiffness and compliance. Mechanical forces can be transmitted from cell surface receptors into the downstream signaling cascades. This has been described by Chester as direct centralized mechanotranduction (Chester A.H. et al., 2014). In this way, mechanical forces are transmitted by integrin-based cell focal adhesion complexes that connect ECM proteins to the cell actino-myosin cytoskeleton through transmembrane integrins and several types of adaptor proteins that are involved in this reaction (i.e. talin, vinculin, α-actinin, filamin A, p130Cas, tensin, zyxin, paxillin). Actin filaments are connected to the intracellular part of focal adhesions by filamin A. This process is influenced by several growth factors (i.e. fibroblast growth factor - FGF, transforming growth factor - TGF, vasodilatator-stimulated phosphoprotein - VASP, vascular endothelial growth factor - VEGF) (Zaidel-Bar R., Geiger B., 2010; Atherton P. et al., 2015; Nieves B. et al., 2010; Hoffman L.M., et al., 2012). Several proteins also change the enzyme activity in these integrin-based functions of focal adhesion mechanosignaling, including small GTPase proteins, such as RhoA kinase (ROCK), Rac1 and Srs, focal adhesion kinase (FAK), the receptor protein tyrosin phosphatase α (RPTPα), extracellular signal regulated kinase (ERK), mitogen-activated protein kinase (MAPK), or they become phosphorylated during mechanical stretching (i.e. substrate domain p130Cas by Src family kinase in vitro) (Jiang G. et al., 2006; Sawada Y., et al., 2006). Activated ROCK and RhoA kinase play a role in several cellular processes, most notably in the organization of actin filaments and in phosphorylation of myosin light chains (MLC) under dynamic conditioning (Holle A.W., Engler A.J., 2011). Several enzymes, e.g. FAK, are involved in mechanical stretch signaling through integrins in cell migration and proliferation (Mitra S.K. et al., 2005). The intermediate filament protein vimentin, which binds to filamin A and protein kinase C epsilon, influences cell adhesion and spreading on collagen due to interaction with actin and with β_1 integrins (Kim H., et al. 2010). Myosin light chain kinase (MLCK) also plays a role in cell spreading by phosphorylating the regulatory light chain of myosin II, which binds to F-actin (Yang C.X. et al., 2006).

The increase in membrane tension and stress fibre contraction may also increase the activity of mechanosensitive (stretch-activated) ion channels, such as MscL (Ca^{2+}) or the two-pore domain K^+ channels TRAAK, TREK1, and TREK2. Mechanosensitive eukaryotic cation-selective ion channels sense and respond to mechanical forces, transducing them into an ionic current across cellular membranes. This modifies the electrical potential of the cell and participates with the actin cytoskeleton in plasma membrane remodeling (Sbrana F. et al., 2008; Kocer A., 2015; Brohawn S.G., 2015).

Merely removing VICs from their ECM environment is enough to transform them to an aVIC phenotype (Schoen F.J., 2008). Stretching experiments conducted on these cells *in vitro* have also shown that they readily become activated by low levels of cell membrane and cytoplasm deformation. In response to mechanical 14% or 20% stretch activation of VICs, there was an increase in mesenchymal stem cells and adipose-derived stem cells *in vitro*, [(3)H]-proline, collagen synthesis and in the expression of gene products such as collagen III (COL3A1 gene) or collagen I (COL1A1 gene). It is also hypothesized that similar gene expression and secretion of MMPs or their tissue inhibitors (TIMPs) takes place (Colazzo F. et al., 2011; Ku C.H. et al., 2006). This may be an example showing that mechanical forces can also be transmitted without central signaling pathways to the nucleoskeleton. This process has been described by Chester et al. as decentralized mechanotransduction (Chester A.H. et al., 2014). The F-actin cytoskeleton and the mechanical connections formed by the LINC complex (Linker of the Nucleoskeleton to the Cytoskeleton) have a key role in propagating the mechanical forces from integrin receptors to the nuclear surface. The LINC complex consists of two protein families in the nuclear envelope: the SUN (Sad1p, UNC-84) domain proteins, which are located in the inner nuclear membrane (INM) and transluminally bind the KASH (Klarsicht/ANC-1/Syne Homology) domain proteins, i.e. nesprins, which occur in the outer nuclear membrane (Alam S. et al., 2014). Deformations at subcellular scale (microtubule motors like dynein and kinesin, actomyosin, intermediate filaments) with changes in nuclear shape may induce changes in gene transcriptional activity (Wang N. et al., 2009), i.e. α-SMA corresponds with gene ACTA2 expression (Gould R.A. et al., 2012). Recently, a description was made of nuclear membrane proteins called lamins, which are mechanosensitive. Lamin A and cytoskeletal myosin II seem to be mechanically coupled to the collagenous matrix, and these coiled-coil proteins assemble into specific networks that are responsible for transducing mechanical signals from ECM to the nucleus (Dingal P.C., Discher D.E., 2014). Additional research is needed to provide a better understanding of the pathways and the mechanisms of mechanotransduction.

In vitro mechanical conditioning induces changes and modification of the human pericardial tissue and improves its histological structure. After conditioning, human pericardium contains live PICs and well-developed and crimped collagen fibers interspersed with fibers of elastin and GAGs. As in the aortic heart valve, interactions between these components of the ECM are responsible for the mechanical properties of the tissue. Elastin (which extends in diastole) allows pericardial valve cusps to stretch, while collagen crimping unfolds during valve closure in diastole. Once unfolded, the collagen resists any further stretching of the valve cusp tissue, providing strength and preventing cusp prolapse. During systole, elastin contracts (recoils) the valve cusps, and the collagen fibers refold to allow for smooth valve opening.

The 3D mechanotransduction of the human pericardial valve construct, supported by the holding system in a specific physiological shape, causes strain and stress to act differently at different points on the pericardial heart valve replacement, which stimulates the differentiation of PICs and the formation of new ECM. The anatomical 3D stent serves to remodel human pericardium to the desired shape of the heart valve and prevents retraction of the pericardial heart valve leaflets, which could lead to regurgitation. This could be due to the tissue contraction that occurs with new ECM production, and due to collagen and elastin cross-linking. Our results indicate that sufficient tissue remodeling occurs with shorter conditioning periods (shorter than 4 weeks).

Uniaxial tensile tests confirmed that human pericardium is a suitable material for heart valve replacements. In one part of the study, the secant elastic modulus of the native pericardial specimens was compared with native aortic heart valves. The differences were not statistically significant, and may have been could be caused by variability between the donors. Uniaxial tensile tests also confirmed the mechanical integrity of the pericardial tissue-engineered valves. The secant elastic modulus of human pericardial conditioned specimens was found to be slightly lower than the secant elastic modulus of native aortic heart valves. However, the differences were not statistically significant. After conditioning, a tendency toward anisotropy in the pericardial tissue was observed, Es was larger in circumferential direction than in radial direction. However, the differences were not statistically significant. Soft tissues frequently exhibit nonlinear and anisotropic mechanical behavior. In such a situation, unlike for linearly elastic materials, there is no simple parameter that is suitable for forming a basis for comparing material properties. The correct procedure is to specify the stress or strain level, the loading rate, and the direction in which the elastic modulus was obtained.

Depending on these conditions, the Young elastic modulus of aortic valves is usually in the range of units and tens of MPa (Balguid A. et al., 2007). Kalejs et al. (Kalejs M. et al., 2009) found the elastic modulus in the circumferential direction (under a stress level of 1 MPa) for human NAV to be 15.3 ± 4 MPa. Anssari-Benam et al. (Anssari-Benam A. et al., 2011) reported a monotonically increasing elastic modulus from 5 MPa to 35 MPa for engineering strains from 0 to 0.2 at a strain rate of 0.1 s^{-1} in porcine samples. However, our assessment of the culturing procedure is based on the secant modulus. The estimated circumferential E_s, fig. 2 in Anssari-Benam et al. (Anssari-Benam A. et al., 2011), gives ≈ 10 MPa. Figure 1 in Stradins et al. (Stradins P. et al., 2004) gives E_s ≈ 5 MPa for human aortic valve in the circumferential direction (at ε = 0.1). In comparison with bovine pericardium, which is frequently used as a scaffold, our data suggest that human tissue has lower stiffness. The reported elastic moduli for fixed and fresh bovine tissue range between 20 and 70 MPa (Sung H.W. et al., 1999; Zioupos P., Barbenel J.C., 1994). However, tissue engineered heart valves based on biodegradable scaffolds seeded with cells such as a polyglycolic acid (PGA) scaffold coated with polyhydroxybutyrate (PH4B) and seeded with human venous myofibroblast cells, have a generally lower secant elastic modulus than a native aortic heart valve ~ 3 - 6 MPa and conditioned human pericardium (Mol A. et al., 2006).

Proper function of the human pericardial heart valve construct was confirmed visually and by echocardiography at systemic pressures. The well-developed ECM, pericardial tissue is strong enough to withstand such pressures. The soft and pliable characteristics of human pericardial valve leaflets allow the leaflet motion to have a close resemblance to the motion of normal aortic heart valve leaflets, and the measured pressure gradients and EOA are comparable with those of a normal aortic heart valve of similar annulus size (19-21 mm). However, these gradients and EOA are superior to currently used xenogeneic heart valvular prostheses of similar size 19 - 21 mm: maximum gradient 17 - 47 mmHg, mean gradient 9 - 24 mmHg, effective orifice area 1.1 - 1.8 cm^2 (Zoghbi W.A. et al., 2009).

Our results suggested that the surface mesothelial cells of the pericardium were released from the pericardial tissue during conditioning. It can be assumed that, after implantation of the autologous pericardial heart valve replacement *in vivo*, the surface of the valve may again endothelialize and become covered by the patient's own endocardial and endothelial cells (originating from circulating endothelial progenitor cells). We consider it very probable that endothelial cells will attach and anchor onto the surface of the pericardial heart valve replacement. We base this assumption on the presence of specific amino acid sequences and other ligands for cellular adhesion receptors on the surface of the pericardial extracellular

ECM, which is considered to be an attractive substrate for endothelial cell adhesion (Li S., Henry J.J. 2011; Hristov M. et al., 2003).

The physiological shape of the stent or holding system for 3D conditioning should be designed according to the patient's aortic root or valve geometry, using computer-aided drafting (CAD) and rapid prototyping techniques (Lee M., Wu B.M., 2012). The best method for forming a custom-designed pericardial heart valve replacement that "fits the patient" is based on CT angiography, MR or 3D echocardiography imaging of the patient's heart, because the dimensions of the aortic root and the aortic valves are not uniform, and vary in individual patients (Jatene M.B. et al, 1999). The shape of the stent of the aortic heart valve can be created on the basis of a 3D CT scan of the patient's heart. This model is exported to STL format (an abbreviation based on 3D printing technology called STereoLithography) for 3D printing. An example of this method is shown in Fig. 23 (Krupa P., Straka F., Poslednik J., 2016, unpublished data).

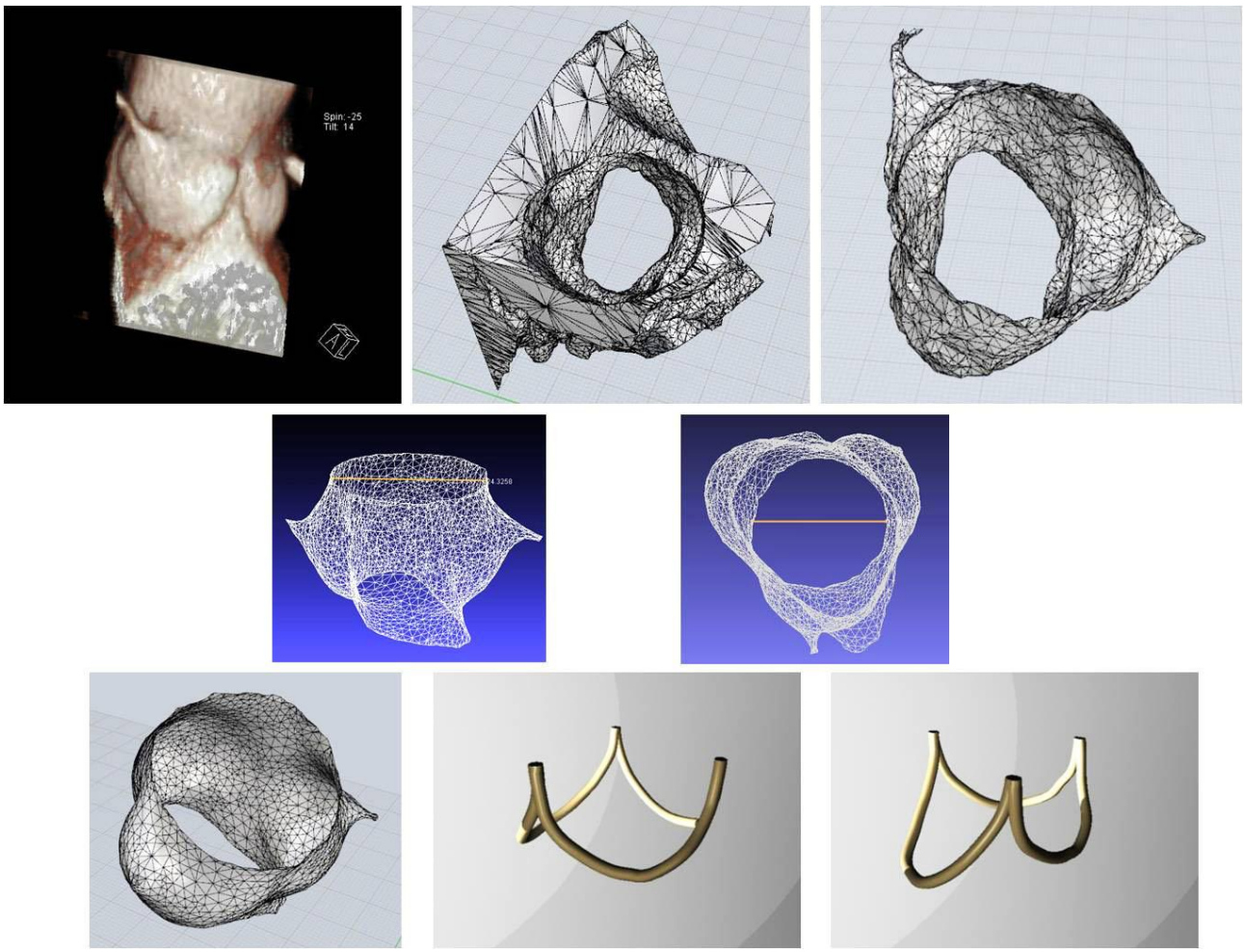


Fig. 23. The anatomically shaped 3D stent, based on the CT of the patient's heart - CAD models, format of STL files for 3D printing.

Autologous human pericardium is a complex tissue. Due to its inherently natural cellular and ECM organization, which closely resembles the normal aortic heart valve, and has similar mechanical properties, autologous human pericardium may be a promising tissue (scaffold) from which to construct a living heart valve replacement. Mini-invasive harvesting of autologous pericardial tissue from an individual patient can be performed using minimally invasive (thoracoscopic) techniques, or using a specially-designed catheter with optics.

In vitro modification of autologous human pericardial tissue by 3D dynamic conditioning (3D mechanotransduction) may offer a new approach for preparing an autologous heart replacement for potential clinical use. The valve can be stentless or stented. The stent may be manufactured from biodegradable materials (for example polylactide, polyurethane, etc.) The advantage of an absorbable stent is that it will temporarily support the pericardial heart valve replacement in the anatomical position of the native heart valve until the sutured margins grow into the wall of the heart or into the aortic root.

An autologous human pericardial tissue scaffold may also avoid a negative immune response, which results in the development of degenerative structural changes in heart valve replacements made from allogeneic, xenogeneic or biocompatible synthetic materials after *in vivo* implantation (Barone A. et al., 2014). It can be assumed that there will be a lower incidence of degenerative changes and a longer functional lifetime for an autologous heart valve replacement of this kind after *in vivo* implantation than for currently-used heart valve substitutes.

9. Conclusions

The research has demonstrated the potential of autologous human pericardium for mimicking the natural structure, the mechanical properties and the interstitial cell behavior of the normal aortic heart valve.

In vitro modification of autologous human pericardial tissue by dynamic conditioning (3D mechanotransduction) may offer a new approach for preparing an autologous heart replacement for potential clinical use. A dynamic culture system (a bioreactor) with pulse flow of the culture medium was prepared for this purpose.

Normal human pericardium consists of vimentin-positive PICs that have properties similar to those of VICs of the aortic heart valve. These cells are able to respond to mechanical stresses (3D mechanotransduction) by proliferating and differentiating into an activated

phenotype (aPICs) capable of producing a significant amount of new ECM (collagen I, elastin and GAGs) and remodeling the histological structure of the tissue.

Uniaxial tensile tests were performed in order to compare the mechanical properties of conditioned pericardium with the mechanical properties of native aortic heart valve. The results of our assessment of the mechanical properties indicate that the secant elastic modulus of the pericardium after conditioning is comparable to that of the native aortic heart valve. Such a pericardial heart valve may possess optimal hemodynamic properties, physiological pressure gradients and flow parameters, and may be capable of remodeling and regenerating *in vivo*, and there may be no negative immune response reaction. This makes human pericardium a promising tissue for preparing an autologous living heart valve substitute by tissue engineering modification.

Mini-invasive harvesting of autologous pericardial tissue from an individual patient can be performed using minimally invasive (thoracoscopic) techniques, or using a specially-designed catheter with optics**.** An autologous human pericardial tissue scaffold may avoid a negative immune response, which results in the development of degenerative structural changes in heart valve replacements made from allogeneic, xenogeneic or biocompatible synthetic materials after *in vivo* implantation. We assume that there will be a lower incidence of degenerative changes and a longer functional lifetime for an autologous heart valve replacement of this kind after *in vivo* implantation than for currently-used heart valve substitutes.

10. Summary

1. The research has confirmed that the cellular content, extracellular matrix (ECM) structure and the mechanical properties of human pericardium are very similar to the structure of the normal human aortic heart valve.

2. A bioreactor has been constructed that is capable of generating a pulse flow of culture medium for three dimensional (3D) conditioning of human pericardial tissue.

3. A pilot study was perfomed with the use of a new method for preparing a three-cusp heart valve replacement from autologous human pericardium based on human pericardial tissue 3D dynamic conditioning (mechanotransduction).

4. The histological structure of the conditioned pericardium is very similar to that of the normal aortic heart valve, and 3D dynamic conditioning has been proven to be important for PIC activation and tissue remodeling. Autologous human pericardium may be a promising tissue from which to construct a living heart valve substitute. A pericardial heart valve replacement of this type may possess optimal mechanical and hemodynamic properties, and there may be no negative immune response after implantation *in vivo*.

Souhrn

1. Výzkum prokázal, že buněčné složení, struktura mezibuněčné hmoty a mechanické vlastnosti lidského perikardu jsou velmi podobné normální aortální chlopni.

2. Zkonstruovali jsme bioreaktor generující pulsní průtok kultivačního média pro trojrozměrné (3D) kondicionování tkáně lidského perikardu.

3. Provedli jsme pilotní studii s použitím nové metody přípravy trojcípé náhrady srdeční chlopně z lidského perikardu, založené na 3D kondicionování (mechanotransdukci) této tkáně.

4. Histologická struktura kondiciovaného perikardu se velmi podobá tkáni normální aortální chlopně a 3D dynamické kondicionování bylo prokázáno jako významné pro aktivaci PIC buněk a tkáňovou remodelaci. Autologní lidský perikard může být slibnou tkání pro vytvoření živé srdeční chlopenní náhrady. Perikadiální chlopenní náhrada tohoto typu vykazuje optimální mechanické a hemodynamické vlastnosti a nebude vyvolávat nežádoucí imunitní reakci po implantaci *in vivo*.

11. References

1. Akhyari, P., Kamiya, H., Gwanmesia, P., Aubin, H., Tschierschke, R., Hoffmann, S., Karck, M., Lichtenberg, A. In vivo functional performance and structural maturation of decellularised allogenic aortic valves in the subcoronary position. Eur J Cardiothorac Surg 2010; 38(5): 539-46

2. Alam, S., Lovett, D.B., Dickinson, R.B., Roux, K.J., Lele, T.P. Nuclear forces and cell mechanosensing. Prog Mol Biol Transl Sci. 2014; 126: 205-15.

3. Anssari-Benam, A., Bader, D.L., Screen, H.R. A combined experimental and modelling approach to aortic valve viscoelasticity in tensile deformation. J Mater Sci Mater Med. 2011; 22(2): 253-62.

4. Atherton, P., Stutchbury, B., Wang, D.Y., Jethwa, D., Tsang, R., Meiler-Rodriguez, E., Wang, P., Bate, N., Zent, R., Barsukov, I.L., Goult, B.T., Critchley, D.R., Ballestrem, C. Vinculin controls talin engagement with the actomyosin machinery. Nat Commun. 2015; 6: 10038.

5. Balguid, A., Rubbens, M.P., Mo,l A., Bank, R.A., Bogers, A.J., van Kats, J.P., de Mol, B.A., Baaijens, F.P., Bouten, C.V. The role of collagen cross-links in biomechanical behavior of human aortic heart valve leaflets--relevance for tissue engineering. Tissue Eng 2007; 13(7): 1501-11.

6. Barone, A., Benktander, J., Teneberg, S., Breimer, M.E. Characterization of acid and non-acid glycosphingolipids of porcine heart valve cusps as potential immune targets in biological heart valve grafts. Xenotransplantation. 2014; 21(6): 510-22.

7. Bax, D.V., Mahalingam, Y., Cain, S., Mellody, K., Freeman, L., Younger, K., Shuttleworth, C.A., Humphries, M.J., Couchman, J.R., Kielty, C.M. Cell adhesion to fibrillin-1: identification of an Arg-Gly-Asp-dependent synergy region and a heparin-binding site that regulates focal adhesion formation. J Cell Sci. 2007; 120: 1383-92.

8. Berry, J.L., Steen, J.A., Koudy Williams, J., Jordan, J.E., Atala, A., Yoo, J.J. Bioreactors for the development of tissue engineered heart valves. Annals Biomed Eng 2010; 38(11): 3272-79.

9. Brohawn, S.G. How ion channels sense mechanical force: insights from mechanosensitive K2P channels TRAAK, TREK1, and TREK2. Ann N Y Acad Sci. 2015; 1352: 20-32.

10. Campagnola, P.J., Millard, A.C., Terasaki, M., Hoppe, P.E., Malone, C.J., Mohler, W.A. Three-dimensional high-resolution second-harmonic generation imaging of endogenous structural proteins in biological tissues. Biophys J. 2002; 82: 493-508.

11. Claiborne, T.E., Slepian, M.J., Hossainy, S., Bluestein, D. Polymeric trileaflet prosthetic heart valves: evolution and path to clinical reality. Expert Rev Med Devices 2012; 9(6): 577-94.

12. Colazzo, F., Sarathchandra, P., Smolenski, R.T., Chester. A.H., Tseng, Y.T., Czernuszka, J.T., Yacoub, M.H., Taylor, P.M. Extracellular matrix production by adipose-derived stem cells: implications for heart valve tissue engineering. Biomaterials 2011; 32(1):119-27.

13. Crapo, P.M., Gilbert, T.W., Badylak, S.F. An overview of tissue and whole organ decellularization processes. Biomaterials 2011; 32(12) :3233-43.

14. Dijkman, P.E., Driessen-Mol, A., Frese, L., Hoerstrup, S.P., Baaijens, F.P. Decellularized homologous tissue engineered heart valves as off-the-shelf alternatives to xeno- and homografts. Biomaterials 2012; 33(18): 4545-54.

15. Dingal, P.C., Discher, D.E. Systems mechanobiology: tension-inhibited protein turnover is sufficient to physically control gene circuits. Biophys J. 2014; 107(11): 2734-43.

16. Duan, B., Kapetanovic, E., Hockaday, L.A., Butcher, J.T. Three-dimensional printed trileaflet valve conduits using biological hydrogels and human valve interstitial cells. Acta Biomater 2014; 10(5): 1836-46.

17. Engelmayr Jr., G.C., Sacks, M.S. Prediction of extracellular matrix stiffness in engineered heart valve tissues based on nonwoven scaffolds. Bimech Model Mechanobiol. 2008; 7(4): 309-21.

18. Fallahiarezoudar, E., Ahmadipourroudposht, M., Idris, A., Mohd Yusof, N. A review of: Application of synthetic scaffold in tissue engineering heart valves. Mater Sci Eng C Mater Biol Appl. 2015; 48: 556-65.

19. Fan, R., Bayoumi, A.S., Chen, P., Hobson, C.M., Wagner, W.R., Mayer, J.E. Jr., Sacks, M.S. Optimal elastomeric scaffold leaflet shape for pulmonary heart valve leaflet replacement. J Biomech 2013; 46(4): 662-69.

20. Filova, E., Straka, F., Mirejovsky, T., Masin, J., Bacakova, L. Tissue-engineered heart valves. Physiol Res 2009; 58: S141-158.

21. Flameng, W., De Visscher, G., Mesure, L., Hermans, H., Jashari, R., Meuris, B. Coating with fibronectin and stromal cell- derived factor-1a of decellularized homografts used for right ventricular outflow tract reconstruction eliminates immune response - related degeneration. J Thorac Cardiovasc Surg 2014; 147(4): 1398-1404.

22. Flanagan, T.C., Cornelissen, C., Koch, S., Tschoeke, B., Sachweh, J.S., Schmitz-Rode, T., Jockenhoevel, S. The in vitro development of autologous fibrin-based tissue-engineered heart valves through optimised dynamic conditioning. Biomaterials 2007; 28(23): 3388-97.

23. Garcia, D., Kadem, L. What do you mean by aortic valve area: geometric orifice area, effective orifice area, or gorlin area? J Heart Valve Dis. 2006; 15(5): 601-8.

24. Garcia, D., Pibarot, P., Landry, C., Allard, A., Chayer, B., Dumesnil, J.G., Durand, L.G. Estimation of aortic valve effective orifice area by Doppler echocardiography: effects of valve inflow shape and flow rate. J Am Soc Echocardiogr. 2004; 17(7): 756-65.

25. Ghanbari, H., Viatge, H., Kidane, A.G., Burriesci, G., Tavakoli, M., Seifalian, A.M. Polymeric heart valves: new materials, emerging hopes. Trends Biotechnol. 2009; 27(6): 359-67.

26. Gould, R.A., Chin, K., Santisakultarm, T.P., Dropkin, A., Richards, J.M., Schaffer, C.B., Butcher, J.T. Cyclic strain anisotropy regulates valvular interstitial cell phenotype and tissue remodeling in three-dimensional culture. Acta Biomater. 2012; 8(5): 1710-9.

27. Hockaday, L.A., Kang, K.H., Colangelo, N.W., Cheung, P.Y., Duan, B., Malone, E., Wu, J., Girardi, L.N., Bonassar, L.J., Lipson, H., Chu, C.C., Butcher, J.T. Rapid 3D printing of anatomically accurate and mechanically heterogeneous aortic valve hydrogel scaffolds. Biofabrication. 2012; 4(3): 035005.

28. Hoffman, L.M., Jensen, C.C., Chaturvedi, A., Yoshigi, M., Beckerle, M.C. Stretch-induced actin remodeling requires targeting of zyxin to stress fibers and recruitment of actin regulators. Mol Biol Cell. 2012; 23(10):1846-59.

29. Holle, A.W., Engler, A.J. More than a feeling: discovering, understanding, and influencing mechanosensing pathways. Curr Opin Biotechnol. 2011; 22(5): 648-54.

30. Hristov, M., Erl, W., Weber, P.C. Endothelial progenitor cells: mobilization, differentiation, and homing. Arterioscler Thromb Vasc Biol. 2003; 23(7): 1185-9.

31. Chester, A.H., El-Hamamsy, I., Butcher, J.T., Latif, N., Bertazzo, S., Yacoub, M.H. The living aortic valve: From molecules to function. Glob Cardiol Sci Pract. 2014, 29(1): 52-77.

32. Chester, A.H., Taylor, P.M. Molecular and functional characteristics of heart-valve interstitial cells. Philos Trans R Soc Lond B Biol Sci. 2007; 362(1484): 1437- 43.

33. Cheung, D.Y., Duan, B., Butcher, J.T. Current progress in tissue engineering of heart valves: multiscale problems, multiscale solutions. Expert Opin Biol Ther. 2015; 15(8): 1155-72.

34. Imanishi, Y., Lodowski, K.H., Koutalos, Y. Two-photon microscopy: shedding light on the chemistry of vision. Biochemistry 2007; 46: 9674–84.

35. Jatene, M.B., Monteiro, R., Guimaraes, M.H., Veronezi, S.CH., Koike, M.K., Jatene, F.B., Jatene, A.D. Aortic valve assessment. Anatomical study of 100 healthy human hearts. Arq Bras Cardiol. 1999; 73(1): 75-86.

36. Jiang, G., Huang, A.H., Cai, Y., Tanase, M., Sheetz, M.P. Rigidity sensing at the leading edge through alphavbeta3 integrins and RPTPalpha. Biophys J. 2006; 90(5): 1804-9.

37. Jiao. T., Clifton, R.J., Converse, G.L., Hopkins, R.A. Measurements of the effects of decellularization on viscoelastic properties of tissues in ovine, baboon, and human heart valves. Tissue Eng Part A 2012; 18(3-4): 423-431.

38. Kalejs, M., Stradins, P., Lacis, R., Ozolanta, I., Pavars, J., Kasyanov, V. St Jude Epic heart valve bioprostheses versus native human and porcine aortic valves - comparison of mechanical properties. Interact Cardiovasc Thorac Surg. 2009; 8(5): 553-6.

39. Kallio, M., Pihkala, J., Sairanen, H., Mattila, I. Long-term results of the Ross procedure in a population-based follow-up. Eur J Cardiothorac Surg. 2015; 47(5): e164-70.

40. Kasimir, M.T., Rieder, E., Seebacher, G., Nigisch, A., Dekan, B., Wolne,r E., Weigel, G., Simon, P. Decellularization does not eliminate thrombogenicity and inflammatory stimulation in tissue-engineered porcine heart valves. J Heart Valve Dis. 2006; 15(2): 278-86.

41. Kim, H., Nakamura, F., Lee, W., Hong, C., Pérez-Sala, D., McCulloch, C.A. Regulation of cell adhesion to collagen via beta1 integrins is dependent on interactions of filamin A with vimentin and protein kinase C epsilon. Exp Cell Res. 2010; 316(11): 1829-44.

42. Kishimoto, S., Takewa, Y., Nakayama, Y., Date, K., Sumikura, H., Moriwaki, T., Nishimura, M., Tatsumi, E. Sutureless aortic valve replacement using a novel autologous tissue heart valve with stent (stent biovalve): proof of concept. J Artif Organs. 2015; 18(2): 185-90.

43. Knight, R.L., Wilcox, H.E., Korossis, S.A., Fisher, J., Ingham, E. The use of acellular matrices for the tissue engineering of cardiac valves. Proc Inst Mech Eng H. 2008; 222(1): 129-43.

44. Kocer, A. Mechanisms of mechanosensing-mechanosensitive channels, function and re-engineering. Curr Opin Chem Biol. 2015; 29: 120-7.

45. Ku, C.H., Johnson, P.H., Batten, P., Sarathchandra, P., Chambers, R.C., Taylor, P.M., Yacoub, M.H., Chester, A.H. Collagen synthesis by mesenchymal stem cells and aortic valve interstitial cells in response to mechanical stretch. Cardiovasc Res 2006; 71(3): 548-56.

46. Kunzelman, K.S., Cochran, R.P., Murphree, S.S., Ring, W.S., Verrier, E.D., Eberhart, R.C. Differential collagen distribution in the mitral valve and its influence on biomechanical behaviour. J Heart Valve Dis. 1993; 2(2): 236-44.

47. Kurpinski, K., Chu, J., Hashi, C., Li, S. Anisotropic mechanosensing by mesenchymal stem cells. Proc Natl Acad Sci U S A. 2006; 103(44): 16095-100.

48. Latif, N., Sarathchandra, P., Taylor, P.M., Antoniw, J., Brand, N., Yacoub, M.H. Characterization of molecules mediating cell-cell communication in human cardiac valve interstitial cells. Cell Biochem Biophys. 2006; 45(3): 255-64.

49. Lee, M., Wu, B.M. Recent advances in 3D printing of tissue engineering scaffolds. Method Mol Biol. 2012; 868: 257-67.

50. Li, S., Henry, J.J. Nontrombogenic approaches to cardiovascular bioengineering. Annu Rev Biomed Eng. 2011, 13: 451-75.

51. Lincoln, J., Lange, A.W., Yutzey, K.E. Hearts and bones: shared regulatory mechanisms in heart valve, cartilage, tendon, and bone development.Dev Biol. 2006; 294(2): 292-302.

52. Liu, A.C., Joag, V.R., Gotlieb, A.I. The emerging role of valve interstitial cell phenotypes in regulating heart valve pathobiology. Am J Pathol. 2007; 171(5) :1407-18.

53. Martin, C., Sun, W. Biomechanical characterization of aortic valve tissue in humans and common animal models. J Biomed Mater Res A. 2012; 100(6): 1591-99.

54. Masoumi, N., Larson, B.L., Annabi, N., Kharaziha, M., Zamanian, B., Shapero, K.S., Cubberley, A.T., Camci-Unal, G., Manning, K.B., Mayer, J.E. Jr., Khademhosseini, A. Electrospun PGS:PCL microfibers align human valvular interstitial cells and provide tunable scaffold anisotropy. Adv Healthc Mater. 2014; 3(6): 929-39.

55. Mitra, S.K., Hanson, D.A., Schlaepfer, D.D. Focal adhesion kinase: in command and control of cell motility. Nat Rev Mol Cell Biol. 2005; 6(1): 56-68.

56. Mol, A., Rutten, M.C., Driessen, N.J., Bouten, C.V., Zünd, G., Baaijens, F.P., Hoerstrup, S.P. Autologous human tissue-engineered heart valves: prospects for systemic application. Circulation. 2006; 114(1 Suppl): I152-8.

57. Neidert, M.R., Tranquillo, R.T. Tissue-engineered valves with commissural alignment. Tissue Eng. 2006; 12(4): 891-903.

58. Neumann, A., Sarikouch, S., Breymann, T., Cebotari, S., Boethig, D., Horke, A., Beerbaum, P., Westhoff-Bleck, M., Bertram, H., Ono, M., Tudorache, I., Haverich, A., Beutel, G. Early systemic cellular immune response in children and young adults receiving decellularized fresh allografts for pulmonary valve replacement. Tissue Eng Part A. 2014; 20(5-6): 1003-11.

59. Nieves, B., Jones, C.W., Ward, R., Ohta, Y., Reverte, C.G., LaFlamme, S.E. The NPIY motif in the integrin beta1 tail dictates the requirement for talin-1 in outside-in signaling J Cell Sci. 2010; 123(Pt 8): 1216-26.

60. Paniagua Gutierrez, J.R., Berry, H., Korossis, S., Mirsadraee, S., Lopes, S.V., da Costa, F., Kearney, J., Watterson, K., Fisher, J., Ingham, E. Regenerative potential of low-

concentration SDS-decellularized porcine aortic valved conduits in vivo. Tissue Eng Part A. 2015; 21(1-2): 332-42.

61. Plant, A.L., Bhadriraju, K., Spurlin, T.A., Elliott, J.T. Cell response to matrix mechanics: focus on collagen. Biochim Biophys Acta. 2009; 1793(5): 893-902.

62. Popelová, J., Benešová, M., Brtko, M., Černý, Š., Krupička, J., Čerbák, R., Dominik, J., Marek, T.. Doporučené postupy pro diagnostiku a léčbu chlopenních vad v dospělosti.. Cor et Vasa 2007; 49 (7-8): K195-K234.

63. Reiser, K., McCormick, R.J., Rucker, R.B. Enzymatic and nonenzymatic cross-linking of collagen and elastin. FASEB J 1992; 6(7): 2439-49.

64. Sant, S., Iyer, D., Gaharwar, A.K., Patel, A., Khademhosseini, A. Effect of biodegradation and de novo matrix synthesis on the mechanical properties of valvular interstitial cell-seeded polyglycerol sebacate-polycaprolactone scaffolds. Acta Biomater. 2013; 9(4): 5963-73.

65. Sawada, Y., Tamada, M., Dubin-Thaler, B.J., Cherniavskaya, O., Sakai, R., Tanaka, S., Sheetz, M.P. Force sensing by mechanical extension of the Src family kinase substrate p130Cas. Cell. 2006; 127(5): 1015-26.

66. Sbrana, F., Sassoli, C., Meacci, E., Nosi, D., Squecco, R., Paternostro, F., Tiribilli, B., Zecchi-Orlandini, S., Francini, F., Formigli, L. Role for stress fiber contraction in surface tension development and stretch-activated channel regulation in C2C12 myoblasts. Am J Physiol Cell Physiol. 2008; 295(1): C160-72.

67. Schneider, C.A., Rasband, W.S., Eliceiri K.W. "NIH Image to ImageJ: 25 years of image analysis". Nat Methods 2012, 9(7): 671-75.

68. Schoen, F.J. Evolving concepts of cardiac valve dynamics: the continuum of development, functional structure, pathobiology, and tissue engineering. Circulation 2008; 118(18): 1864-80.

69. Sodian, R., Loebe, M., Hein, A., Martin, D.P., Hoerstrup, S.P., Potapov, E.V., Hausmann, H., Lueth, T., Hetze,r R. Application of stereolithography for scaffold fabrication for tissue engineered heart valves. ASAIO J. 2002; 48(1): 12-16.

70. Somers, P., Roosens, A., De Somer, F., Cornelissen, M., Van Nooten, G. Non-Cytotoxic Crosslinkers for Heart Valve Tissue Engineering. J Heart Valve Dis. 2015; 24(1): 92-100.

71. Stella, J.A., Sacks, M.S. On the biaxial mechanical properties of the layers of the aortic valve leaflet. J Biomech Eng. 2007; 129(5): 757-66.

72. Stradins, P., Lacis, R., Ozolanta, I., Purina, B., Ose, V., Feldmane, L., Kasyanov, V. Comparison of biomechanical and structural properties between human aortic and pulmonary valve. Eur J Cardiothorac Surg. 2004; 26(3): 634-9.

73. Straka, F., Masin, J., Schornik, D., Pirk, J. A method of constructing a pericardial heart valve replacement, a pericardial heart valve replacement constructed according to this method, and a device for the in vitro conditioning and modification of tissue of autologous pericardium for the construction of a pericardial heart valve replacement. 2013; WIPO/PCT. International Publication Number: WO 2013/182171 A1.

74. Sung, H.W., Chang, Y., Chiu, C.T., Chen, C.N., Liang, H.C. Crosslinking characteristics and mechanical properties of a bovine pericardium fixed with a naturally occurring crosslinking agent. J Biomed Mater Res. 1999; 47(2): 116-26.

75. Takagi, K., Fukunaga, S., Nishi, A., Shojima, T., Yoshikawa, K., Hori, H., Akashi, H., Aoyagi, S. In vivo recellularization of plain decellularized xenografts with specific cell characterization in the systemic circulation: histological nad immunohistochemical study. Artif Organs. 2006; 30(4): 233-41.

76. Tam, H., Zhang, W., Feaver, K.R., Parchment, N., Sacks, M.S., Vyavahare, N. A novel crosslinking method for improved tear resistance and biocompatibility of tissue based biomaterials. Biomaterials. 2015; 66: 83-91.

77. Taylor, P.M., Allen, S.P., Yacoub, M.H. Phenotypic and functional characterization of

interstitial cells from human heart valves, pericardium and skin. J Heart Valve Dis. 2000; 9(1): 150-8.

78. Tseng, H., Grande-Allen, K.J. Elastic fibers in the aortic valve spongiosa: a fresh perspective on its structure and role in overall tissue function. Acta Biomater. 2011; 7(5): 2101-8.

79. Vahanian, A., Alfieri, O., Andreotti, F., Antunes, M.J., Barón-Esquivias, G., Baumgartner, H., Borger, M.A., Carrel, T.P., De Bonis, M., Evangelista, A., Falk, V., Iung, B., Lancellotti, P., Pierard, L., Price, S., Schäfers, H.J., Schuler, G., Stepinska, J., Swedberg, K., Takkenberg, J., Von Oppell, U.O., Windecker, S., Zamorano, J.L., Zembala, M. Guidelines on the management of valvular heart disease (version 2012). Eur Heart J. 2012; 33(19): 2451-96.

80. Van Loosdregt, I.A., Argento, G., Driessen-Mol, A., Oomens, C.W., Baaijens, F.P. Cell-mediated retraction versus hemodynamic loading - A delicate balance in tissue-engineered heart valves. J Biomech. 2014; 47(9): 2064-69.

81. Voges, I., Bräsen, J.H., Entenmann, A., Scheid, M., Scheewe, J., Fischer, G., Hart, C., Andrade, A., Pham, H.M., Kramer, H.H., Rickers, C. Adverse results of a decellularized tissue-engineered pulmonary valve in humans assessed with magnetic resonance imaging. Eur J Cardiothorac Surg. 2013; 44(4): e272-79.

82. Vojáček, J., Kettner, J., a kol. Klinická kardiologie. Hradec Králové: Nucleus HK, 2009, Dysfunkce chlopenních protéz, p.536-541.

83. Wagenseil, J.E., Mecham, R.P. New insights into elastic fiber assembly. Birth Defects Res C Embryo Today. 2007; 81(4): 229-40.

84. Wang, N., Tytell, J.D., Ingber, D.E. Mechanotransduction at a distance: mechanically coupling the extracellular matrix with the nucleus. Nat Rev Mol Cell Biol. 2009; 10(1): 75-82.

85. Welke, K.F., Wu, Y., Grunkemeier, G.L., Ahmad, A., Starr, A. Long-term results after Carpentier-Edwards pericardial aortic valve implantation, with attention to the impact of age. Heart Surg Forum. 2011; 14(3): E160-5.

86. Yacoub, M.H., Takkenberg, J.J. Will heart valve tissue engineering change the world? Nat Clin Pract Cardiovasc Med. 2005; 2(2): 60-1.

87. Yang, C.X., Chen, H.Q., Chen, C., Yu, W.P., Zhang, W.C., Peng, Y.J., He, W.Q., Wei, D.M., Gao, X., Zhu, M.S. Microfilament-binding properties of N-terminal extension of the isoform of smooth muscle long myosin light chain kinase. Cell Res. 2006; 16(4): 367-76.

88. Yap, C.H., Saikrishnan, N., Yoganathan, A.P. Experimental measurement of dynamic fluid shear stress on the ventricular surface of the aortic valve leaflet. Biomech Model Mechanobiol. 2012; 11(1-2): 231-44.

89. Zaidel-Bar, R., Geiger, B. The switchable integrin adhesome. J Cell Sci. 2010; 123(Pt 9): 1385-8.

90. Zhou, J., Fritze, O., Schleicher, M., Wendel, H.P., Schenke-Layland, K., Harasztosi C., Hu, S., Stock, U.A. Impact of heart valve decellularization on 3-D ultrastructure, immunogenicity and thrombogenicity. Biomaterials. 2010; 31(9): 2549-54.

91. Zioupos, P., Barbenel, J.C. Mechanics of native bovine pericardium. I. The multiangular behaviour of strength and stiffness of the tissue. Biomaterials. 1994; 15(5): 366-73.

92. Zoghbi, W.A., Chambers, J.B., Dumesnil, J.G., Foster, E., Gottdiener, J.S., Grayburn, P.A., Khandheria, B.K., Levine, R.A., Marx, G.R., Miller, F.A. Jr., Nakatani, S., Quiñones, M.A., Rakowski, H., Rodriguez, L.L., Swaminathan, M., Waggoner, A.D., Weissman, N.J., Zabalgoiti, M.; American Society of Echocardiography's Guidelines and Standards Committee; Task Force on Prosthetic Valves; American College of Cardiology Cardiovascular Imaging Committee; Cardiac Imaging Committee of the American Heart Association; European Association of Echocardiography; European Society of Cardiology; Japanese Society of Echocardiography; Canadian Society of

Echocardiography; American College of Cardiology Foundation; American Heart Association; European Association of Echocardiography; European Society of Cardiology; Japanese Society of Echocardiography; Canadian Society of Echocardiography. Recommendations for evaluation of prosthetic valves with echocardiography and doppler ultrasound: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Task Force on Prosthetic Valves, developed in conjunction with the American College of Cardiology Cardiovascular Imaging Committee, Cardiac Imaging Committee of the American Heart Association, the European Association of Echocardiography, a registered branch of the European Society of Cardiology, the Japanese Society of Echocardiography and the Canadian Society of Echocardiography, endorsed by the American College of Cardiology Foundation, American Heart Association, European Association of Echocardiography, a registered branch of the European Society of Cardiology, the Japanese Society of Echocardiography, and the Canadian Society of Echocardiography. J Am Soc Echocardiogr. 2009; 22(9): 975-1014.

12. PUBLICATIONS:

12.1 Publications with impact factor

12.1.1 First author:

STRAKA, F., PIRK, J., PINĎÁK, M., MAREK, T., SCHORNÍK, D., ČIHÁK, R., SKIBOVÁ, J. A pilot study of systolic dyssynchrony index by real time three-dimensional echocardiography and Doppler tissue imaging parameters predicting the hemodynamic response to biventricular pacing in the early postoperative period after cardiac surgery. *Echocardiography*. 2012, **29**(7), 827-839. ISSN 0742-2822. **IF 1,261.**

STRAKA, F., SCHORNÍK, D. Letters to the editor. *Pacing and clinical electrophysiology*. 2011, **34**(12), 1705-1706. ISSN 0147-8389. **IF 1,351.**

STRAKA, F., PIRK, J., PINĎÁK, M., SKALSKÝ, I., VANČURA, V., ČIHÁK, R., MAREK, T., LUPÍNEK, P., SCHORNÍK, D., MAŠÍN, J., ZEMAN, M., ŠKROBÁKOVÁ, J., DORAZILOVÁ, Z., SKIBOVÁ, J. Biventricular pacing in the early postoperative period after cardiac surgery. *Physiological research*. 2011, **60**(6), 877-885. ISSN 0862-8408. **IF 1,555.**

STRAKA, F., PIRK, J., PINĎÁK, M., SKALSKÝ, I., VANČURA, V., ČIHÁK, R., MAREK, T., LUPÍNEK, P., MAŠÍN, J., SCHORNÍK, D., ZEMAN, M., ŠKROBÁKOVÁ, J., DORAZILOVÁ, Z., SKIBOVÁ, J. The hemodynamic effect of right ventricle (RV), RT3DE targeted left Ventricle (LV) and Biventricular (BIV) pacing in the early postoperative period after cardiac surgery. *Pacing and clinical electrophysiology*. 2011, **34**(10), 1231-1240. ISSN 0147-8389. **IF 1,351.**

STRAKA, F., SCHORNÍK, D., MAŠÍN, J., FILOVÁ, E., MIŘEJOVSKÝ, T., CHLUP, H., HORNÝ, L., VESELÝ, J., SKIBOVÁ J., PIRK, J., BAČÁKOVÁ, L. A new approach to heart valve tissue engineering based on modifying autologous human pericardium by mechanotransduction. This paper is currently being edited by the journal: Journal of Tissue Engineering and Regenerative Medicine. Impact Factor: 5,199.

STRAKA, F., SCHORNÍK, D., MAŠÍN, J., FILOVÁ, E., BURDÍKOVÁ, Z., MIŘEJOVSKÝ, T., ŠVINDRYCH, Z., CHLUP, H., HORNÝ, L., VESELÝ, J., SKIBOVÁ J., PIRK, J., BAČÁKOVÁ, L. The cellular and extracellular matrix structure of human pericardium, a

promising scaffold for autologous heart valve tissue engineering. This paper is currently being edited by the journal: Journal of Biomedical Materials Research Part A. Impact Factor: 3,369.

12.1.2 Co-author:

FILOVÁ, E., STRAKA, F., MIŘEJOVSKÝ, T., MAŠÍN, J., BAČÁKOVÁ, L. Tissue-engineered heart valves. *Physiological research*. 2009, **58**(suppl. 2), S141-S158. ISSN 0862-8408. **IF 1,430.**

12.2 Publications without impact factor

12.2.1 First author:

STRAKA, F., MÁLEK, I., STANĚK, V., OUHRABKOVÁ, R., GEBAUEROVÁ, M., URBANOVÁ, D., VRUBEL, J., PIRK, J., ŽELÍZKO, M., LÁNSKÁ, V. Koronární nemoc u pacientů po transplantaci srdce. *Cor et Vasa*. 1993, **35**(6), 267-275. ISSN 0010-8650.

12.2.2 Co-author:

MAREK, T., DORAZILOVÁ, Z., STRAKA, F., NETUKA, I., PIRK, J., SKALSKÝ, I. Role echokardiografie v péči o pacienty s mechanickou srdeční podporou HeartMate II. *Cor et Vasa*. 2011, **53**(1-2), 55-59. ISSN 0010-8650.

JONÁK, M., STRAKA, F., LÁNSKÁ, V., PAVEL, P., ROHN, V., SKALSKÝ, I., PIRK, J. Mid-term results with the Sorin Allcarbon mechanical prosthesis in the mitral position. *Cor et Vasa*. 2003, **45**(7-8), 351-356. ISSN 0010-8650.

KŘÍŽOVÁ, B., STANĚK, V., GEBAUEROVÁ, M., STRAKA, F. Kardiální manifestace syndromu karcinoidu. *Cor et Vasa*. 2003, **45**(1), 42-45. ISSN 0010-8650.

PIRK, J., WIENDL, M., ČERVENKA, V., STANĚK, V., KASALICKÝ, J., MAŠÍN, J., STRAKA, F. První zkušenosti s transmyokardiální laserovou revaskularizací s použitím CO2 laseru LUXCOR 500/800. *Cor et Vasa*. 2002, **44**(7-8), 353-355. ISSN 0010-8650.

PIRK, J., ČERNÝ, Š., STRAKA, F., KOVÁČ, J. První zkušenosti s aortokoronárními rekonstrukcemi bez mimotělního oběhu. LAST operace. *Cor et Vasa*. 1998, **40**(2), 68-71. ISSN 0010-8650.

HEJNAL, J., MÁLEK, I., FIRT, P., PIRK, J., KOČANDRLE, V., STRAKA, F. Naše zkušenosti s transplantacemi srdce. Věnováno k 65. narozeninám prof. MUDr. P. Firta, DrSc. *Rozhledy v chirurgii*. 1992, **71**(7), 350-357. ISSN 0035-9951.

12.3 Abstracts

STRAKA, F., SCHORNÍK, D., MAŠÍN, J., FILOVÁ, E., BURDÍKOVÁ, Z., MIŘEJOVSKÝ, T., CHLUP, H., HORNÝ, L., VESELÝ, J., PIRK, J., BAČÁKOVÁ, L. A method of preparing a living autologous pericardial tissue heart valve replacement that is modified and stabilized using in vitro three-dimensional mechanotransduction. *Valves in the Heart of the Big Apple VIII: Evaluation and Management of Valvular Heart Diseases 2014 : Fifth Annual Joint Scientific Session of the Heart Valve Society of*. New York, US, 08.05.2014 - 10.05.2014. Zorg. Heart Valve Society of America. *Cardiology*. 2014, **128**(2), 166-166. ISSN 0008-6312.

FILOVÁ, E., BURDÍKOVÁ, Z., STAŇKOVÁ, L., HADRABA, D., CHLUP, H., GULTOVÁ, E., VESELÝ, J., HORNÝ, L., ŠVINDRYCH, Z., STRAKA, F., SCHORNÍK, D., ŽITNÝ, R., PIRK, J., BAČÁKOVÁ, L. Collagen structures in pericardium and aortic heart valves and their significance for tissue engineering. *4th IEE International Conference on e-Health and Bioengineering EHB 2013*. Iasi, RO. 21.11.2013 - 23.11.2013. Zorg. Grigire T. Popa University of Medicine and Pharmacy. In: *E-Health and Bioengineering Conference (EHB), 2013*. New York: Institute of Electrical and Electronics Engineers, 2013, s. 10.1109/EHB.2013.6707382. ISBN 978-1-4799-2373-1.

STRAKA, F., SCHORNÍK, D., MAŠÍN, J., FILOVÁ, E., BURDÍKOVA, Z., MIŘEJOVSKÝ, T., CHLUP, H., HORNÝ, L., VESELÝ, J., PIRK, J., BAČÁKOVÁ, L. The influence of mechanotransduction on the extracellular matrix structure of autologous human pericardium: Significance for heart valve tissue engineering. Poster. *World Conference on Regenerative Medicine*. Leipzig, DE, 23.10.2013 - 25.10.2013. Zorg. Fraunhofer Institute for Cell Therapy and Immunology. *Regenerative medicine*. 2013, **8**(6s), 255, no. abstr. PP-097. ISSN 1746-0751.

STRAKA, F., PIRK, J., PINĎÁK, M., VANČURA, V., ČIHÁK, R., MAREK, T., MAŠÍN, J., ZEMAN, M., ŠKROBÁKOVÁ, J., SKIBOVÁ, J. Cardiac resynchronization therapy (CRT) in

the early postoperative period after cardiac surgery. Poster. *ESC Congress 2013*. Amsterdam, NL, 31.08.2013 - 04.09.2013. Zorg. European Society of Cardiology. *European heart journal*. 2013, **34**(suppl. 1), 947. ISSN 0195-668X.

STRAKA, F., PIRK, J., PINĎÁK, M., SKALSKÝ, I., ČIHÁK, R., MAREK, T., MAŠÍN, J., ZEMAN, M., SKIBOVÁ, J. The hemodynamic effect of right ventricle, real-time three-dimensional echocardiography targeted left ventricle and biventricular pacing in the early postoperative period after cardiac surgery. *Congress of the European Society of Cardiology (ESC Congress)*. München, 25.08.2012 - 29.08.2012. *European heart journal*. 2012, **33**(suppl. 1), 497. ISSN 0195-668X.

STRAKA, F., SCHORNÍK, D., MAŠÍN, J., FILOVÁ, E., BURDÍKOVÁ, Z., MIŘEJOVSKÝ, T., HONSOVÁ, E., CHLUP, H., PIRK, J., BAČÁKOVÁ, L. Modification of human pericardium based on dynamic loading, a new approach for heart valve tissue engineering. *3rd TERMIS World Congress*. Vienna, AT, 05.09.2012 - 08.09.2012. Zorg. Tissue Engineering and Regenerative Medicine International Society. *Journal of tissue engineering and regenerative medicine*. 2012, **6**(s1), 111-111. ISSN 1932-6254.e-ISSN 1932-7005.

FILOVÁ, E., STRAKA, F., LODEREROVÁ, A., HONSOVÁ, E., MAŠÍN, J., CHLUP, H., HORNÝ, L., VESELÝ, J., KRONEK, J., GULTOVÁ, E., SCHORNÍK, D., PIRK, J., BAČÁKOVÁ, L. Aortic heart valve prepared from human pericardium under static and dynamic conditions. *TERMIS-EU 2011 Annual Meeting*. Granada, ES, 07.06.2011 - 10.06.2011. Zorg. Tissue Engineering and Regenerative Medicine International Society. *Histology and histopathology*. 2011, **26**(suppl. 1), 352, abstr. no. 42.O5. ISSN 0213-3911.

STRAKA, F., FILOVÁ, E., MAŠÍN, J., TSENG, Y. T., SCHORNÍK, D., HONSOVÁ, E., LODEREROVÁ, A., BAČÁKOVÁ, L., PIRK, J. Collagen I scaffold and valve interstitial cells a promising approach for heart valves tissue engineering. *2nd international conference on Cardiology (Cardiology '11)*. Prague, CZ. 26.09.2011 - 28.09.2011. Zorg. North Atlantic University Union. In: *Proceedings of the world medical conference*. Stevens Point: WSEAS Press, 2011, s. 21. ISBN 978-1-61804-036-7.

STRAKA, F., FILOVÁ, E., HONSOVÁ, E., LODEREROVÁ, A., MAŠÍN, J., SCHORNÍK, D., CHLUP, H., HORNÝ, L., VESELÝ, J., KRONEK, J., GULTOVÁ, E., PIRK, J.,

BAČÁKOVÁ, L. Human pericardium, a promising scaffold for heart valve tissue engeneering. *BIT's 4th Annual World Congress of Regenerative Medicine & Stem Cell 2011*. Beijing, CN. 11.11.2011 - 13.11.2011. Zorg. BIT Life Sciences. In: *BIT's 4th annual world congress of regenerative medicine & stem cell 2011*. B.m.n., 2011, s. 222.

STRAKA, F., FILOVÁ, E., MAŠÍN, J., TSENG, Y.T., SCHORNÍK, D., HONSOVÁ, E., LODERÉROVÁ, A., BAČÁKOVÁ, L., PIRK, J. Collagen I scaffold seeded with valve interstitial cells, a promising approach for heart valve tissue engineering. *TERMIS-EU 2011 Annual Meeting*. Granada, ES, 07.06.2011 - 10.06.2011. Zorg. Tissue Engineering and Regenerative Medicine International Society. *Histology and histopathology*. 2011, **26**(suppl. 1), 351-352, abstr. no. 42.O4. ISSN 0213-3911.

FILOVÁ, E., STRAKA, F., BAČÁKOVÁ, L., SVOBODOVÁ, J., JUKLÍČKOVÁ, M., RUBÁČKOVÁ, K. Adhesion and growth of valve interstitial cells on oriented and randomly oriented degradable nanofibres. *Biologic Scaffolds for Regenerative Medicine. 6th Symposium*. Napa Valley, California, US. 25.04.2010 - 27.04.2010. Zorg. University of Pittsburgh. In: *Biologic Scaffolds for Regenerative Medicine. 6th Symposium*. Pittsburgh: McGowan Institute for Regenerative Medicine University of Pittsburgh, 2010, s. 55.

STRAKA, F., PIRK, J., VANČURA, V., ČIHÁK, R., PINĎÁK, M., MAREK, T., LUPÍNEK, P., ZEMAN, M., ŠKROBÁKOVÁ, J., SKIBOVÁ, J. Význym RT3DE v cílené resychronizační léčbě v časném období po kardiochirurgických operacích. *18. výroční sjezd České kardiologické společnosti*. Brno, CZ. 16.05.2010 - 19.05.2010. Zorg. Česká kardiologická společnost. In: *18. výroční sjezd České kardiologické společnosti [on-line]*. Praha: Česká kardiologická společnost, 2010, s. 1.

STRAKA, F., FILOVÁ, E., MAŠÍN, J., TSENG, Y.T., BAČÁKOVÁ, L., HONSOVÁ, E. Novel porous collagen scaffold for heart valve tissue engineering under static and dynamic cell culture condidions. *Biologic Scaffolds for Regenerative Medicine. 6th Symposium*. Napa Valley, California, US. 25.04.2010 - 27.04.2010. Zorg. University of Pittsburgh. In: *Biologic Scaffolds for Regenerative Medicine. 6th Symposium*. Pittsburgh: McGowan Institute for Regenerative Medicine University of Pittsburgh, 2010, s. 18.

STRAKA, F. Valve interstitial cells growth and differentation upon porous collagen / scaffold under static and dynamic culture conditions for heart valve tissue engineering. *5th World Congress on Preventive and Regenerative Medicine*. Hannover, DE. 05.10.2010 - 07.10.2010. In: *5th World Congress on Preventive and Regenerative Medicine*. B.m.n., 2010, s. 9.

FILOVÁ, E., STRAKA, F., BAČÁKOVÁ, L., MUNZAROVÁ, M., SVOBODOVÁ, J. Growth of valve interstitial cells on aligned and randomly oriented electrospun nanofibres. Poster. *5th Biennial Meeting of the SHVD*. Berlin, DE. 27.06.2009 - 30.06.2009. In: *5th Biennial Meeting 2009. Joint Meeting of the Society for Heart Valve Disease and Heart Valve Society of America. Abstracts*. Rodgau: Keller Congress Organisation, 2009, s. nestr., č. abstr. P155.

MAREK, T., ŽELÍZKO, M., JANEK, B., KOTULÁK, T., MALÝ, J., NETUKA, I., STRAKA, F. Perkutánní náhrada aortální chlopně - časné echokardiografické výsledky. *Echodny 2009*. Špindlerův Mlýn, CZ, 18.09.2009 - 19.09.2009. *Cor et Vasa*. 2009, **51**(9), 646. ISSN 0010-8650.

STRAKA, F., FILOVÁ, E., CHLUPÁČ, J., BAČÁKOVÁ, L., PIRK, J. Bilayer of vascular smooth muscle cells (SMC) and endothelial cells (EC) on a nanofibre mesh in a pulse bioreactor. Poster. *5th Biennial Meeting of the SHVD*. Berlin, DE. 27.06.2009 - 30.06.2009. In: *5th Biennial Meeting 2009. Joint Meeting of the Society for Heart Valve Disease and Heart Valve Society of America. Abstracts*. Rodgau: Keller Congress Organisation, 2009, s. nestr., č. abstr. P156.

STRAKA, F., PIRK, J., VANČURA, V., ČIHÁK, R., PINĎÁK, M., MAREK, T., LUPÍNEK, P., ZEMAN, M., ŠKROBÁKOVÁ, J., DORAZILOVÁ, Z., WURMOVÁ, Š., SKIBOVÁ, J. Parametry RT3DE v predikci respondence k CRT v časném období po kardiochirurgických operacích. *Echodny 2009*. Špindlerův Mlýn, CZ, 18.09.2009 - 19.09.2009. *Cor et Vasa*. 2009, **51**(9), 648. ISSN 0010-8650.

STRAKA, F., PIRK, J., VANČURA, V., ČIHÁK, R., PINĎÁK, M., MAREK, T., LUPÍNEK, P., ZEMAN, M., WURMOVÁ, Š., SCHORNÍK, D. The hemodynamic effect of right ventricle (RV), left ventricle (LV) and biventricular (BIV) pacing in the early postoperaive period after cardiac surgery. *Venice Arrhythmias 2009. 11th International Workshop on*

Cardiac Arrhythmias. Venice, IT, 04.10.2009 - 07.10.2009. *Journal of cardiovascular electrophysiology*. 2009, **20**(suppl. 1), S90, č. abstr. 8.14. ISSN 1045-3873.e-ISSN 1540-8167.

STRAKA, F., PIRK, J., VANČURA, V., ČIHÁK, R., PINĎÁK, M., MAREK, T., LUPÍNEK, P., ZEMAN, M., ŠKROBÁKOVÁ, J., DORAZILOVÁ, Z., WURMOVÁ, Š., SKIBOVÁ, J. RT3DE cílená resynchronizační léčba v časném období po kardiochirurgických operacích - srovnání hemodynamického efektu pravokomorové (RV), levokomorové (LV) a biventrikulární stimulace (BIV). *7. konference pracovní skupiny akutní kardiologie České kardiologické společnosti*. Karlovy Vary, CZ, 03.12.2009 - 05.12.2009. Zorg. Pracovní skupina akutní kardiologieČKS. *Intervenční a akutní kardiologie*. 2009, **8**(suppl. B), B14. ISSN 1213-807X.

MAREK, T., DORAZILOVÁ, Z., STRAKA, F., ŠKROBÁKOVÁ, J., PIRK, J., SKALSKÝ, I., NETUKA, I., MALÝ, J. Mechanická srdeční podpora heartmate II, echokardiografický obraz. *Echodny 2008*. Český Krumlov, CZ, 19.09.2008 - 20.09.2008. Zorg. Česká kardiologická společnost. *Cor et Vasa (Kardio)*. 2008, **50**(7-8), K135. ISSN 0010-8650.

VANČURA, V., WICHTERLE, D., LEFFLEROVÁ, K., BYTEŠNÍK, J., STRAKA, F., KAUTZNER, J. Ovlivňuje energie stimulace z pravé komory srdeční šíři komplexu QRS?. *16. výroční sjezd České kardiologické společnosti*. Brno, CZ. 24.05.2008 - 27.05.2008. Zorg. Česká kardiologická společnost. In: *16. výroční sjezd České kardiologické společnosti. Program a abstrakta*. Praha: Medical Tribune CZ, 2008, s. 71.

KRAUSOVÁ, R., KAUTZNER, J., MALÝ, J., PIRK, J., SKALSKÝ, I., STRAKA, F., ŠKROBÁKOVÁ, J. Atrial arrhythmias after surgical treatment of atrial fibrillation using radiofrequeny energy. *Europace 2007*. Lisbon, PT, 24.06.2007 - 27.06.2007. *Europace*. 2007, **9**(suppl. 3), iii41-iii42. ISSN 1099-5129.

LUPÍNEK, P., STRAKA, F., MAREK, T. Kvantifikace objemů a ejekční frakce levé komory trojrozměrnou echokardiografií: schůdnost, časová náročnost, reprodukcibilita. *Echodny 2007*. Olomouc, CZ, 20.09.2007 - 22.09.2007. *Cor et Vasa (Kardio)*. 2007, **49**(9), K 277. ISSN 0010-8650.

LUPÍNEK, P., STRAKA, F., MAREK, T. 3rozměrná echokardiografie v reálném čase - první zkušenosti. *Echodny 2006*. Darovanský Dvůr u Rokycan, CZ, 21.09.2006 - 23.09.2006. Zorg. Česká kardiologická společnost. *Cor et Vasa (Kardio)*. 2006, **48**(9), 173-178. ISSN 0010-8650.

KRAUSOVÁ, R., KAUTZNER, J., MALÝ, J., PIRK, J., STRAKA, F. Jaký rytmus mají nemocní po perioperační radiofrekvenční ablaci fibrilace síní?. *3. slovenské a české sympózium o arytmiách a kardiostimulácii*. Piešťany, SK, 23.02.2005 - 25.02.2005. Zorg. Pracovná skupina pre arytmie a kardiostimuláciu Slovenskej kardiologickej spoločnosti. *Kardiológia*. 2005, **14**(2), K/C 59. ISSN 1210-0048.

KRAUSOVÁ, R., KAUTZNER, J., MALÝ, J., PIRK, F., STRAKA, F., ŠKROBÁKOVÁ, J. What kind of rhythm have patients after radiofrequency maze procedure?. *Europace 2005*. Prague, CZ, 26.06.2005 - 29.06.2005. Zorg. European Heart Rhythm Association. *Europace [suppl.]*. 2005, **7**(suppl. 1), 218, č. abstr. 900. ISSN 1099-6044.

KRAUSOVÁ, R., MALÝ, J., KAUTZNER, J., PIRK, J., STRAKA, F., ŠKROBÁKOVÁ, J. Srdeční rytmus po perioperační radiofrekvenční ablaci fibrilace síní - dlouhodobé výsledky. *13.výroční sjezd České kardiologické společnosti*. Brno, CZ, 08.05.2005 - 11.05.2005. Zorg. Česká kardiologická společnost. *Cor et Vasa*. 2005, **47**(4, suppl.), 54. ISSN 0010-8650.

MARTINČA, T., PIRK, J., STRAKA, F., ŠKROBÁKOVÁ, J., MALÝ, J. Náhrada aortální chlopně biologickou protézou SJM Biocor - střednědobé výsledky. *13.výroční sjezd České kardiologické společnosti*. Brno, CZ, 08.05.2005 - 11.05.2005. Zorg. Česká kardiologická společnost. *Cor et Vasa*. 2005, **47**(4, suppl.), 67. ISSN 0010-8650.

PIRK, J., MARTINČA, T., STRAKA, F., JUŠTÍK, P., ŠKROBÁKOVÁ, J. An easy method to avoid the patient - valve mismatch. *23rd International Cardiovascular Surgical Symposium*. Zürs am Arlberg, AT. 26.02.2005 - 05.03.2005. In: *23rd International Cardiovascular Surgical Symposium. Abstracts*. B.m.: b.n., 2005, s. nestr.

SENTIVANOVÁ, L., HEŘMANOVÁ, J., STRAKA, F., PIRK, J., MAŠÍN, J., MARTINČA, T., BEŠÍK, J. Síňový defibrilátor - nový způsob léčby fibrilace a flutteru síní u nemocných po kardiochirurgických operacích. *13.výroční sjezd České kardiologické společnosti. Sesterská*

sekce. Brno, CZ, 08.05.2005 - 11.05.2005. Zorg. Česká kardiologická společnost. *Cor et Vasa*. 2005, **47**(4), 96. ISSN 0010-8650.

KRAUSOVÁ, R., KAUTZNER, J., PIRK, J., MALÝ, J., STRAKA, F. The treatment of atrial fibrillation using radiofrequency MAZE procedure - heart rhythm during follow up. *11th International Symposium on Progress in Clinical Pacing*. Rome, IT. 30.11.2004 - 03.12.2004. In: *Giornale Italiano di aritmologia e cardiostimulazione*. Torino: Centro Scientifico Editore, 2004, s. 10.

MALÝ, J., KRAUSOVÁ, R., KAUTZNER, J., STRAKA, F., ŠKROBÁKOVÁ, J., NETUKA, I., TĚŠÍNSKÝ, L., PIRK, J. Chirurgická léčba fibrilace síní v IKEM. *1.sjezd České společnosti kardiovaskulární chirurgie*. Brno, CZ. 11.11.2004 - 12.11.2004. Zorg. Česká společnost kardiovaskulární chirurgie. In: *1.sjezd České společnosti kardiovaskulární chirurgie. Sborník abstrakt*. B.m.: b.n., 2004, s. 58, č.abstr. 40.

MALÝ, J., KRAUSOVÁ, R., KAUTZNER, J., STRAKA, F., ŠKROBÁKOVÁ, J., NETUKA, I., TĚŠÍNSKÝ, L., PIRK, J. Chirugická léčba fibrilace síní v IKEM. *12.výroční sjezd České kardiologické společnosti*. Brno, 09.05.2004 - 12.05.2004. Zorg. Česká kardiologická společnost. *Cor et Vasa*. 2004, **46**(4, suppl.), 56. ISSN 0010-8650.

MARTINČA, T., PIRK, J., STRAKA, F., ŠKROBÁKOVÁ, J. Střednědobé výsledky nemocných po náhradě aortální chlopně biologickou protézou SJM Biocor. *1.sjezd České společnosti kardiovaskulární chirurgie*. Brno, CZ. 11.11.2004 - 12.11.2004. Zorg. Česká společnost kardiovaskulární chirurgie. In: *1.sjezd České společnosti kardiovaskulární chirurgie. Sborník abstrakt*. B.m.: b.n., 2004, s. 40, č.abstr. 22.

KRAUSOVÁ, R., KAUTZNER, J., MALÝ, J., PIRK, J., SKALSKÝ, I., STRAKA, F. The treatment of atrial fibrillation using MAZE like procedure- heart rhythm during follow up. *Europace 2003*. Paris, FR, 14.12.2003 - 17.12.2003. *Europace [suppl.]*. 2003, **4**(suppl.B), B 168. ISSN 1099-6044.

MALÝ, J., KAUTZNER, J., KRAUSOVÁ, R., BYTEŠNÍK, J., STRAKA, F., SKALSKÝ, I., ROHN, V., PAVEL, P., NETUKA, I., PIRK, J. Chirurgická léčba supraventrikulárních tachyarytmií v IKEM. *1.slovenské a české sympózium o arytmiách a kardiostimulácii.*

5.slovenské arytmologické a kardiostimulačné dni. 9.české sympozium o arytmiích a kardiostimulaci. Stará Lesná, 06.02.2002 - 08.02.2003. *Kardiológia*. 2003, **12**(1), K/C 8A. ISSN 1210-0048.

PIRK, J., MALÝ, J., KAUTZNER, J., KRAUSOVÁ, R., BYTEŠNÍK, J., STRAKA, F., SKALSKÝ, I., NETUKA, I. Surgical treatment of supraventricular tachyarrhythmias. *6th Biennial Meeting of the Society of Cardiothoracic Surgeons*. Kruger National Park. 13.06.2003 - 16.06.2003. Zorg. Society of Cardiothoracic Surgeons of South Africa. In: *6th Biennial Meeting of the Society of Cardiothoracic Surgeons*. b.m.: Society of Cardiothoracic Surgeons of South Africa, 2003, s. 33.

PIRK, J., STRAKA, F., HÝŽA, Z., STUPKA, J., MAŠÍN, J., ROHN, V., BEŠÍK, J., SKIBOVÁ, J. Atrial defibrillator - new way of fibrillation and flutter therapy of patients after cardiosurgical operations. *21st International Cardiovascular Srugical Symposium Zürs*. Zürs am Arlberg. 22.02.2003 - 01.03.2003. Zorg. 1st Surgical Dept. of Hospital Vienna. In: *21st International Cardiovascular Surgical Symposium Zürs. Abstracts*. b.m.: b.n., 2003, s. nestr.

ČERNÝ, Š., PAVEL, P., PIRK, J., HOLÁ, I., BŘEZINA, A., SEDLÁČEK, J., STRAKA, F., HEGAROVÁ, M., MÁLEK, I. Donor myocardial protection using secondary blood cardioplegia during heart transplantation: prospective randomized study in 100 patients. *22nd Annual Meeting and Scientific Sessions*. Washington, US, 10.04.2002 - 13.04.2002. Zorg. International Society fot Heart and Lung Transplantation. *Journal of heart and lung transplantation*. 2002, **21**(1), 61, č.abstr.19. ISSN 1053-2498.e-ISSN 1557-3117.

JONÁK, M., STRAKA, F., PIRK, J. Protéza Sorin Allcarbon v mitrální pozici - střednědobé sledování. *10. výroční sjezd České kardiologické společnosti*. Brno, CZ, 19.05.2002 - 22.05.2002. Zorg. Česká kardiologická společnost. *Cor et Vasa*. 2002, **44**(4, suppl.), 51, č.abstr.238. ISSN 0010-8650.

PIRK, J., PAVEL, P., FRÍDL, P., JANDOVÁ, R., STRAKA, F. Zkušenosti s plastikou mitrální chlopně v IKEM. *10. výroční sjezd České kardiologické společnosti*. Brno, CZ, 19.05.2002 - 22.05.2002. Zorg. Česká kardiologická společnost. *Cor et Vasa*. 2002, **44**(4, suppl.), 92-93, č.abstr.103. ISSN 0010-8650.

PIRK, J., JONÁK, M., STRAKA, F., LÁNSKÁ, V. Sorin allcarbon mechanical prosthesis in the mitral position. *20th International Cardiovascular Surgical Symposium Zürs*. Zurs am Arlberg, AT. 02.03.2002 - 09.03.2002. Zorg. 1st Surgical Dept. of Hospital Vienna-Lainz. In: *20th International Cardiovascular Surgical Symposium Zürs : abstracts*. B.m.: b.n., 2002, s. nestr.

PIRK, J., KAUTZNER, J., STRAKA, F., KRAUSOVÁ, R., BYTEŠNÍK, J. Surgical linear ablation of chronic atrial fibrilation using radiofrequency energy (RF). *10th Annual Meeting of the Asian Society for Cardiovascular Surgery*. Jeju Island, KR. 17.04.2002 - 19.04.2002. Zorg. Asian Society for Cardiovascular Surgery. In: *10th Annual Meeting of the Asian Society for Cardiovascular Surgery : program and abstracts*. Jeju Island: Asian Society for Cardiovascular Surgery, 2002, s. 169, č.abstr.S05-08.

PIRK, J., KAUTZNER, J., STRAKA, F., KRAUSOVÁ, R., MAŠÍN, J., BYTEŠNÍK, J. Chirugická lineární ablace při chronické fibrilaci síní pomocí radiofrekvenční energie. *10. výroční sjezd České kardiologické společnosti*. Brno, CZ, 19.05.2002 - 22.05.2002. Zorg. Česká kardiologická společnost. *Cor et Vasa*. 2002, **44**(4, suppl.), 93, č.abstr.290. ISSN 0010-8650.

ČERNÝ, Š., PAVEL, P., PIRK, J., HOLÁ, I., DRLÍK, M., KOLESÁR, A., BŘEZINA, A., SEDLÁČEK, J., STRAKA, F. Ochrana dárcovského myokardu sekundární krevní kardioplegií při transplantaci srdce. *27. den mladých kardiologů*. Praha, CZ. . Zorg. Česká kardiologická společnost. In: *27. den mladých kardiologů : abstrakta*. Praha: Česká kardiologická společnost, 2001, s. 42.

ČERNÝ, Š., PAVEL, P., PIRK, J., HOLÁ, I., DRLÍK, M., KOLESÁR, A., BŘEZINA, A., SEDLÁČEK, J., STRAKA, F. Ochrana dárcovského myokardu sekundární krevní kardioplegií při transplantaci srdce. *9. výroční sjezd České kardiologické společnosti*. Brno, CZ, 13.05.2001 - 16.05.2001. Zorg. Česká kardiologická společnost. *Cor et Vasa*. 2001, **43**(suppl. k 4), 10. ISSN 0010-8650.

ČERNÝ, Š., PAVEL, P., PIRK, J., HOLÁ, I., DRLÍK, M., KOLESÁR, A., BŘEZINA, A., SEDLÁČEK, J., STRAKA, F., VAVŘÍNKOVÁ, H., HONSOVÁ, E. Sekundární krevní kardioplegie při transplantaci srdce. *Kardiologické dny*. Praha, CZ, 22.11.2001 - 23.11.2001.

Zorg. Česká kardiologická společnost. *Cor et Vasa (Kardio)*. 2001, **43**(9), K237-K238. ISSN 0010-8650.

KAUTZNER, J., PIRK, J., STRAKA, F., KRAUSOVÁ, R., BYTEŠNÍK, J. Chirugická lineární ablace chronické fibrilace síní pomocí radiofrekvenční energie. *8. české sympozium o arytmiích a kardiostimulaci*. Olomouc, CZ. 24.10.2001 - 26.10.2001. In: *8. české sympozium o arytmiích a kardiostimulaci : sborník abstrakt*. B.m.: b.n., 2001, s. 8.

PIRK, J., ČERNÝ, Š., PAVEL, P., HOLÁ, I., BŘEZINA, A., SEDLÁČEK, J., STRAKA, F. Donor myocardial protection by secondary blood cardioplegia during heart transplantation. *11th World Congress the World Society of Cardio-Thoracic Surgeons*. Sao Paulo, BR. 12.08.2001 - 15.08.2001. Zorg. World Society of Cardio-Thoracic Surgeons. In: *11th World Congress the World Society of Cardio-Thoracic Surgeons : program and abstracts*. Sao Paulo: b.n., 2001, s. 33, č.abstr.12.

BEŠÍK, J., PIRK, J., ČERNÝ, Š., ROHN, V., MUSILOVÁ, B., STRAKA, F. Chirurgická revaskularizace myokardu z minithoraktomie - naše zkušenosti. *5.kardiochirurgické dny*. Milovy, CZ. 02.11.2000 - 04.11.2000. Zorg. Kardiochirurgická klinika FN LF UK v Hradci Král.. In: *5.kardiochirurgické dny : sborník přednášek*. B.m.: HK Credit,s.r.o., 2000, s. 41, č.abstr.41. ISBN 80-902753-1-1.

JONÁK, M., STRAKA, F., PIRK, J., SKALSKÝ, I. Naše zkušenosti s náhradami MI chlopní protézou Sorin Allcarbon. *Mitrální vady. 2.sympozium*. Kroměříž, CZ. 24.02.2000 - 26.02.2000. Zorg. Prac.sk.pro chlop.a vroz.srdeční vady v dospěl.ČKS. In: *2.sympozium pracovní skupiny pro chlopenní a vrozené srdeční vady v dospělosti. Mitrální vady : Sborník abstrakt*. B.m.: b.n., 2000, s. 36.

STRAKA, F., PIRK, J., HÝŽA, Z., STUPKA, J., MAŠÍN, J., BEŠÍK, J., ROHN, V., SKIBOVÁ, J. Síňový defibrilátor - nový způsob léčby fibrilace a flutteru síní u nemocných po kardiochirurgických operacích. *8.výroční sjezd*. Brno, CZ, 28.05.2000 - 31.05.2000. Zorg. Česká kardiologická společnost. *Cor et Vasa*. 2000, **42**(4,suppl.), 63. ISSN 0010-8650.

PIRK, J., ČERNÝ, Š., BŘEZINA, A., SEDLÁČEK, J., STRAKA, F. Advantages of the MIDCABG procedure compared with single CABG to LAD with ECC. *17th International*

Cardiovascular Surgical Symposium Zürs. Zürs am Arlberg, AT. 06.03.1999 - 13.03.1999. Zorg. 1st Surgical Dept. of Hospital Vienna-Lainz. In: *17th International Cardiovascular Surgical Symposium Zürs : Abstracts*. B.m.n., 1999, s. nestr.

MÁLEK, I., STANĚK, V., GEBAUEROVÁ, M., FRÍDL, P., KAKÁČ, J., LOMOVÁ, J., PIRK, J., STRAKA, F., URBANOVÁ, D., STANĚK, V., VÍTKO, Š., ŽELÍZKO, M. Aktuální problémy transplantace srdce. *Weberův večer. Přednáškový večer IKEM*. Praha, CZ, 23.10.1995 - 23.10.1995. *Časopis lékařů českých*. 1996, **135**(6), 195. ISSN 0008-7335.

12.4 Oral presentations or posters

BURDÍKOVÁ, Z., FILOVÁ, E., FILOVÁ, E., ŠEPITKA, J., LUKEŠ, J., ŠVINDRYCH, Z., STRAKA, F., HADRABA, D., LIŠKOVÁ, J., STAŇKOVÁ, L., BAČÁKOVÁ, L. SHG imaging in tissue engeering. Poster. *Focus on Microscopy 2013*. Maastricht, NL. 24.03.2013 - 27.03.2013. Zorg. Maastricht University.

STRAKA, F., SCHORNÍK, D., MAŠÍN, J., FILOVÁ, E., BURDÍKOVÁ, Z., MIŘEJOVSKÝ, T., CHLUP, H., HORNÝ, L., VESELÝ, J., PIRK, J., BAČÁKOVÁ, L. A new approach for heart valve tissue engineering based on modifying autologous human pericardium by dynamic loading. Poster. *7th Biennial Congress*. Venice, IT. 22.06.2013 - 25.06.2013. Zorg. Society for Heart Valve Disease.

FILOVÁ, E., VANDROVCOVÁ, M., STRAKA, F., SVOBODOVÁ, J., JUKLIČKOVÁ, M., RUBÁČKOVÁ, K., BAČÁKOVÁ, L. Polymeric nanomats in cultures with heart valve interstitial cells. *3rd International NanoMedicine Conference*. Sydney, AU. 02.07.2011 - 04.07.2011. Zorg. Australian Centre for NanoMedicine (ACN).

SCHORNÍK, D., STRAKA, F., FILOVÁ, E., MAŠÍN, J., BURDÍKOVÁ, Z., MIŘEJOVSKÝ, T., LODERER OVÁ, A., CHLUP, H., HORNÝ, L., PIRK, J., BAČÁKOVÁ, L. The significance and organization of extracellular matrix of autologous human pericardium for heart valve tissue engineering. *7th Symposium on Biologic Scaffolds for Regenerative Medicine*. Napa Valley, US. 26.04.2012 - 28.04.2012. Zorg. McGowan Institute for Regenerative Medicine.

SCHORNÍK, D., STRAKA, F., BURDÍKOVÁ, Z., MAŠÍN, J., FILOVÁ, E., MIŘEJOVSKÝ, T., LODERÉROVÁ, A., CHLUP, H., HORNÝ, L., PIRK, J., BAČÁKOVÁ, L. Extracellular matrix structure of autologous human pericardium and significance for heart valve tissue engineering. Poster. *3rd TERMIS World Congress*. Vienna, AT. 05.09.2012 - 08.09.2012.

SCHORNÍK, D., STRAKA, F., MAŠÍN, J., FILOVÁ, E., BURDÍKOVÁ, Z., MIŘEJOVSKÝ, T., LODERÉROVÁ, A., CHLUP, H., HORNÝ, L., PIRK, J., BAČÁKOVÁ, L. The cellular and extracellular matrix structure of human pericardium for heart valve tissue engineering. Poster. *5th Biennial Conference on Heart Valve Biology and Tissue Engineering*. Myconos, GR. 18.05.2012 - 20.05.2012. Zorg. Society for Heart Valve Disease.

STRAKA, F., SCHORNÍK, D., FILOVÁ, E., MAŠÍN, J., BURDÍKOVÁ, Z., MIŘEJOVSKÝ, T., HONSOVÁ, E., LODERÉROVÁ, A., CHLUP, H., HORNÝ, L., VESELÝ, J., PIRK, J., BAČÁKOVÁ, L. Autologous human pericardium, a new approach for heart valve tissue engineering. Poster. *7th Symposium on Biologic Scaffolds for Regenerative Medicine*. Napa Valley, US. 26.04.2012 - 28.04.2012. Zorg. McGowan Institute for Regenerative Medicine.

STRAKA, F., SCHORNÍK, D., MAŠÍN, J., FILOVÁ, E., BURDÍKOVÁ, Z., MIŘEJOVSKÝ, T., HONSOVÁ, E., LODERÉROVÁ, A., CHLUP, H., HORNÝ, L., VESELÝ, J., PIRK, J., BAČÁKOVÁ, L. A new approach to heart valve tissue engineering based on the modification of human pericardial tissue. *5th Biennial Conference on Heart Valve Biology and Tissue Engineering*. Myconos, GR. 18.05.2012 - 20.05.2012. Zorg. Society for Heart Valve Disease.

STRAKA, F., SCHORNÍK, D., MAŠÍN, J., FILOVÁ, E., BURDÍKOVÁ, Z., MIŘEJOVSKÝ, T., HONSOVÁ, E., LODERÉROVÁ, A., CHLUP, H., HORNÝ, L., VESELÝ, J., PIRK, J., BAČÁKOVÁ, L. Modification of human pericardium based on dynamic loading, a new approach for heart valve tissue engineering. *3rd TERMIS World Congress*. Vienna, AT. 05.09.2012 - 08.09.2012.

FILOVÁ, E., STRAKA, F., MAŠÍN, J., TSENG, Y. T., SCHORNÍK, D., HONSOVÁ, E., LODERÉROVÁ, A., PIRK, J., BAČAKOVÁ, L. Valve interstitial cells on porous collagen scaffold for heart valve tissue engineering. *24th European Conference on Biomaterials*. Dublin, IR. 04.09.2011 - 08.09.2011. Zorg. European Society for Biomaterials.

STRAKA, F., PIRK, J. Časná resynchronizační léčba po kardiochirurgických operacích. *České kardiologické dny 2011*. Praha, CZ. 24.11.2011 - 25.11.2011. Zorg. Česká kardiologická společnost.

PIRK, J., MARTINČA, T., STRAKA, F., JUŠTÍK, P., ŠKROBÁKOVÁ, J., VINDUŠKA, V. An easy method to avoid the patient - valve mismatch. *3rd Biennial Meeting of The Society for Heart Valve Disease*. Vancouver, CA. 17.06.2005 - 20.06.2005.

MALÝ, J., KRAUSOVÁ, R., KAUTZNER, J., STRAKA, F., ŠKRABÁKOVÁ, J., ROKOŠNÝ, S., NETUKA, I., ROHN, V., PIRK, J. Výsledky střednědobého sledování po chirurgické léčbě fibrilace síní. *12. výroční sjezd České kardiologické společnosti*. Brno, CZ. 09.05.2004 - 12.05.2004. Zorg. Česká kardiologická společnost.

STRAKA, F., PIRK, J., HÝŽA, Z., STUPKA, J., MAŠÍN, J., BEŠÍK, J., ROHN, V., SKIBOVÁ, J. Síňový defibrilátor - nový způsob léčby fibrilace a flutteru síní u nemocných po kardiochirurgických operacích. *Seminář KKCH a KAR*. Praha. 10.02.2003 - 10.02.2003. Zorg. IKEM.

PIRK, J., ČERNÝ, Š., PAVEL, P., HOLÁ, I., BŘEZINA, A., SEDLÁČEK, J., STRAKA, F. Donor myocardial protection by secondary blood cardioplegia during heart transplantation. *11th World Congress of the Society Cardio-Thoracic Surgery*. Sao Paulo, BR. 12.08.2001 - 15.08.2001. Zorg. Society of the Cardio-thoracic Surgery.

STRAKA, F. Síňový defibrilátor - nový způsob léčby fibrilace a flutteru síní u nemocných po kardiochirurgických výkonech. *Seminář KKCH*. Praha, CZ. 19.02.2001 - 19.02.2001. Zorg. IKEM.

MAŠÍN, J., STRAKA, F., HÝŽA, Z. Přímá síňová defibrilace, nová metoda léčby pooperačních arytmií. *3.výroční schůze České společnosti pro mimotělní oběh a podpůrné systémy a 5.společná konference českých a slovenských perfuziologů*. Třeboň, CZ. 30.03.2000 - 01.04.2000. Zorg. Česká společnost pro mimotělní oběh a podpůrné systémy.

SKALSKÝ, I., PIRK, J., PAVEL, P., ROHN, V., STRAKA, F., MUSILOVÁ, B. Náhrada mitrální chlopně bioprotézou. *Mitrální vady. 2.sympozium*. Kroměříž, CZ. 24.02.2000 - 26.02.2000. Zorg. Prac. skupina pro chlopenní a vrozené srdeční vady v dospělosti.

OUHRABKOVÁ, R., STRAKA, F., MÁLEK, I., ŽELÍZKO, M., URBANOVÁ, D., HEJNAL, J. Nálezy na věnčitých tepnách u pacientů po orthotopické transplantaci srdce. *6. dny zobrazovací diagnostiky v dětském věku a sympózium o pooperačních stavech*. Praha, CZ. 14.04.1993 - 16.04.1993. Zorg. FN v Motole.

STRAKA, F., MÁLEK, I., OUHRABKOVÁ, R., GEBAUEROVÁ, M., PIRK, J., URBANOVÁ, D., VRUBEL, J., ŽELÍZKO, M. Koronární nemoc u pacientů po transplantaci srdce. *1. výroční sjezd ČKS*. Zlín, CZ. 19.05.1993 - 21.05.1993. Zorg. Česká kardiologická společnost.

12.5 Anthology contribution

STRAKA, F., MÁLEK, I., STANĚK, V., OUHRABKOVÁ, R., GEBAUEROVÁ, M., URBANOVÁ, M., VRUBEL, J., PIRK, J., ŽELÍZKO, M., MÁLEK, I., LÁNSKÁ, V. Koronární nemoc u pacientů po transplantaci srdce. *Transplantace orgánů a tkání '94*. Praha, CZ. 19.10.1994 - 20.10.1994. In: *Transplantace*. 1995, s. 27-28.

12.6 Chapters in monographs

STRAKA, F., SCHORNÍK, D. Optimizing hemodynamic performance after heart surgery, the role of cardiac resynchronization therapy. In: Senzaki, H. *Hemodynamics: Monitoring, Theory and applications*. 1. vyd. Hauppauge, NY: Nova Science Pub Incorporated, 2012, s. 231-248. ISBN 978-1-62257-361-5.

PIRK, J., STRAKA, F. Echokardiografie v kardiochirurgii. In: *Echokardiografie. 1. díl, Echokardiografie dospělých*. 1. vyd. Praha: Triton, 2002, s. 343-350. ISBN 80-7254-281-8.

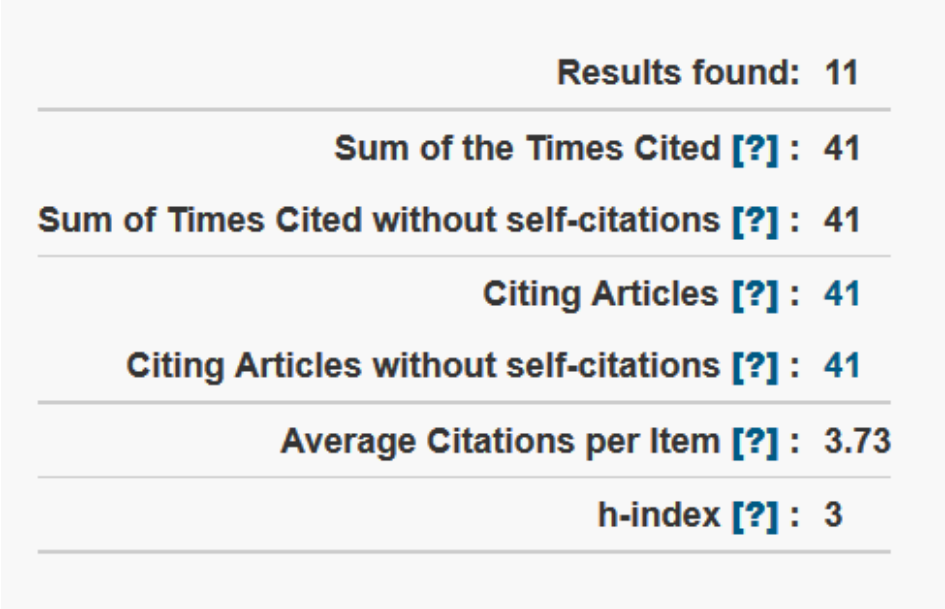
12.7 International Patent

Inventors/Applicants: STRAKA, F., MAŠÍN, J., SCHORNÍK, D., PIRK, J. A method of constructing a pericardial heart valve replacement, a pericardial heart valve replacement

constructed according to this method, and a device for the in vitro conditioning and modification of tissue of autologous pericardium for the construction of a pericardial heart valve replacement. 2013; WIPO/PCT. International Publication Number: WO 2013/182171 A1.

STRAKA, F., MAŠÍN, J., SCHORNÍK, D., PIRK, J. Pericardial heart valve replacement and methods of constructing the same. 2015; U.S. Patent Number 14,406,024**.**

12.8 Web of Science h-index: 3



Results found:	11
Sum of the Times Cited [?] :	41
Sum of Times Cited without self-citations [?] :	41
Citing Articles [?] :	41
Citing Articles without self-citations [?] :	41
Average Citations per Item [?] :	3.73
h-index [?] :	3

13. SCHOLARSHIPS

John Radcliffe Hospital, Cardiac Clinical Center, Headley Way, Oxford, UK, June, 2006.

3rd Biennial Meeting on Heart Valve Biology&Tissue Engineering (London), The Society for Heart Valve Disease, London Heart Valve Meeting Heart Science Centre, 5.-7.5. 2008.

Postgraduate Course on Percutaneous Valve Replacement (London), The Society for Heart Valve Disease, London Heart Valve Meeting Heart Science Centre, 4.5. 2008.

5th Biennial Meeting : Joint Meeting of The Society for Heart Valve Disease&Heart Valve Society of America (Berlin), 27.-30.6. 2009.

Valves in the Heart of the Big Apple VIII. Evaluation&Management of Valvular Heart Disease 2014. *Fifth Annual Joint Scientific Session of the Heart Valve Diseases*, New York City, 8.5.-10.5. 2014.

14. APPENDICES

1. STRAKA, F., PIRK, J., PINĎÁK, M., MAREK, T., SCHORNÍK, D., ČIHÁK, R., SKIBOVÁ, J. A pilot study of systolic dyssynchrony index by real time three-dimensional echocardiography and Doppler tissue imaging parameters predicting the hemodynamic response to biventricular pacing in the early postoperative period after cardiac surgery. *Echocardiography*. 2012, **29**(7), 827-839. ISSN 0742-2822. **IF 1,261.**

2. STRAKA, F., PIRK, J., PINĎÁK, M., SKALSKÝ, I., VANČURA, V., ČIHÁK, R., MAREK, T., LUPÍNEK, P., SCHORNÍK, D., MAŠÍN, J., ZEMAN, M., ŠKROBÁKOVÁ, J., DORAZILOVÁ, Z., SKIBOVÁ, J. Biventricular pacing in the early postoperative period after cardiac surgery. *Physiological research*. 2011, **60**(6), 877-885. ISSN 0862-8408. **IF 1,555.**

3. STRAKA, F., PIRK, J., PINĎÁK, M., SKALSKÝ, I., VANČURA, V., ČIHÁK, R., MAREK, T., LUPÍNEK, P., MAŠÍN, J., SCHORNÍK, D., ZEMAN, M., ŠKROBÁKOVÁ, J., DORAZILOVÁ, Z., SKIBOVÁ, J. The hemodynamic effect of right ventricle (RV), RT3DE targeted left Ventricle (LV) and Biventricular (BIV) pacing in the early postoperative period after cardiac surgery. *Pacing and clinical electrophysiology*. 2011, **34**(10), 1231-1240. ISSN 0147-8389. **IF 1,351.**

4. FILOVÁ, E., STRAKA, F., MIŘEJOVSKÝ, T., MAŠÍN, J., BAČÁKOVÁ, L. Tissue-engineered heart valves. *Physiological research*. 2009, **58**(suppl. 2), S141-S158. ISSN 0862-8408. **IF 1,430.**

5. STRAKA, F., SCHORNÍK, D., MAŠÍN, J., FILOVÁ, E., MIŘEJOVSKÝ, T., CHLUP, H., HORNÝ, L., VESELÝ, J., SKIBOVÁ J., PIRK, J., BAČÁKOVÁ, L. A new approach to heart valve tissue engineering based on modifying autologous human pericardium by mechanotransduction. This paper is currently being edited by the journal: Journal of Tissue Engineering and Regenerative Medicine. Impact Factor: 5,199.

6. STRAKA, F., SCHORNÍK, D., MAŠÍN, J., FILOVÁ, E., BURDÍKOVÁ, Z., MIŘEJOVSKÝ, T., ŠVINDRYCH, Z., CHLUP, H., HORNÝ, L., VESELÝ, J.,

SKIBOVÁ J., PIRK, J., BAČÁKOVÁ, L. The cellular and extracellular matrix structure of human pericardium, a promising scaffold for autologous heart valve tissue engineering. This paper is currently being edited by the journal: Journal of Biomedical Materials Research Part A. Impact Factor: 3,369.

7. International Patent:

Inventors/Applicants: STRAKA, F., MAŠÍN, J., SCHORNÍK, D., PIRK, J. A method of constructing a pericardial heart valve replacement, a pericardial heart valve replacement constructed according to this method, and a device for the in vitro conditioning and modification of tissue of autologous pericardium for the construction of a pericardial heart valve replacement. 2013; WIPO/PCT. International Publication Number: WO 2013/182171 A1.