

# Collagen structures in pericardium and aortic heart valves and their significance for tissue engineering.

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**Abstract-** Biological prostheses of human heart valves are prepared from autologous heart valves and xenogeneic heart valves or pericardium. Xenogenous and allogeneic biological prostheses are associated with adverse immune reactions, thrombosis and degeneration and thus they have a high rate of reoperation. Finding an optimum autologous tissue for heart valve grafts is required. As a potential source human pericardium was assessed and compared with native human heart valves and porcine and bovine pericardium and heart valves. The tissues have been evaluated for their mechanical properties, collagen content and structure and histological structure. We observed differences in mechanical properties, collagen fibre orientation, shape of collagen bundles, and collagen content among the pericardium samples or heart valves of different origin.

**Keywords:** collagen, second harmonic generation, pericardium, aortic heart valve, mechanical properties.

## I. INTRODUCTION

Allogenic heart valves from human donors (i.e. homografts), xenogeneic bovine pericardial valves, and porcine xenograft valves are currently used as heart valve biological prostheses [1]. These tissues, however, evoke immune reaction, thrombosis, and undergo degeneration. The harvesting and implantation of autologous prostheses often requires complicated surgery with an increased risk of mortality. Finding a suitable autologous source of tissue for heart valve prostheses is therefore required. Human pericardium has been evaluated in terms of its potential use for heart valve surgery.

Collagen is responsible for solidity of tissues. An aortic heart valve consists of three layers; collagen is present mainly in lamina fibrosa, which is closest to the outflow layer. The lamina fibrosa contains predominantly circumferentially aligned, macroscopically crimped, densely packed collagen [2]. Lamina ventricularis, closest to the inflow surface is rich in radially aligned elastin fibers. Lamina spongiosa is located in the central part and is rich in glycosaminoglycans (GAGs) and loosely packed collagen [3]. This valve structure is responsible for excellent mechanical properties and resistance to strain loading. The total collagen content, orientation of collagen

fibres may vary among tissues and species. In the present study, collagen fibres were visualised using second harmonic generation imaging (SHG). SHG imaging is a non-invasive scanning optical method based on nonlinear properties of ordered non-centrosymmetric molecules (such as collagen fibres) under extremely intense illumination [4]. Fibrous collagen can be observed in native state without staining. High resolution nonlinear optical microscopy can serve in imaging and quantifying tissue structural changes. Human pericardium, human aortic heart valve, bovine pericardium, bovine heart valve, porcine pericardium and porcine heart valve have been studied using SHG imaging, histological staining, hydroxyproline assay for quantification of both collagen and elastin content and biomechanical evaluation.

## II. MATERIALS AND METHODS

### A. Histology

The samples of human aortic heart valves, and pericardium from patients of any age, bovine aortic heart valves, and pericardium (1-year old healthy cows) and porcine aortic heart valve and pericardium (6-month old healthy pigs) were fixed in 10% formalin and embedded in paraffin. Then the samples were cut into 3-4  $\mu\text{m}$  sections and stained with Hematoxylin-eosin or Masson's trichrome stains. Micrographs were taken under an Olympus IX 71 epifluorescence microscope, DP71 digital camera.

### B. SHG imaging

All images were acquired by a Leica TCS SP2 acousto-optical beamsplitter (AOBS) multiphoton confocal laser scanning microscope based on 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 Ti:Sapphire Chameleon Ultra laser (Coherent Inc., Santa Clara, California), tuneable from 690 to 1040 nm for TPE. Both outer and inner layers and cross-section of human, bovine and porcine pericardium were evaluated. In

addition, lamina fibrosa and lamina ventricularis and crosssections of human, porcine and bovine heart valves have been evaluated. The structure of collagen fibres and orientation of collagen bundles were observed.

### C. Mechanical Evaluation

The specimens dimensions were measured by Laser profile sensor ScanControl 2800 (Micro-Epsilon, Ortenburg, Germany). Uniaxial tensile tests were performed on the customer specific biaxial testing machine (Zwick/Roell). Experiments were recorded by a videoextensometer which provides for online detection of the deformation. The tests were controlled according following scheme: four cycles of loading with a constant limit deformation (for preconditioning of material) followed by stress relaxation, were completed by fifth loading cycle till a failure. Mechanical response of the material during the experiment was visualized by means of  $\sigma$ - $\epsilon$  graphs, where  $\sigma$  denotes Cauchy stress and  $\epsilon$  engineering strain. From the values measured, so called secant modulus  $E_s$  was evaluated. It is defined as a ratio of maximum stress and maximum deformation achieved during elastic phase of loading.

### D. Hydroxyproline content

The samples of pericardium and heart valves of human, porcine and bovine origin (100  $\mu$ g) were cut into small pieces and have been incubated in 3 ml of solution containing collagenase III (298 U/ml) and neutral protease (0,56U/ml) (Worthington Biochemical Corp., U.S.A., Cat No. LS004208 and LS02104) for 24 hours. The final suspension was mechanically homogenized and total hydroxyproline content was measured using an Hydroxyproline assay kit (Biovision, U.S..A.) according to the manufacturer protocol.

## III. RESULTS AND DISCUSSION

### A. Histology

Human, porcine, and bovine aortic heart valves and pericardium samples have been stained by Masson's trichrome staining. Collagen, which was stained blue or green was present throughout the tissues (Fig. 1).

### B. Shg imaging

Aortic heart valves contains 50 % collagen (74 % type collagen I, 24 % type III collagen), 13 % elastin, proteoglycans, and glycosaminoglycans (Taylor 2007). Both the composition and structure of extracellular matrix in lamina fibrosa, lamina ventricularis, and lamina spongiosa imparts strength and elasticity to the heart valve. The knowledge about collagen structure, fiber orientation and shape is important for tissue engineers to evaluate and design new bioartificial scaffolds for cell seeding. We evaluated properties of human pericardium as possible material for autologous biological heart valves and

compared them with bovine and porcine xenogeneic tissues presently available.

SHG confocal microscopy depicts native collagen structures without staining and photobleaching (Fig. 2, 3). A different arrangement of collagen fibers was observed in different parts of aortic heart valve. In lamina fibrosa, more dense and wavy pattern of collagen fibres was found, in contrast to relatively straight collagen fibers irregularly

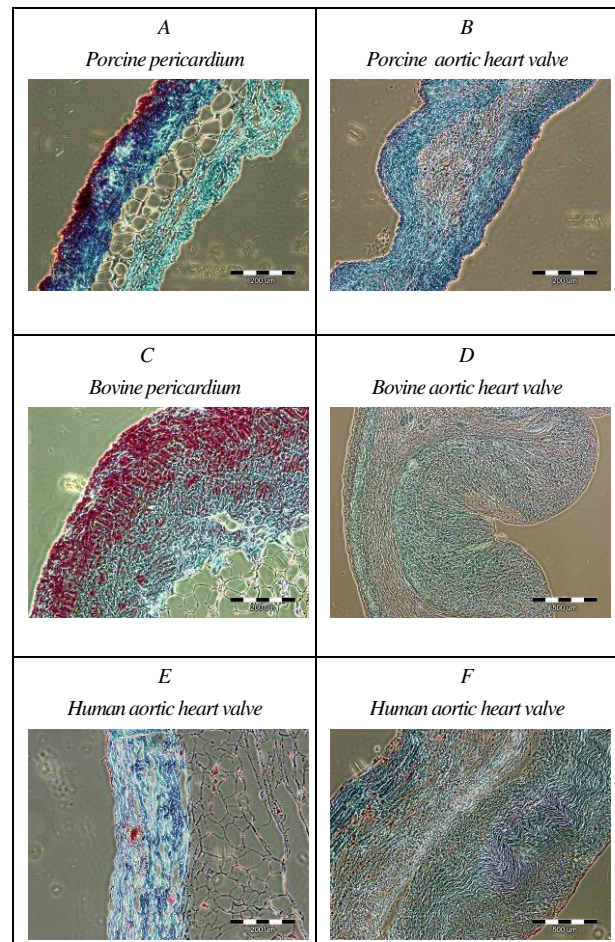


Fig. 1. Masson's trichrome staining of porcine pericardium (A), porcine aortic heart valve (B), bovine pericardium (C), bovine aortic heart valve (D), human pericardium (E), and human aortic heart valve (F). Keratin and muscle fibers are stained red, collagen is blue or green, cytoplasm is coloured light red or pink, cell nuclei are dark brown to black.

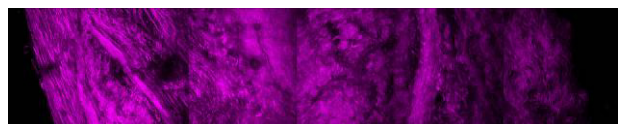


Fig. 2 SHG image of human aortic heart valve, transverse projection, Leica TCS SP2 microscope, .obj. 63x.

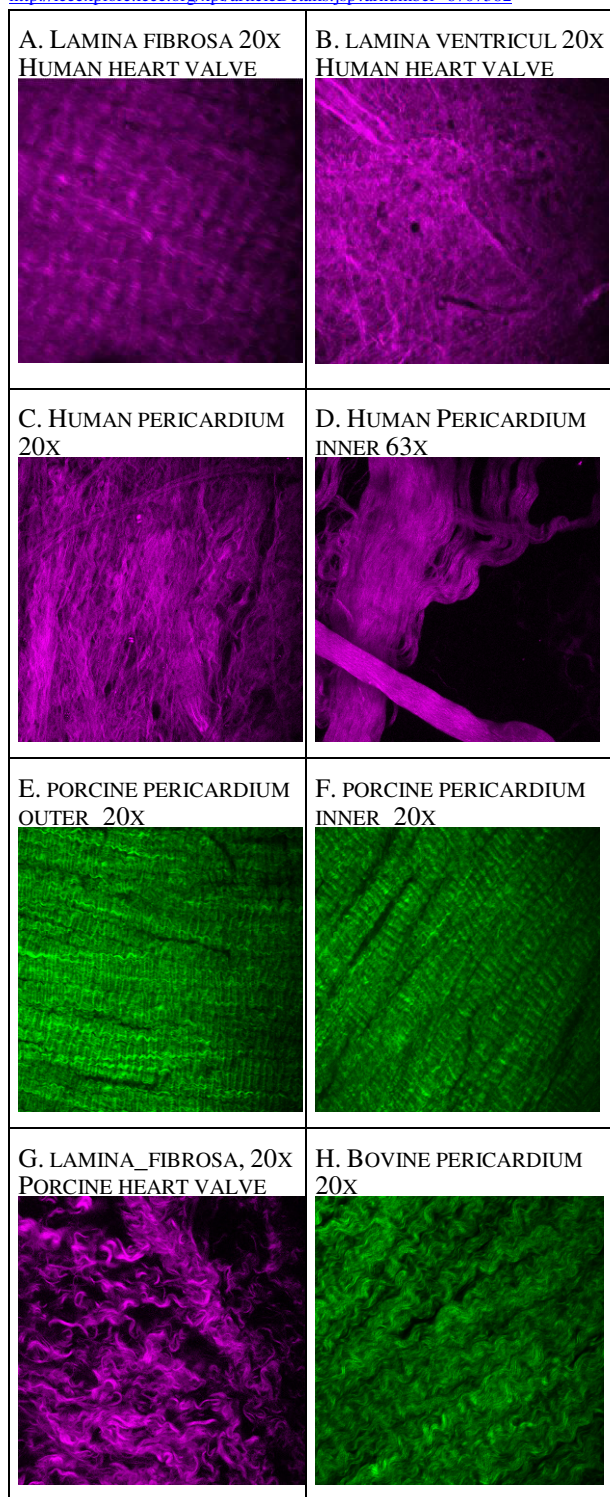


Fig. 3. SHG images of human aortic heart valve (A, B), human pericardium (C, D), porcine pericardium (E, F), porcine aortic heart valve (G), and bovine pericardium (H), Leica TCS SP2 microscope.

### C. Mechanical evaluation

Mechanical properties of all pericardium samples were measured in two orientations, transversal and medial; heart valves also in two directions, radial and circumferential. Preliminary results indicate an increased secant modulus of bovine pericardium compared to bovine heart valves. In addition, anisotropy of the tissue properties has been found in bovine pericardium, but not in human samples. Human and porcine pericardium and heart valve tissues did not differ in mechanical properties. Mechanical evaluation of the tissues are still in progress.

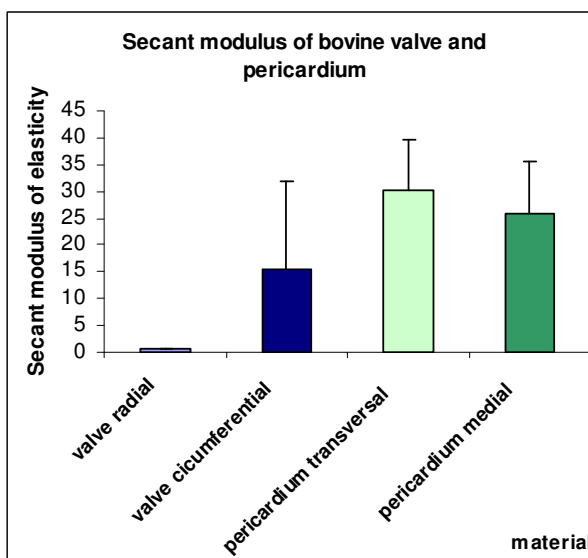


Fig. 4. Secant modulus of bovine pericardium and bovine aortic heart valve in two orthogonal orientations.

### D. Hydroxyproline content

Significantly higher content of hydroxyproline was measured on bovine pericardium. Hydroxyproline measures total content of collagen and elastin. This findings are in agreement with secant modulus of the tissue studied.

dispersed in lamina ventricularis. There was similar wavy shape of collagen fibres on both outer and inner side of pericardium of all species, more intense curly appearance in bovine pericardium.



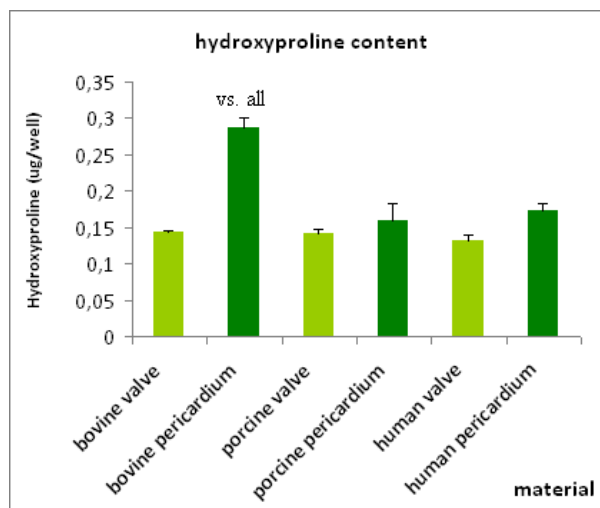


Fig. 5. The content of hydroxyproline in bovine aortic heart valve and pericardium, porcine aortic heart valve and pericardium, and human aortic heart valve and pericardium.

#### IV. CONCLUSIONS

In the present study we evaluated pericardium and aortic heart valve tissue of human, porcine. and bovine origin. Organisation of collagen fibres, collagen concentration, and secant modulus were similar in human and porcine tissues. Among the tissues tested, bovine pericardium contained highly organised collagen fibres, highest secant modulus of elasticity, and the hydroxyproline content. This confirms its warranted usage for preparation of biological heart valves. Despite these excellent properties it represents xenogeneic materials and makes scientists to look for similar autologous tissues.

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