Comparison of biomechanical and structural properties between human aortic and pulmonary valve[☆]

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Abstract

Objective: Pulmonary valve autografts have been reported as clinically effective for replacement of diseased aortic valve (Ross procedure). Published data about pulmonary valve mechanical and structural suitability as a long-term substitute for aortic valve are limited. The aim of this study was to compare aortic and pulmonary valve properties. **Methods**: Experimental studies of biomechanical properties and structure of aortic and pulmonary valves were carried out on pathologically unchanged human heart valves, collected from 11 cadaveric hearts. Biomechanical properties of 84 specimens (all valve elements: cusps, fibrous ring, commissures, sinotubular junction, sinuses) were investigated using uniaxial tensile tests. Ultrastructure was studied using transmission and scanning electron microscopy. **Results**: Ultimate stress in circumferential direction for pulmonary valve cusps is higher than for aortic valve (2.78 ± 1.05 and 1.74 ± 0.29 MPa, respectively). Ultimate stress in radial direction for pulmonary and aortic cusps is practically the same (0.29 ± 0.06 and 0.32 ± 0.04 MPa, respectively). In ultrastructural study, different layout and density in each construction element are determined. The aortic and pulmonary valves have common ultrastructural properties. **Conclusions**: Mechanical differences between aortic and pulmonary valve are minimal. Ultrastructural studies show that the aortic and pulmonary valves have similar structural elements and architecture. This investigation suggests that the pulmonary valve can be considered mechanically and structurally suitable for use as an aortic valve replacement.
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Keywords: Aortic valve; Pulmonary valve; Biomechanical properties; Structural properties; Ross procedure

1. Introduction

Research for a better substitute of human aortic valve is continuing worldwide. Replacement of diseased aortic valve by a pulmonary artery valve complex (autograft) is considered as clinically effective in reconstruction of aortic valve (Ross procedure) [1–3]. At the beginning of previous century, Gross and Kugel [4] described human heart aortic valve cusp structure indicating that it is a composite, laminate material. In 1962, the clinical use of aortic valve homografts was reported by Ross [5] and Barratt-Boyes [6]. Then studies of structural and mechanical properties were generally performed on aortic valve cusps [7–9]. Only few articles examine structure and mechanical properties of aortic and pulmonary valves [10–12]. The first pulmonary autotransplant was performed by Ross at Guy's Hospital on June 8, 1967. In the 1990s, surgeons around the world accepted advantages of the Ross procedure. Published data about pulmonary valve mechanical and structural suitability as a long-term substitute for aortic valve are limited [13–16]. The aim of our study was to compare aortic and pulmonary valve properties. We will integrate biomechanical and structural investigations within our complex studies on use of pulmonary valve in aortic position [17].

2. Materials and methods

The protocol of the study was approved by ethics committee on human research of Riga Stradins University.

Our experimental studies of biomechanical properties and structure were carried out on pathologically unchanged human aortic and pulmonary heart valves. These valves were collected from 11 cadaveric hearts within 24 h of death. Donors' age ranged from 20 to 50 years. Explored aortic and pulmonary valves were stored in a physiological sodium chloride solution at $T = 20 \pm 1$ °C.

All aortic and pulmonary valve construction elements—cusps, commissures, fibrous ring, sinotubular junction, sinuses—were investigated using uniaxial tensile tests with universal testing machine INSTRON 4301. The number of specimens in each group was the following: 22 aortic cusps (11 circumferential, 11 radial), 22 pulmonary cusps (11 circumferential, 11 radial), 5 aortic and 5 pulmonary commissures, 5 aortic and 5 pulmonary fibrous rings, 5 aortic and 5 pulmonary sinotubular junctions, 5 aortic and 5 pulmonary sinuses. Specimens were cut 3.0 mm wide and up to 20 mm long. Specimens of cusps were cut both in radial and circumferential directions. Uniaxial tensile tests were performed to examine the deformability and strength of the tissues. Initial thickness of samples of valve cusps was measured by cathetometer MK-6 (LOMO). The precision of measurements is ± 0.005 mm.

A parallel study was carried out on the same material by light microscopy. We illuminated each aortic and pulmonary valve cusp and took pictures of outflow and inflow surfaces. We performed a total valve cut through one aortic and pulmonary valve cusp, their commissure and fibrous ring. For light microscopy, the samples were fixed in 10% formaldehyde solution, placed in paraffin bloc and cut by 5 μm thick sections in element longitudinal and transverse direction. Prepared sections were stained with hematoxylin–eosin to gain preliminary information about constructive elements of the connective tissue. The collagen fibers were dyed by van Gieson and elastin fibers by Weigert.

Ultrastructure was investigated using transmission and scanning electron microscopy. The micro relief of the surfaces of valve elements (after removal of endothelium) and structure of the deep layers (after dissection of samples) were studied by scanning electron microscope. Samples were fixed in 3% glutaraldehyde, post-fixed in 1% osmium tetra-oxide solution, dehydrated in ethanol of increasing concentration and dried at critical point. Thereafter, specimens were coated in a vacuum chamber with 50–60 nm gold. Specimens were studied on a JEM-100C (Japan) electron microscope with ASJD-4D scanning attachment at 40 kV acceleration voltage and magnification from $800 \times$ to $30,000 \times$. Ultrastructure of valve elements was studied by transmission electron microscope using ultra thin slice method. Samples were fixed in 3% glutaraldehyde in phosphate buffer, post-fixed in 1% osmium tetra-oxide solution, dehydrated in ethanol of increasing concentration, poured and polymerized. The slices were prepared using ultra microtome and contrasted with lead citrate. The prepared ultra thin slices were studied on JEM-100C electron microscope with 80 kV accelerating voltage and magnification from $5000 \times$ to $50,000 \times$.

Experimental data were analyzed by single-factor ANOVA. For pair-wise comparisons, heteroscedastic *t*-test was used to determine significance in differences between population means. Statistically, different pairs were defined as having $P < 0.05$.

3. Results

3.1. Biomechanical properties

Experimental results show that modulus of elasticity of pulmonary and aortic valve cusps in circumferential direction at the level of stress 1.0 MPa is not essentially different between each other: 16.05 ± 2.02 and 15.34 ± 3.84 MPa, respectively ($P > 0.2$). Ultimate stress of pulmonary valve cusps in circumferential direction is higher than for aortic valve in the same direction: 2.78 ± 1.05 and 1.74 ± 0.29 MPa, respectively ($P = 0.049$). There is no difference between ultimate strain in circumferential direction for pulmonary and aortic valve cusps: 19.40 ± 3.91 and $18.35 \pm 7.61\%$, respectively ($P > 0.2$) (Fig. 1).

Modulus of elasticity of pulmonary valve cusps in the radial direction at the level of stress 1.0 MPa is essentially less than modulus of elasticity of aortic valve cusps: 1.32 ± 0.93 and 1.98 ± 0.15 MPa ($P = 0.002$). The ultimate stress of pulmonary and aortic valve cusps in the radial direction is almost the same: 0.29 ± 0.06 and 0.32 ± 0.04 MPa, respectively ($P > 0.2$). Nevertheless, the ultimate strain of pulmonary valve cusps in radial direction is higher than ultimate strain of aortic valve cusps: 29.67 ± 4.41 and $23.92 \pm 3.94\%$ ($P = 0.043$) (Fig. 1).

Biomechanical differences between other aortic and pulmonary valve construction elements are minimal. Modulus of elasticity of pulmonary and aortic valve elements at the level of stress 1.0 MPa does not essentially differ between each other ($P > 0.05$) (Table 1).

We determined the thickness of all the cusps of aortic and pulmonary valve. The average thickness h_0 of the aortic valve cusp is 0.605 ± 0.196 mm. The average thickness h_0 of the pulmonary valve cusp is 0.397 ± 0.114 mm.

3.2. Morphology and ultrastructure

During our morphological and ultrastructural study, we found that the aortic valve and the pulmonary valve have identical construction elements and practically
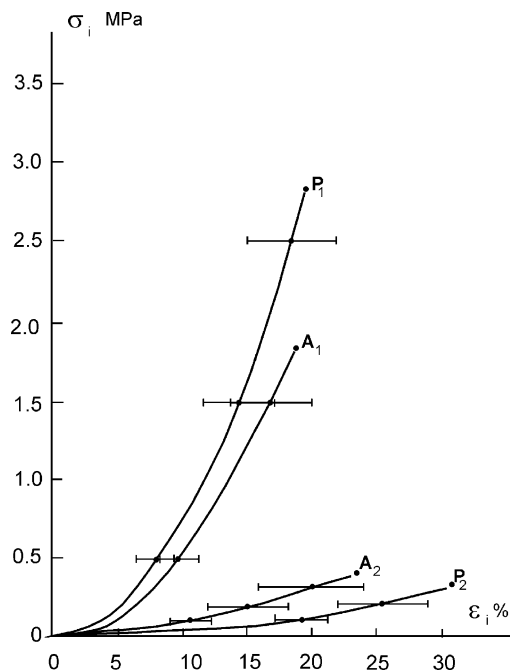
Fig. 1. Stress–strain relationship for pulmonary and aortic valve cusps in circumferential and radial directions (P_1, pulmonary cusps in circumferential direction; A_1, aortic cusps in circumferential direction; P_2, pulmonary cusps in radial direction; A_2, aortic cusps in radial direction).

the same structure. Structural properties of pulmonary and aortic valve elements are shown in Table 2.

Support elements of aortic and pulmonary valves—fibrous ring and commissures (Fig. 2)—contain much collagen fibers and bundles, orientated in the direction of loading. In order to ensure strength of the valve during continuous working, collagen fibers and bundles are wrapped with twisted collagen fibrils. These fibrils build a lattice between collagen bundles and their layers and connect to collateral construction elements forming side branch or making loops around fibrous ring.

Damping elements of aortic and pulmonary valves—cusps (Fig. 3), sinotubular junction (Fig. 4) and sinuses—have more elastic fibers and their lamina orientated in radial and circumferential direction. This ensures good deformation in both directions during the load.

All parts of aortic and pulmonary valves contain cells, mostly fibroblast, which produce fibrous structures and restore the valves (Fig. 5). The concentration of these cell

Table 1
Modulus of elasticity E (MPa) of pulmonary and aortic valve elements

	Commissures	Fibrous ring	Sinotubular junction	Sinuses
Pulmonary valve	10.04 ± 2.82	10.06 ± 2.64	5.85 ± 1.62	14.28 ± 3.48
Aortic valve	13.80 ± 3.16	12.50 ± 2.98	7.41 ± 2.34	10.53 ± 3.22
	$P = 0.07$	$P > 0.2$	$P > 0.2$	$P = 0.12$

Table 2
Structural properties of pulmonary and aortic valve elements

Elements of aortic and pulmonary valves	Biomechanical function	Description
Fibrous ring	SE	Formed by densely and circularly moving massive bundles of CF (diameter 2.4–4.0 μm). They are slightly wavy and twisted. In longitudinal direction of fibrous ring, there are elastic fibers oriented in different directions.
Commissures	SE	Formation of commissures starts 2–3 mm above sinotubular junction, from sinus walls, below they split and each is creating its own free edge consisting of massive and twisted bundles of CF, which connect by their sideline to sinus walls.
Cusps	DE	Connective tissue varies along the cusp. Under endothelia layer of the cusp there are large bundles of CF, several EF. CF are mainly orientated in circumferential direction. They come out from commissures and form the free edge of the cusps. The main concentration of CF is located in nodule of Arantius. Under the nodule they form flaky layout of fibers and bundles, which further overpass in circumferential direction of the cusp. In middle part of the cusp, there are all connective tissue elements. Near the fibrous ring where the cusp is distinctly loaded for flexural strength and compression, EF are more abundant.
Sinotubular junction	DE	Connective tissue thickening, which is orientated to the inner part of arterial wall under endothelia layer. EF orientated at angle of 45°.
Sinuses	DE	EF bundles are orientated in radial and circumferential directions.

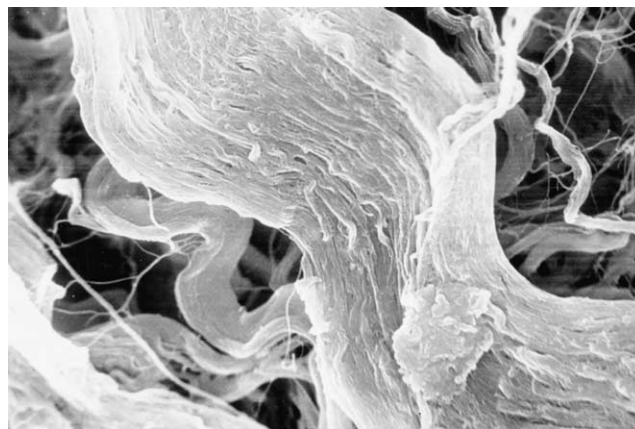SE, support element; DE, damping element; CF, collagen fibers; EF, elastic fibers.

Fig. 2. Scanning electron micrograph of the pulmonary valve deep structures of commissure. We see long, wavy collagen fibers and specifically fixed side branches allowing to 'swing' during the diastole.
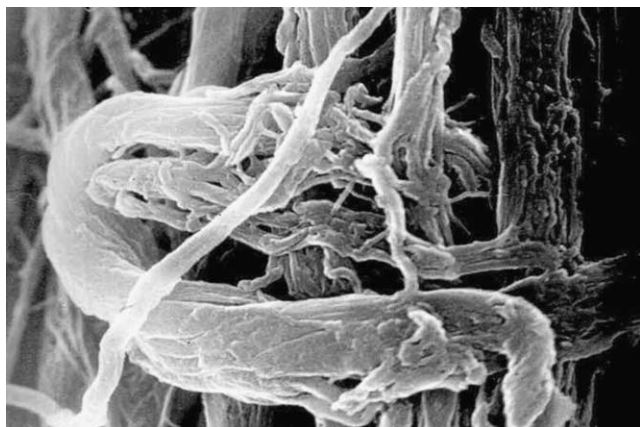
Fig. 3. Scanning electron micrograph of the aortic valve cusp in its connection spot with fibrous ring. Part of collagen fibers change their direction and settle around bundles of collagen fibers of commissures and fibrous ring. We see a massive bundle of collagen fibers wraps circularly going bundle of collagen in the inner part of fibrous ring.

elements is maximal in cusps near commissures and in sinotubular junction, which take the most important load.

4. Discussion

Our study shows that mechanical properties of pulmonary and aortic valves are nearly the same. During biomechanical investigations of the elements of aortic and pulmonary valves under uniaxial tension, a considerable anisotropy of the material is observed. Mechanical properties of aortic and pulmonary valve cusps are non-linear and different in circumferential and radial directions. At the beginning of the loading of samples at low stress the tissue has a large strain. During loading the wavy structure of tissue becomes straight and with increasing stress, the strain of the tissue decreases drastically. This phenomena leads to the concave form of
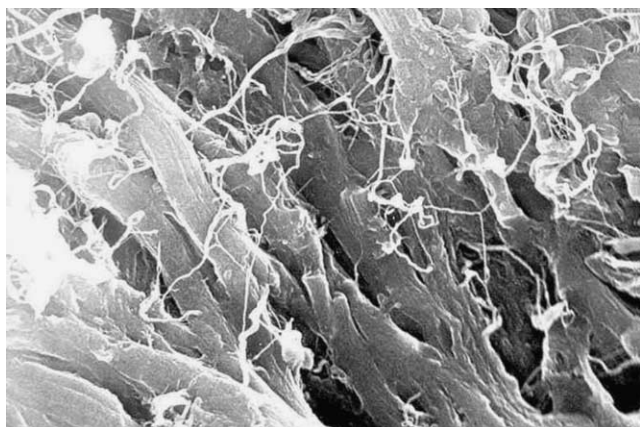

Fig. 4. Scanning electron micrograph of the aortic valve sinotubular junction. We demonstrate elastic fibers orientated at angle of 45°, which are splitting when moving down to the next layer of fibers and creating tissue structure orientated strictly in one direction.
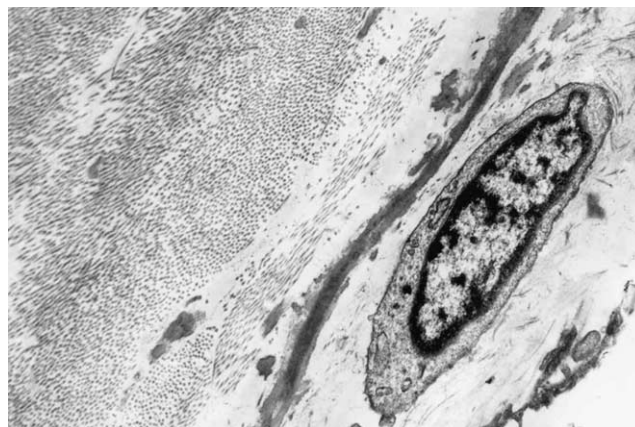

Fig. 5. Transmission electron micrograph of the pulmonary valve cusp showing all elements of connective tissue—collagen fibers in transverse and longitudinal direction, elastic fibers in transverse section, fibroblasts.

curves, and it is specific for soft biological tissue. Ultimate stress in circumferential direction for pulmonary valve cusps is slightly higher than for aortic valve, but in radial direction it is almost the same. Such alike mechanical properties of pulmonary and aortic heart valves are explained by nearly the same structure. Our thickness measurements show differences in cusps thickness—the aortic valve cusps are thicker than pulmonary valve cusps. Our morphological studies demonstrate that the aortic and pulmonary valves have similar structural elements and architecture—cusps, commissures, fibrous ring, sinotubular junction and sinuses. In ultrastructural study with electron microscope, different layout and density in each construction element are determined. Each element has a particular mode of functioning; however, they all have common additive and orientation principles of construction:

1. All aortic and pulmonary valve construction elements have collagen and elastic fibers, their bundles, ground substance and cells.
2. Collagen fibers and their bundles, elastic fibers and their lamina are correlative with fine and twisted collagen fibrils.
3. Both collagen and elastic fibers of each construction element are orientated and impacted in direction of loads during semilunar valve functioning, and they are distinctly wavy.

In summary, our findings show that aortic and pulmonary valves tissues have similar mechanical characteristics. We established slight differences between structure of aortic and pulmonary valve elements and their thickness, as well as insignificant distinctions between density and composition of structural elements. We conclude that the pulmonary valve can be considered mechanically and structurally suitable as a long-term substitute for aortic valve.

The results of present study are in good agreement with previous reports regarding comparison of biomechanical

and morphological properties of aortic and pulmonary valve. Our investigations confirm the aortic valve tissue to be thicker than pulmonary valve tissue, as noted by Gross and Kugel [4]. In studies of mechanical properties, Vesely [15] compared cryopreserved aortic and pulmonary homografts and Leeson-Dietrich [16] made the comparison of porcine pulmonary and aortic valves. They both came to conclusion that mechanical differences between valves are minimal and the pulmonary valve can substitute the aortic valve. Up to now there is no evidence of complete studies where the comparison of biomechanical properties of human aortic and pulmonary valves is complemented with the evaluation of their structural properties.

The efficiency of Ross procedure is justified by clinical results. A long-term report by Chambers [3] demonstrates that the autograft was free of replacement 88% at 10 years and 75% at 20 years and pulmonary homograft was free of replacement 69% at 25 years. However, further to the larger use of Ross procedure and after summarization of post-operative results, some questions remain open. A number of authors report about neo-aortic root dilatations leading to progression of aortic regurgitation [18,19]. Neo-aortic valve competence depends on the valve annulus and sinotubular junction. Thus, if the annulus dilatates, the base of the cusps extends and causes valve incompetence. Similarly, if the sinotubular junction dilatates, the commissures extend and disturb cusps coapting. Svensson [20] notes that anatomical mismatch of the pulmonary autograft in the aortic root may be the reason of neo-aortic insufficiency and recommends the careful patient selection and the intraoperative correction of anatomical mismatch. Much less post-operative aortic insufficiency is found, if the annulus is fixed or narrowed prior to anastomosing [21,22]. Adjustment of the diameter of the aortic annulus and the sinotubular junction by plicating or supporting by a synthetic graft in order to fix the annulus at the desired measured size probably is very effective, but should be considered only in older patients, because the autograft has ability to grow in children. Hokken [23] in morphological study points out the structural differences of pulmonary and aortic valve root: pulmonary root is hardly supported by right ventricular myocardium, whereas the aortic root is supported by its wedged position between the left and right atrioventricular annuli and the thick left ventricular myocardium; pulmonary autograft should be inserted as proximally as possible to get support of the fibrous structures of the left ventricular outflow tract and surrounding ventricular and atrial myocardium. The above-mentioned is confirmed by clinical results— there is evidence that the subcoronary implantation technique has a higher failure rate in comparison with root replacement technique.

Despite the results of our biomechanical and structural studies, which demonstrate the similarity of aortic and pulmonary valves, we see that clinical data show some differences after functioning of pulmonary valve in aortic position. We still have insufficient information about biomechanical and structural properties of outflow tract of left and right ventricle. Likewise, further studies should include investigations of biomechanical and structural properties of explanted pulmonary autografts after their functioning in aortic position in order to compare them with unchanged pulmonary valves and to define the potential changes—Carr-White [24] shows adaptation of explanted 4-month-old autograft in histological and mechanical behavior. This kind of studies is restricted due to the low availability of research material.

Summarizing existing studies on morphology and biomechanics of aortic and pulmonary valves, as well as clinical investigations of Ross procedure, we believe that Ross procedure should be recommended for children and young adults, females of childbearing age, patients with aortic endocarditis, and for patients with congenital aortic stenosis and left outflow tract obstruction. The great advantage of the Ross procedure is superior hemodynamic, little or no thromboembolism despite no anticoagulation therapy. In addition, follow-up in children post-Ross procedure has remarkably shown that the autograft can grow with the child [25]. Changes in implantation techniques transitioning from subcoronary to root replacement, intraoperative correction of anatomical mismatch, and creation of the support for annular and sinotubular junction can decrease the incidence of neo-aortic regurgitation. However, overall, results of the Ross procedure are excellent and highly demonstrative.

Acknowledgements

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Appendix A. Conference discussion

Dr A. Corno (*Lausanne, Switzerland*): You are supporting with your study our clinical observation, and the patients after Ross procedure are doing extremely well.

You compared the normal native pulmonary valve leaflets versus normal native aortic valve leaflets. Do you think that you'll be able to find differences in the structure if you analyze pulmonary valve leaflets after a long period in the aortic position, with a different exposure to higher diastolic and systolic pressures, and also different oxygen tension?

Dr Stradins: I think it's maybe the most important question about our study.

We made our investigations only for fresh human valves. During the last years, there were reports summarizing long-term results of Ross procedure. And we see that some problems still remain open.

Many authors report about neo-aortic insufficiency after Ross procedure, and maybe here are two possible reasons. The first is maybe due to technical problems of Ross operation performance and geometrical differences in perioperative period between aortic and pulmonary root.

But on the other hand, here are maybe some structural and biomechanical changes in pulmonary autograft after functioning in human's body in higher pressure. It's very important to continue this work, in this aspect, to investigate properties of explanted pulmonary autografts and to compare this data with fresh pulmonary and aortic valves.