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Mechanical and structural properties of human aortic and pulmonary allografts do not deteriorate in the first 10 years of cryopreservation and storage in nitrogen

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Abstract

The aortic and pulmonary allograft heart valves (AHV) are used in the cardiac surgery for replacing the impaired semilunar valves. They are harvested from donor hearts and cryostored in tissue banks. The expiration period was set to 5 years arbitrarily. We hypothesized that their mechanical and structural properties do not deteriorate after this period. A total of 64 human AHV (31

aortic and 33 pulmonary) of different length of cryopreservation (fresh, 0–5, 5–10, over 10 years) were sampled to different tissue strips (artery, leaflet, ventriculo-arterial junction) and tested by tensile test with loading velocity 10 mm/min until tissue rupture. Neighbouring regions of tissue were processed histologically and evaluated for elastin and collagen area fraction. The results were evaluated statistically. In aortic AHV, the physical deformation response of wall samples to stress did not changed significantly neither during the process of cryopreservation nor during the first 10 years of storage. In pulmonary AHV, the ultimate strain dropped after 5 years of cryopreservation indicating that pulmonary artery was significantly less deformable at the time of rupture. On the other hand, the ultimate stress was equal during the first 10 years of cryostorage. The changes in collagen and elastin amount in the tissue samples were not associated with mechanical impairment. Neither elasticity, stiffness and solidity nor morphology of aortic and pulmonary AHV did not change reasonably with cryopreservation and in the first 10 years of cryostorage. This evidence suggests that the expiration period might be extended in the future.

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Keywords

    Heart valve allograft
    Homograft
    Cryopreservation
    Tissue banking
    Mechanical characteristics
    Structural changes

Electronic supplementary material

The online version of this article (https://doi.org/10.1007/s10561-019-09762-x) contains supplementary material, which is available to authorized users.

Introduction

Despite the undisputable progress in vascular and heart valve prosthesis research and development, the ideal substitute remains an unmet clinical need. Among wide range and designs of manufactured mechanical and tissue (biological) heart valve prosthesis exists a special group of human donor valves—allografts. The use of allograft heart valves (AHV) was developed by Duran and Gunning on animal model in Oxford, UK. They introduced the subcoronary aortic allograft valve transplantation into the subcoronary position (Duran and Gunning 1962).

The history of clinical introduction of the method was fascinating. Three surgeons, in three continents replaced the diseased aortic valve by aortic AHV transplantation independently: Raymond Heimbecker in Toronto, Canada (Heimbecker et al. 1962) Donald Ross in London, UK (Ross 1962) and Sir Brian Barratt-Boyes, Aucland, New Zealand (Barrat-Boyes 1964) from March to August 1962. Pulmonary valve replacement with AHV was introduced subsequently by Donald Ross (Ross 1967; Ross and Somerville 1966). Nevertheless, clinical use of AHV remains controversial up to now. Nowadays, in many cardiac surgery centres of excellence aortic and pulmonary AHV are routinely used. AHV were proved to be extremely useful in congenital heart defects surgery. In heart valve surgery of adolescents and adults they became popular for repair of particular acquired pulmonary and aortic valve disease. Most often for infective endocarditis and for aortic valve replacement by means of pulmonary autograft (Ross 1967) in young and middle-aged adults and in women of childbearing age. AHV offer an excellent hemodynamic performance by restoring nearly normal anatomy, have higher resistance to infection compared to all other valves, do not require anticoagulation, but their use is limited by long-term durability issues (Barron et al. 2010; Da Costa et al. 2006; Stoliński et al. 2006). The most important drawback of the use of allografts remains their availability (Antunes 2018).

The AHV are harvested from cadaveric donor hearts not suitable for transplantation (for any reason). The morphological suitability of AHV strongly depends on the age of donors. The age limit for donor is usually set to 65 years (Jashari et al. 2004). Some facilities have raised the age limit to 70 years following the findings that especially pulmonary valves are not affected by age-related tissue changes (Grosse et al. 2008). The further utilization depends on the time of transplantation. Previously used "homovital" AHV were harvested in the regime of organ transplant from the brain-dead organ donors or cardiac transplant recipients, stored at 4 °C in tissue culture medium and kept unprocessed until their implantation within 48 h (Yacoub et al. 1995). Present legislation has led to abandoning this method in many countries.

The "fresh" AHV can be harvested either at the time of standard multiorgan harvesting or from "non-heart-beating donors" with a warm ischemia time of less than 6 h. The hearts are processed under sterile conditions in a laminar flow cabinet. Fresh AHV are sterilized by a solution containing a combination of antibiotics in which they remain at 4 °C for 7 days. Thorough quality and safety testing (microbiological and histological) take place within this period. Fresh AHV have to be transplanted within 6 weeks after the decontamination period (Anastasiadis et al. 2004) and therefore do not allow longer storage and wider availability.

Current method of choice is antibiotic decontamination and subsequent cryopreservation. The AHV are harvested and processed in the same way as fresh and thereafter frozen (programme cooled) in liquid nitrogen and stored in tissue banks. The main advantage of cryopreservation and the existence of a tissue bank is the on-call availability for cardiac surgery centre. However, there can be an imbalance between supply and demand. Shelf life of cryopreserved aortic and pulmonary allografts was arbitrarily set on 5 years in most cardiovascular tissue banks around the world. Expired heart valves are discarded. Hence the number of available AHV can be limited (Spatenka and Burkert 2018).

Aortic and pulmonary (semilunar) AHV structure

Semilunar AHV consist of the arterial root, valve leaflets and a corresponding artery. Despite their similar function, aortic and pulmonary roots differ in their gross anatomy and histology, reflecting the different hemodynamic condition they have to act in. Aortic root exposed to the systemic pressure is thicker, stouter and contains more fibro-elastic tissue than the pulmonary one (Muresian 2018). The base of the aortic heart valve that supports the valve leaflets (annulus) is formed by dense collagenous tissue. According to Muresian's work, the pulmonary valve leaflets has no annulus in the sense of a fibrous ring. They take off directly from the muscles of the right ventricular infundibulum (Muresian 2016). Both aortic and pulmonary valves have 3 leaflets of semilunar shape. The leaflets of aortic valve are commonly named by their relationship to the coronary arteries—left coronary, right coronary and non-coronary. The pulmonary valve leaflets are classified as left, right and anterior according to usual nomenclature (Nomina anatomica), although their real-life positions are rather posterior, right anterior and left anterior (Muresian 2016). Human valve leaflets are normally pliable and thinner than 1 mm. Aortic leaflets are slightly thicker than the pulmonary ones as they belong to left-side of the heart maintaining significantly higher pressures than the right side (Hinton and Yutzey 2011).

The structure of valve leaflets consists of three layers—the fibrosa, spongiosa and ventricularis. Their histological structure is composed by endothelium, connective tissue cells and extracellular matrix. The main constituents of the connective tissue are collagen fibrils, elastic fibres and proteoglycans (Hopkins 2005). Collagen is the major extracellular matrix (ECM) component of the valves forming approximately 50% of the total valve by dry weight, elastin comprises 13% of the ECM (Bashey et al. 1967; Kubíková et al. 2017). Elastic arteries (ascending aorta, pulmonary artery) consists of three layers as follows: the tunica intima (endothelium and subendothelial layer), the tunica media

(elastic membranes and smooth muscle cells) and the tunica adventitia (elastic and collagenous fibres, connective tissue cells, blood vessels, nerve fibres) (Kubíková et al. 2016).

Aortic and pulmonary valve mechanics

The heart valve's function is to maintain unimpeded unidirectional blood flow. While the heart ventricles propel the blood into aorta and pulmonary artery in systole, the semilunar valves placed in the left and right ventricular outlets prevent reversal blood flow to the ventricles in diastole. Therefore, their biomechanical properties are very important, essential to life. The dynamics anatomy of the aortic root is very complex (Vojacek et al. 2018). In a simplified interpretation the leaflets are passive elements that open and close rapidly to blood flow. They are pushed to open as soon as the pressure in the ventricle exceeds the pressure in the artery and closed by the backflow of the blood when the pressure in the ventricle drops at the end of systole. The valve leaflets are fully loaded in diastole (when the valve is closed) and relaxed in systole. It is believed that biomechanical behaviour is dominated by collagen and elastin under high tensile loading (Eckert et al. 2013). Collagen and elastin are curved fibres that change their shape according to the valve movement—crimping when the tissue is relaxed and flattening when the tissue is under stress. The collagen provides strength to the leaflets, while the purpose of the elastin is to maintain a specific collagen fibre configuration and return the fibres to this "resting" state between loading cycles (Vesely 1998). The single layers of valve leaflets are bonded by transverse collagen fibres and work as one unit (Buchanan and Sacks 2014).

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Study aims

The aim of our study is to assess the mechanical and structural properties of aortic (AAHV) and pulmonary (PAHV) allograft heart valves according to the duration of cryopreservation and compare them to the fresh (not cryopreserved) grafts. We hypothesize that mechanical and structural attributes of AHV do not deteriorate after 5 years of cryopreservation period reasonable. We wonder if there are any signs of mechanical and/or structural deterioration detectable after cryostorage longer than 5 years and whether the mechanical and structural parameters of AAHV and PAHV differ between fresh and less than 10 years cryopreserved ones.

Materials and methods

Specimens

A total of 64 AHV (31 aortic and 33 pulmonary) were studied. The control group (group 0) consisted of fresh AHV. The rest of AHV of different duration of cryopreservation were divided into three groups as follows:

Group I: cryopreservation period 0.1–4.9 years.

Group II: cryopreservation period 5–9.9 years.

Group III: cryopreservation period more than 10 years.

The group demographics and cryostorage characteristics are summarized in Table 1.

Table 1

Demographics and cryostorage characteristics

Variable	Group 0 (fresh)	Group I (cryo 0.1–4.9 years)	Group II (cryo 5–9.9 years)	Group III (cryo > 10 years)
Aortic AHV				
n	3	9	11	8
Gender				
Male	2	4	2	3
Female	1	5	9	5
Donor age (years) (median, interquartile range)	51.35 (33.07–58.14)	49.08 (40.73–51.91)	45.64 (40.59–50.33)	44.36 (35.43–47.37)
Cryopreservation (years) (median, interquartile range)	N/A	3.89 (0.94–4.48)	5.26 (5.10–5.32)	12.74 (10.51–14.45)
Pulmonary AHV				
n	4	12	8	9
Gender				
Male	3	5	3	5
Female	1	7	5	4
Donor age (years) (median, interquartile range)	54.74 (37.64–62.93)	49.99 (45.23–54.96)	53.36 (51.28–56.69)	46.66 (38.91–53.79)

AHV allograft heart valve

Variable	Group 0 (fresh)	Group I (cryo 0.1–4.9 years)	Group II (cryo 5–9.9 years)	Group III (cryo > 10 years)
Cryopreservation (years) (median, interquartile range)	N/A	2.98 (1.11–4.54)	7.48 (6.80–8.85)	15.21 (11.81–17.28)
AHV allograft heart valve				

All samples of fresh and cryopreserved AHV were obtained from the National AHV Bank of the Department of Transplantations and Tissue Bank, Motol University Hospital, Prague, Czech Republic. They were harvested from the pool of heart beating multiorgan donors in case when the donor heart was not used for transplantation as an organ for any reason. All AHV were dissected from the donor hearts and in the time of the quality control assessed as suitable for clinical use. They were decontaminated by an antibiotic cocktail according to the established protocol of the tissue bank (Spatenka et al. 1997). Subsequently, all AHV were moved into the cryoprotectant solution—10% dimethylsulfoxide (DMSO)—and packed by double layer technique (sealed in Gambro Hemofreeze bags, NPBI BV, Gambro, Netherlands). Packed AHV were programme cooled (at a rate of − 1 °C/min) and stored in cryocontainer in the liquid phase of liquid nitrogen at − 196 °C in compliance with the Czech legislation (Czech Law on Quality and Safety of Human Tissue and Cells for Application in Humans 296/2008 of Czech Legal Code).

Randomly selected samples in their original packaging were transported in the cryocontainers (CXR 500, Wharton-Tylor International LLC, USA, with a digital thermometer and datalogger) to the research facility. After removal they were left at room temperature for at least 15 min to equilibrate. Packages were then immersed directly into a water bath at + 37 °C for another 15 min at least. This thawing protocol was selected based on previous own unpublished experimental work (protocol validation) and is still routinely used in our tissue establishment.

After completing the thawing protocol, the allografts were unpacked and processed. AHV was opened longitudinally by incision between the leaflets. Custom made devices with two longitudinally placed razor blades in the distance of 5 and 10 millimetres were then used for tissue strips cutting to ensure the samples homogeneity. From each allograft we cut one specimen of the ventriculo-arterial junction (10 mm in width), prepared two leaflet specimens (5 mm in width) and 1–3 (depending on the AHV size) artery specimens (10 mm in width) as shown in Fig. 1. The ventriculo-arterial junction was selected as the composite specimen containing both arterial wall and leaflet tissue. The number

of specimens is summarized in Table 2. Neighbouring region of each sample was used for histological assessment. All samples were washed in saline solution (0.9% solution of sodium chloride) before further evaluation. The mechanical testing was performed on the day of sampling. Tissue samples for histological analysis were fixed with 4% buffered formalin and underwent standard histological processing.

Fig. 1

a Scheme for the preparation of tissue specimens from the allograft heart valve (AHV) under study: Two 5 mm wide transversal cuts in the middle of the leaflets and one 10 mm wide longitudinal cut through the ventriculo-arterial junction (V-A junction) with adjacent part of the leaflet were made in each AHV. The 10 mm wide samples of arterial wall were cut longitudinally out of the ascending aorta. **b** Pulmonary AHV opened by longitudinal incision between leaflets. **c** Aortic AHV sampling was performed in coronary leaflets, V-A junction in non-coronary leaflet
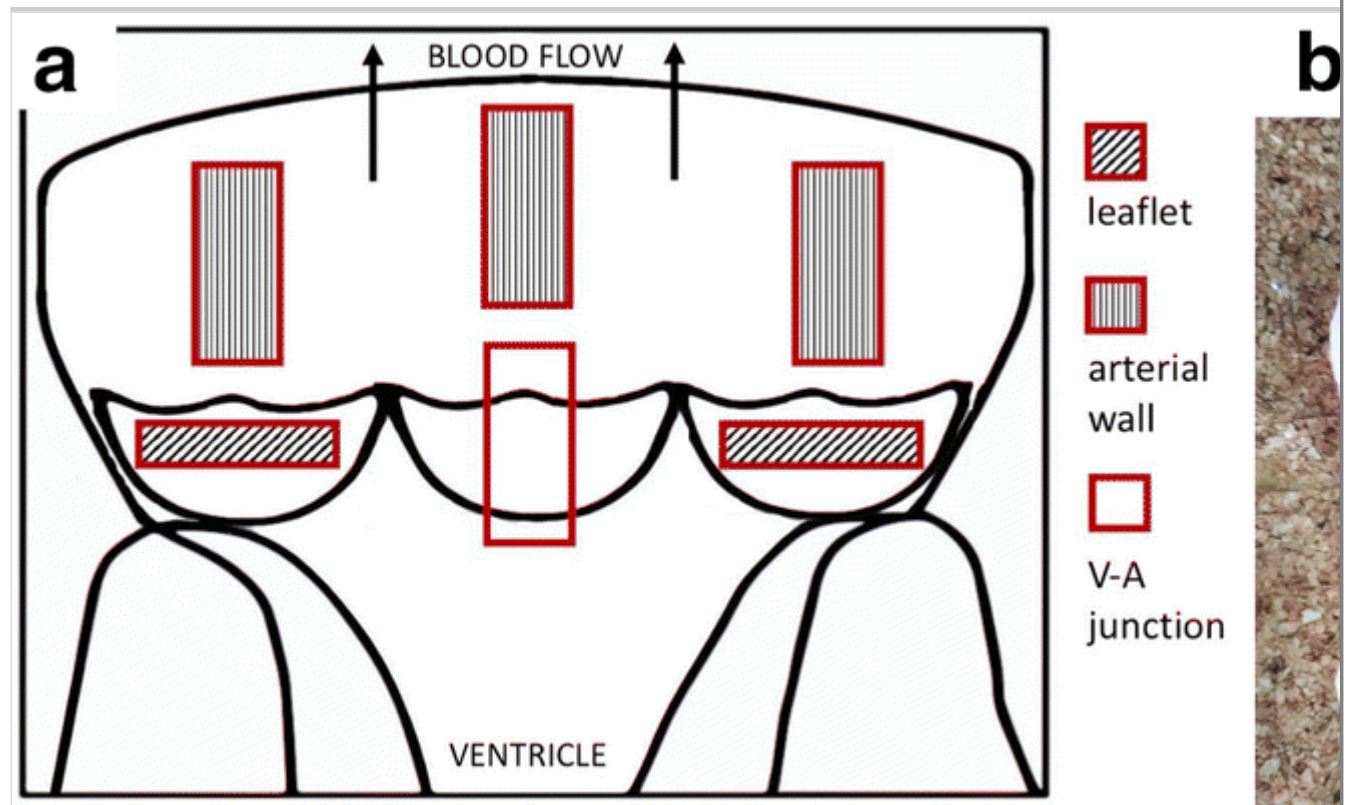

Table 2

Number of tissue samples in different groups according to their duration of cryopreservation

	Group 0	Group I	Group II	Group III

|	Group 0	Group I	Group II	Group III
Aortic AHV	3	9	11	8
Artery	6	11	26	6
Leaflet	6	18	21	16
Junction	3	9	11	8
Pulmonary AHV	4	12	8	9
Artery	9	21	10	11
Leaflet	7	21	16	14
Junction	4	12	8	9

AHV, allograft heart valve; group 0, fresh; group I, cryopreserved for 0.1–4.9 years; group II, cryopreserved for 5–9.9 years; group III, cryopreserved for more than 10 years

Mechanical loading

Mechanical properties, namely Young's moduli of elasticity (E), ultimate stresses and strains, of all AHV samples were assessed by tensile test. Tissue strips were tested using Zwick/Roel Z50 traction machine (Zwick GmbH & Co, Germany) equipped with a 1 kN load cell. Geometrical measurements of sample thickness were obtained using a calliper. The specimens were clamped into the jaws of measurement device at the initial length of 10 mm (Fig. 2a). The specimens were first preconditioned using 20 loading and unloading cycles up to 10% of the initial specimen's length with the velocity 10 mm/min. After preconditioning the specimens were loaded by strain velocity 10 mm/min until the tissue rupture (Fig. 2b). All specimens were tested at room temperature and held moistened by saline solution during the experiment. The mechanical measurement and evaluation protocol were described previously in our own studies (Kubíková et al. 2016, 2017).

Fig. 2

Mechanical measurement: **a** The aortas wall specimen in the jaws of the measurement device Zwick/Roell Z050. **b** A rupture of the aortas wall specimen. **c** An example of a nonlinear stress–strain curve of the aortic wall. The small deformation region (blue line) with a low stiffness and the linear region of large deformations (red line) with a higher stiffness. The star marks the beginning of the rupture of the tissue characterized by ultimate stress and strain
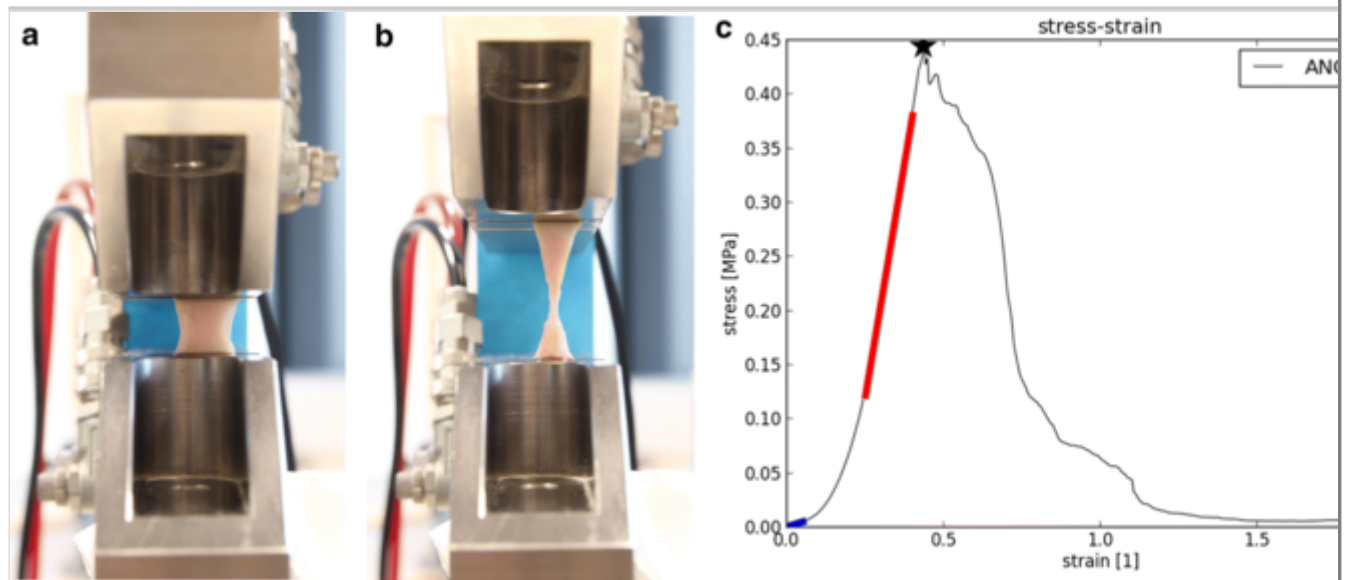
The result of mechanical measurement was the relation between loading and deformation: the stress–strain curve (Fig. 2c). The strain was defined as the actual specimen elongation divided by initial length of the specimen. The stress was defined as the actual force divided by the initial cross-sectional area. The Young's moduli of elasticity in the small deformation region (E_0) as well as in the large deformation region (E_1) were determined using linear regression of the stress–strain curve. The strains intervals of the small and large deformation regions were approximately 0–4% and 20–40% for the elastic arteries, 0–2% and 10–20% for the leaflets, 0–2% and 10–20% for the ventriculo-arterial junctions, respectively, depending on the shape of the curve. The ultimate stresses and the ultimate strains were determined at the start of the rupture. Note: the Young's modulus of elasticity characterizes physiological stiffness of the tissue. The in-house software enabling semiautomatic evaluation of mechanical measurements (Elfpy) was used for the evaluation (Elfpy2018, http://docs.sfepy.org/elfpy/doc-devel/index.html#).

Histological processing and assessment

The 5-μm-thick histological sections obtained from each formalin-fixed, paraffin-embedded tissue block were used for assessment. Two sections were stained with haematoxylin–eosin and two sections were stained with Verhoeff's haematoxylin and green trichrome to visualize the connective tissue. Two sections were stained with orcein to visualize the elastic fibres, two sections were stained with picrosirius red to visualize the type I collagen using circularly polarized light. In total, 849 slides and 5094 micrographs were examined histologically (Table 3, Fig. 3).

Table 3

Sampling of micrographs for measuring the structural parameters of the artery, leaflet and ventriculo-arterial junction

Evaluated parameter	Staining	Number of microphotographs per specimen	Used objective in microscope
Whole wall thickness (MT)	Verhoeff's haematoxylin and green trichrome	2	4×
Area fraction of elastin within wall [A_A(elastin)]	Orcein	8	40×
Area fraction of collagen within wall [A_A(collagen)]	Picrosirius red	8	20×

A_A area fraction, *MT* medium thickness

Fig. 3

An example of histological staining for the aortic wall (group II): **a** Verhoeff's haematoxylin and green trichrome to visualize the connective tissue (collagen I—green, elastin—black). Scale bar = 500 μm. **b** Visualization of the red–brown elastic fibres with orcein. Scale bar = 50 μm. **c** Visualization of the red collagen I fibres with picrosirius red in polarized light. Scale bar = 50 μm
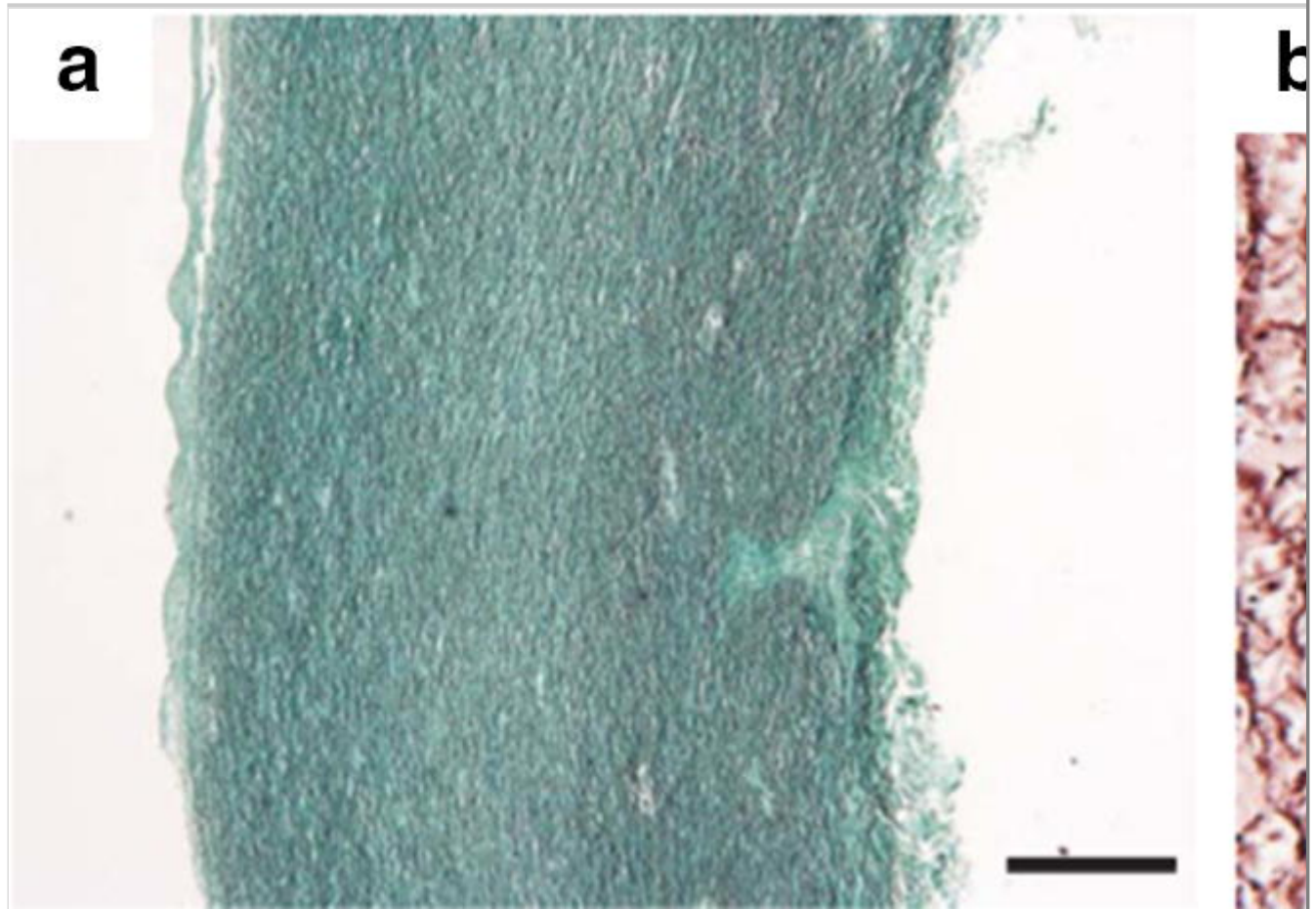
The area fraction (A_A) of elastin and collagen were evaluated by stereological morphometry using a stereological grid point test system based on the Cavalieri's method (Kochová et al. 2012; Kubíková et al. 2017; Mouton 2002; Tonar et al. 2015).

Statistics

The data were processed using Statistica Base 10 (StatSoft, Inc., Tulsa, OK, USA). Normality of mechanical and structural parameters was tested by the Shapiro–Wilk's W test, for the differences in parameters between groups the Kruskal–Wallis ANOVA and Man-Whitney U tests were used. The Spearman's rank correlation test was used to find relation between duration of cryopreservation and mechanical and structural parameters. The significance level was set at the p value of 0.05 or less. The resultant parameters are shown as median (interquartile range).

Results

Mechanical and structural properties of aortic AHV (AAHV)

The mechanical and structural properties of AAHV are summarized in Table 4 and Figs. 4, 5, 6. The complete primary data from the analyses are provided in

Supplement 1.

Table 4

The mechanical and structural properties of the aortic allograft heart valves in different time groups

	Group 0	Group I	Group II	Group III
E_0 (MPa)				
Wall	0.24 (0.08–0.34)	0.34 (0.16–1.54)	0.18 (0.13–0.25)	0.10 (0.06–0.12)
Leaflet	0.87 (0.46–2.56)	2.33 (0.69–4.99)	1.59 (0.80–4.04)	1.16 (0.20–2.15)
V-A junction	0.29 (0.07–0.38)	0.23 (0.22–0.85)	0.16 (0.09–0.24)	0.10 (0.07–0.18)
E_1 (MPa)				
Wall	2.31 (1.01–3.97)	3.94 (1.28–9.95)	1.82 (1.19–4.34)	1.26 (0.44–1.59)
Leaflet	20.53 (15.77–47.35)	19.55 (12.61–29.01)	24.17 (16.63–34.72)	13.02 (10.16–22.38)
V-A junction	0.84 (0.58–1.80)	2.21 (0.86–2.85)	1.17 (1.00–1.27)	0.93 (0.80–1.32)
Ultimate strain				
Wall	0.36 (0.21–0.45)	0.45 (0.35–0.67)	0.50 (0.47–0.59)	0.38 (0.31–0.72)
Leaflet	0.19 (0.17–0.29)	0.33 (0.20–0.37)	0.22 (0.16–0.31)	0.34 (0.27–0.40)
V-A junction	0.25 (0.13–0.56)	0.43 (0.23–0.51)	0.32 (0.26–0.43)	0.45 (0.38–0.56)
Ultimate stress (MPa)				
Wall	0.49 (0.30–0.60)	0.83 (0.37–5.69)	0.54 (0.36–1.03)	0.16 (0.05–0.50)
Leaflet	3.38 (2.22–4.14)	4.76 (2.34–8.13)	3.98 (3.04–4.24)	3.32 (2.33–4.75)
V-A junction	0.18 (0.16–0.22)	0.34 (0.31–0.48)	0.25 (0.18–0.31)	0.28 (0.25–0.41)
A_A (elastin)				
Wall	0.47 (0.46–0.60)	0.43 (0.38–0.44)	0.45 (0.40–0.49)	0.44 (0.41–0.50)
Leaflet	0.15 (0.11–0.16)	0.14 (0.08–0.18)	0.11 (0.08–0.16)	0.18 (0.13–0.22)

Median values (interquartile range). E_0 and E_1—Young's modulus of elasticity in the small and large deformation region, respectively

A_A, area fraction; MT, medium thickness; V-A junction, ventriculo-arterial junction; group 0, fresh, group I, cryopreserved for 0.1–4.9 years, group II, cryopreserved for 5–9.9 years, group III, cryopreserved for more than 10 years

	Group 0	Group I	Group II	Group III
V-A junction	0.23 (0.20–0.30)	0.29 (0.22–0.35)	0.29 (0.25–0.34)	0.33 (0.25–0.40)
A_A(collagen)				
Wall	0.07 (0.02–0.18)	0.18 (0.10–0.25)	0.03 (0.01–0.11)	0.12 (0.08–0.33)
Leaflet	0.21 (0.13–0.33)	0.38 (0.31–0.48)	0.27 (0.12–0.41)	0.48 (0.41–0.58)
V-A junction	0.12 (0.11–0.29)	0.27 (0.17–0.32)	0.13 (0.06–0.40)	0.32 (0.25–0.37)
MT (µm)				
Wall	1941.14 (1681.14–2137.03)	1763.41 (1682.01–2116.60)	1914.17 (1638.27–2207.02)	2230.03 (1717.23–2803.79)
Leaflet	479.33 (417.00–540.00)	540.52 (431.42–637.44)	501.00 (365.00–668.00)	506.69 (470.34–602.42)
V-A junction	911.18 (702.89–1458.44)	1209.07 (997.10–1395.08)	1052.06 (897.63–1370.51)	1081.31 (896.78–1191.41)

Median values (interquartile range). E_0 and E_1—Young's modulus of elasticity in the small and large deformation region, respectively

A_A, area fraction; MT, medium thickness; V-A junction, ventriculo-arterial junction; group 0, fresh, group I, cryopreserved for 0.1–4.9 years, group II, cryopreserved for 5–9.9 years, group III, cryopreserved for more than 10 years

Fig. 4

The mechanical and structural parameters of the arterial wall samples of aortic allograft heart valves (AAHV): the Young's modulus of elasticity in the small deformation region (E_0, **a**), the Young′s modulus of elasticity in the large deformation region (E_1, **b**), ultimate strain (**c**), ultimate stress (**d**), area fraction of elastin [A_A(elastin), **e**], area fraction of collagen I [A_A(collagen I), **f**], and medium thickness (MT, **g**). The square dot denotes the median, the rectangle spans the interquartile range, the whiskers denote the limit values, p value denote the significant differences between groups (Kruskal–Wallis ANOVA), and $*p < 0.05$; $**p < 0.01$; $***p < 0.001$ (Mann–Whitney U test). The results showing significant differences between groups I–II, I–III and II–III in E_0 and no differences in E_1. Ultimate strain differed between groups 0–II, ultimate stress differed between groups I–III and II–III. A_A(collagen I) differed between groups I–II and II–III. No differences were found in A_A(elastin) and MT
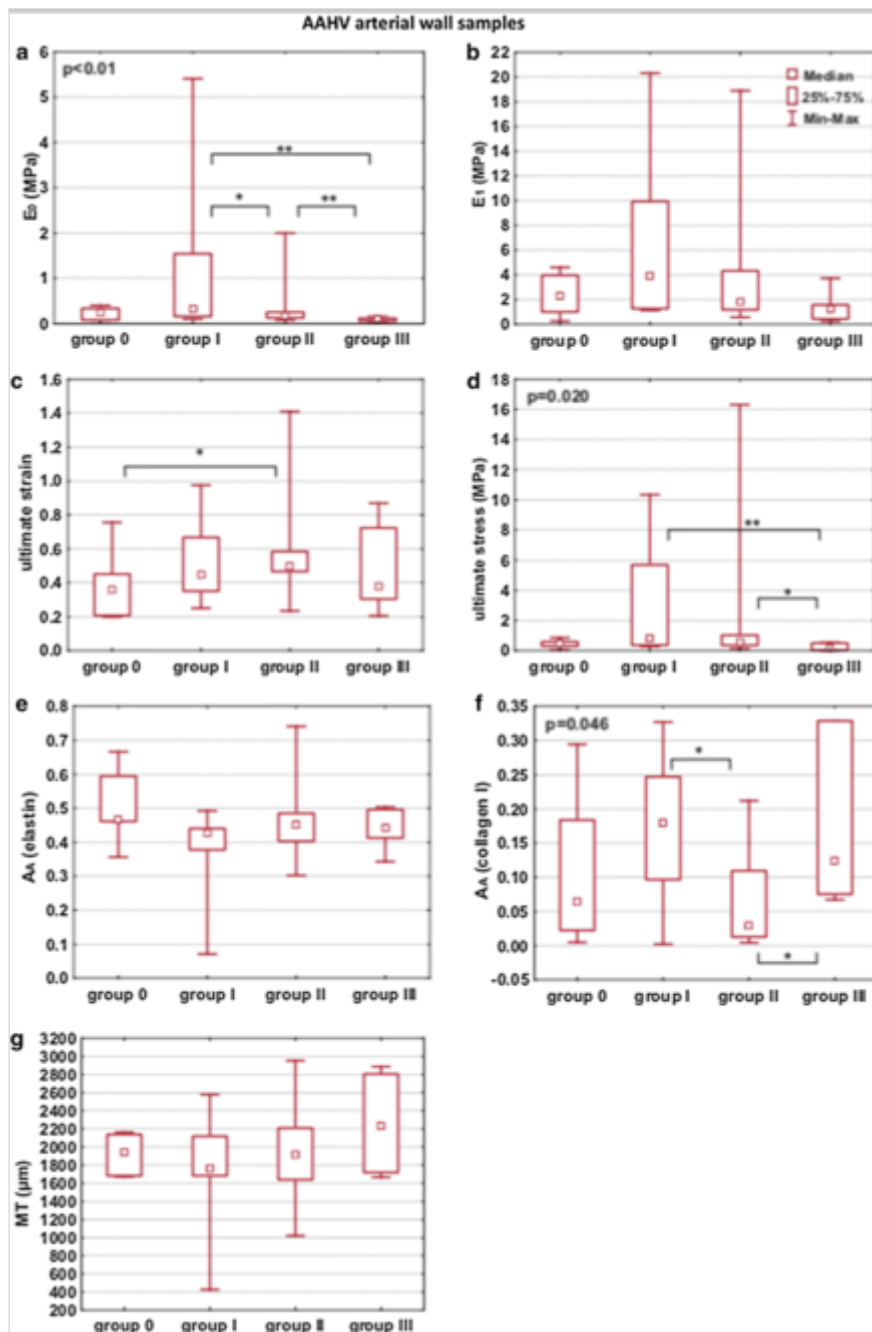
Fig. 5

The mechanical and structural parameters of the leaflet samples of aortic allograft heart valves (AAHV): the Young's modulus of elasticity in the small deformation region (E_0, **a**), the Young´s modulus of elasticity in the large deformation region (E_1, **b**), ultimate strain (**c**), ultimate stress (**d**), area fraction of elastin [A_A(elastin), **e**], area fraction of collagen I [A_A(collagen I), **f**], and medium thickness (MT, **g**). The square dot denotes the median, the rectangle spans the interquartile range, the whiskers denote the limit values, p value denote the significant differences between groups (Kruskal–Wallis ANOVA), and $*p < 0.05$; $**p < 0.01$; $***p < 0.001$ (Mann–Whitney U test). The results showing significant differences between groups I–III in E_0 and no differences in E_1. Ultimate strain differed between groups 0–III, I–II and II–III. No differences were observed in ultimate

stress. A_A(elastin) increased between group II and III. A_A(collagen I) differed between groups 0–III, I–III and II–III. No differences were found in MT
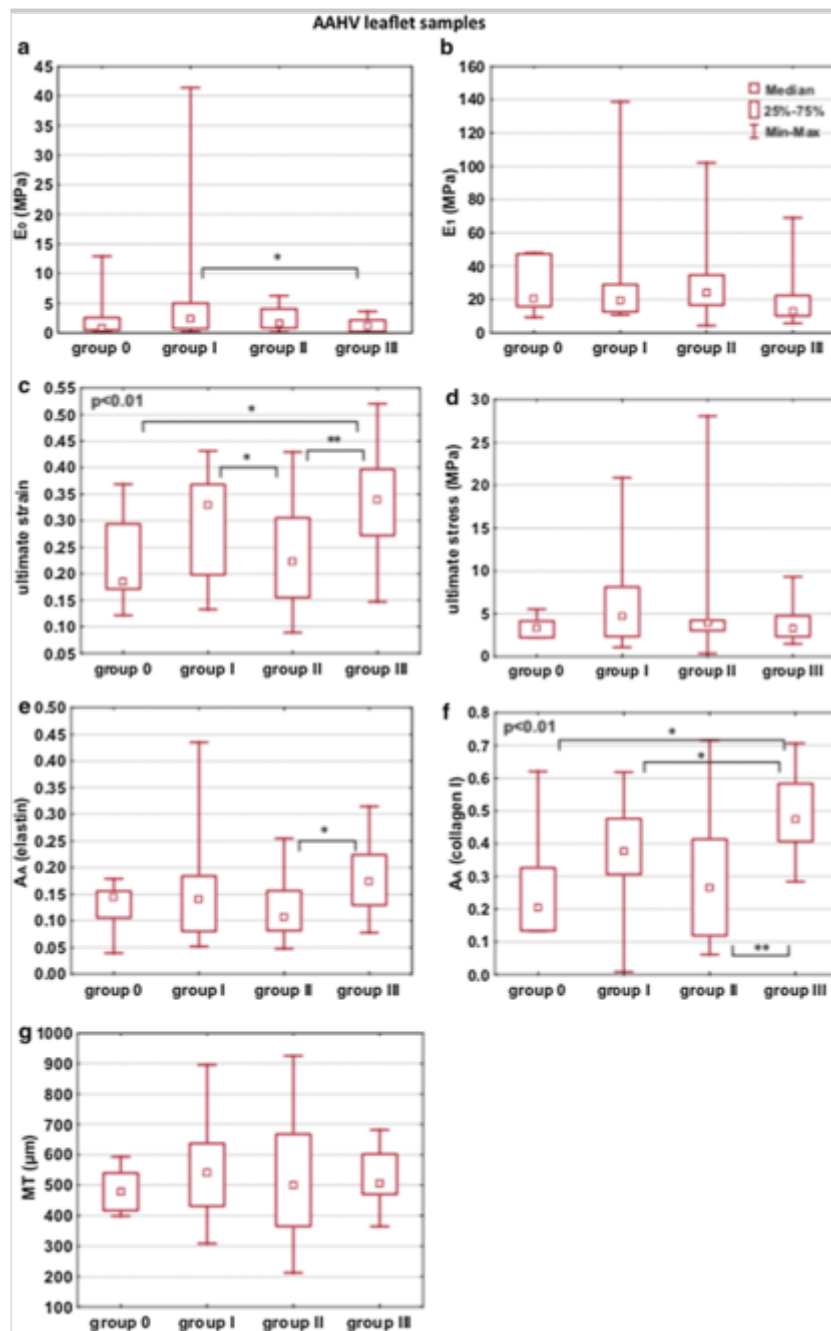

Fig. 6

The mechanical and structural parameters of the ventriculo-arterial junction samples of aortic allograft heart valves (AAHV): the Young's modulus of elasticity in the small deformation region (E_0, **a**), the Young's modulus of elasticity in the large deformation region (E_1, **b**), ultimate strain (**c**), ultimate stress (**d**), area fraction of elastin [A_A(elastin), **e**], area fraction of collagen I [A_A(collagen I), **f**], and medium thickness (MT, **g**). The square dot denotes the median, the rectangle spans the interquartile range, the whiskers denote the limit values, p value denote the significant differences between groups (Kruskal–Wallis ANOVA), and $*p < 0.05$; $**p < 0.01$; $***p < 0.001$ (Mann–Whitney U test). The results showing

significant differences between groups I–III in E_0 and no differences in E_1. Ultimate strain did not differ. Ultimate stress differed between groups 0–I and I–II. No differences were observed in A_A(elastin), A_A(collagen I) and MT
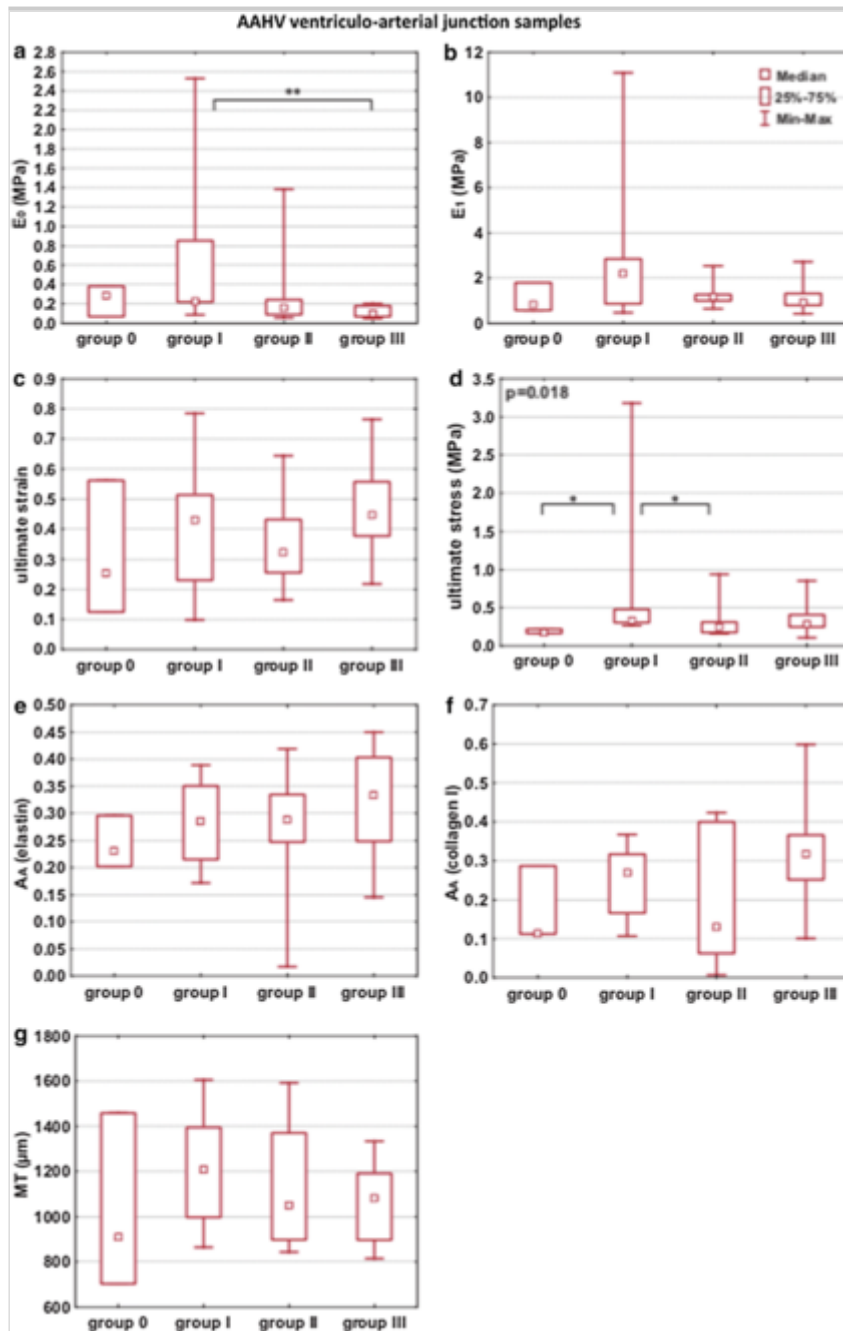
Mechanical and structural properties of pulmonary AHV (PAHV)

The mechanical and structural properties of PAHV are summarized in Table 5 and Figs. 7, 8, 9. The complete primary data from the analyses are provided in Supplement 1.

Table 5

The mechanical and structural properties of the pulmonary allograft heart valves in different time groups

	Group 0	Group I	Group II	Group III
E_0 (MPa)				
Wall	0.09 (0.06–0.12)	0.13 (0.09–0.21)	0.32 (0.20–0.49)	0.14 (0.05–0.22)
Leaflet	1.17 (0.40–6.32)	2.33 (0.90–4.98)	1.61 (0.71–6.77)	2.12 (0.59–7.32)
V-A junction	0.36 (0.19–0.54)	0.21 (0.14–0.33)	0.43 (0.18–0.83)	0.22 (0.15–0.31)
E_1 (MPa)				
Wall	0.97 (0.91–1.17)	0.99 (0.58–1.95)	2.03 (1.18–3.04)	1.23 (1.10–2.07)
Leaflet	15.51 (11.13–30.02)	15.48 (10.77–24.49)	18.49 (8.59–34.02)	22.66 (12.32–43.74)
V-A junction	2.10 (1.21–2.83)	1.54 (0.67–2.09)	2.47 (1.47–4.08)	1.30 (0.64–1.53)
Ultimate strain				
Wall	0.45 (0.34–0.81)	0.46 (0.31–0.67)	0.20 (0.15–0.25)	0.41 (0.33–0.48)
Leaflet	0.14 (0.11–0.23)	0.25 (0.17–0.39)	0.15 (0.12–0.31)	0.29 (0.17–0.37)
V-A junction	0.21 (0.15–0.34)	0.18 (0.16–0.21)	0.13 (0.12–0.18)	0.25 (0.22–0.33)
Ultimate stress (MPa)				
Wall	0.33 (0.27–0.36)	0.31 (0.20–0.45)	0.31 (0.19–0.46)	0.39 (0.34–0.42)
Leaflet	2.23 (1.01–2.95)	3.07 (2.04–3.84)	2.34 (1.08–4.38)	4.69 (3.00–5.50)
V-A junction	0.26 (0.22–0.33)	0.16 (0.09–0.25)	0.23 (0.17–0.38)	0.20 (0.13–0.29)
A_A (elastin)				
Wall	0.37 (0.32–0.39)	0.36 (0.27–0.40)	0.40 (0.37–0.43)	0.41 (0.34–0.42)
Leaflet	0.08 (0.05–0.13)	0.12 (0.10–0.18)	0.13 (0.09–0.16)	0.10 (0.08–0.27)
V-A junction	0.22 (0.19–0.25)	0.21 (0.14–0.29)	0.24 (0.22–0.25)	0.25 (0.25–0.29)

Median values (interquartile range). E_0 and E_1—Young's modulus of elasticity in the small and large deformation region, respectively

A_A, area fraction; MT, medium thickness; V-A junction, ventriculo-arterial junction; group 0, fresh, group I, cryopreserved for 0.1–4.9 years, group II, cryopreserved for 5–9.9 years, group III, cryopreserved for more than 10 years

	Group 0	Group I	Group II	Group III
A_A(collagen)				
Wall	0.08 (0.05–0.19)	0.18 (0.03–0.28)	0.03 (0.02–0.07)	0.14 (0.07–0.17)
Leaflet	0.21 (0.18–0.25)	0.32 (0.26–0.43)	0.36 (0.17–0.43)	0.31 (0.18–0.39)
V-A junction	0.13 (0.08–0.21)	0.36 (0.06–0.43)	0.08 (0.07–0.27)	0.24 (0.10–0.33)
MT (μm)				
Wall	1316.86 (1017.65–1368.02)	1239.31 (1150.27–1701.19)	1370.92 (1160.06–1606.94)	1005.53 (852.56–1395.19)
Leaflet	317.00 (283.00–375.00)	427.91 (329.78–505.36)	325.72 (248.00–416.00)	382.50 (287.00–454.51)
V-A junction	709.33 (662.18–808.45)	823.56 (687.78–888.51)	819.95 (657.74–892.81)	868.60 (822.42–1098.59)
Median values (interquartile range). E_0 and E_1—Young's modulus of elasticity in the small and large deformation region, respectively				
A_A, area fraction; MT, medium thickness; V-A junction, ventriculo-arterial junction; group 0, fresh, group I, cryopreserved for 0.1–4.9 years, group II, cryopreserved for 5–9.9 years, group III, cryopreserved for more than 10 years				

Fig. 7

The mechanical and structural parameters of the arterial wall samples of pulmonary allograft heart valves (PAHV): the Young's modulus of elasticity in the small deformation region (E_0, **a**), the Young's modulus of elasticity in the large deformation region (E_1, **b**), ultimate strain (**c**), ultimate stress (**d**), area fraction of elastin [A_A(elastin), **e**], area fraction of collagen I [A_A(collagen I), **f**], and medium thickness (MT, **g**). The square dot denotes the median, the rectangle spans the interquartile range, the whiskers denote the limit values, p value denote the significant differences between groups (Kruskal–Wallis ANOVA), and *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ (Mann–Whitney U test). The results showing significant differences between groups 0–II, I–II and II–III in E_0 and no differences in E_1. Ultimate strain differed between groups 0–II, I–II and II–III. No differences were found in ultimate stress. A_A(elastin) differed between groups I–II and I–III, A_A(collagen I) differed between groups I–II and II–III, MT between groups I–III
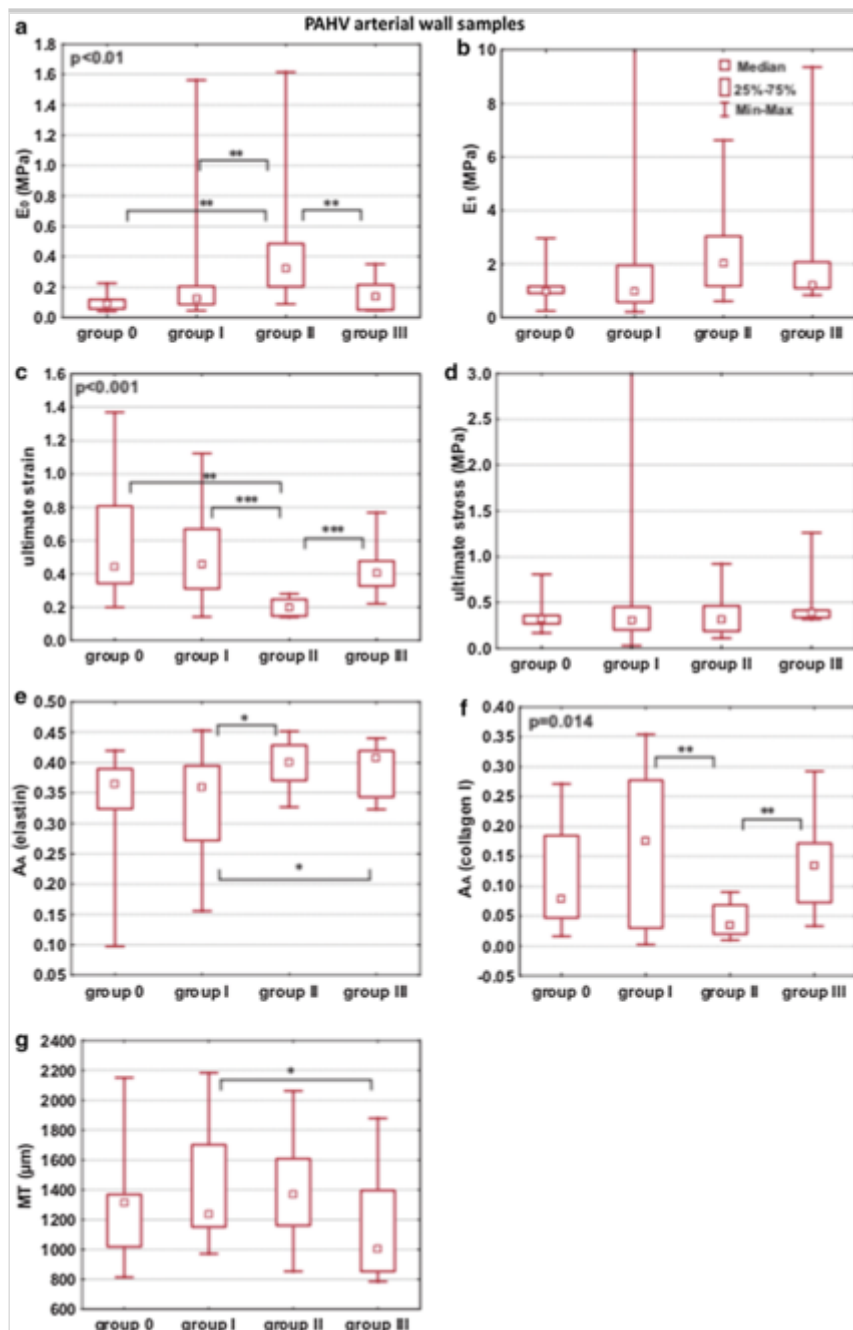
Fig. 8

The mechanical and structural parameters of the leaflet samples of pulmonary allograft heart valves (PAHV): the Young's modulus of elasticity in the small deformation region (E_0, **a**), the Young's modulus of elasticity in the large deformation region (E_1, **b**), ultimate strain (**c**), ultimate stress (**d**), area fraction of elastin [A_A(elastin), **e**], area fraction of collagen I [A_A(collagen I), **f**], and medium thickness (MT, **g**). The square dot denotes the median, the rectangle spans the interquartile range, the whiskers denote the limit values, p value denote the significant differences between groups (Kruskal–Wallis ANOVA), and *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ (Mann–Whitney U test). The results showing no differences in E_0 and E_1. Ultimate strain differed between groups 0–I and 0–III. Ultimate stress differed between groups 0–III, I–III and II–III. No changes were

observed in A_A(elastin). A_A(collagen I) differed between groups 0–I. MT differed between groups I–II
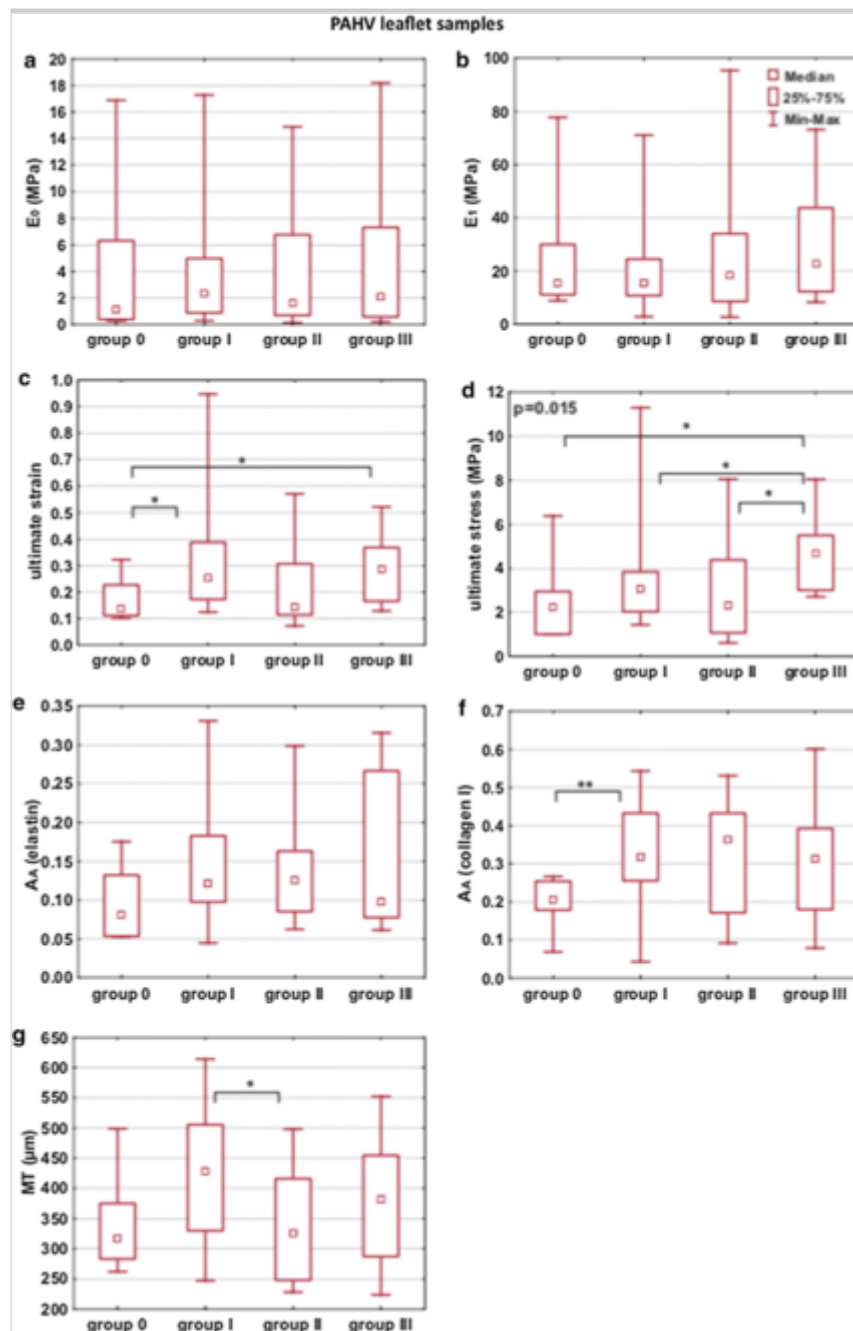

Fig. 9

The mechanical and structural parameters of the ventriculo-arterial samples of pulmonary allograft heart valves (PAHV): the Young's modulus of elasticity in the small deformation region (E_0, **a**), the Young's modulus of elasticity in the large deformation region (E_1, **b**), ultimate strain (**c**), ultimate stress (**d**), area fraction of elastin [A_A(elastin), **e**], area fraction of collagen I [A_A(collagen I), **f**], and medium thickness (MT, **g**). The square dot denotes the median, the rectangle spans the interquartile range, the whiskers denote the limit values, p value denote the significant differences between groups (Kruskal–Wallis ANOVA), and *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ (Mann–Whitney U test). The results showing differences

in ultimate strain between groups I–III. No differences were observed in the other variables
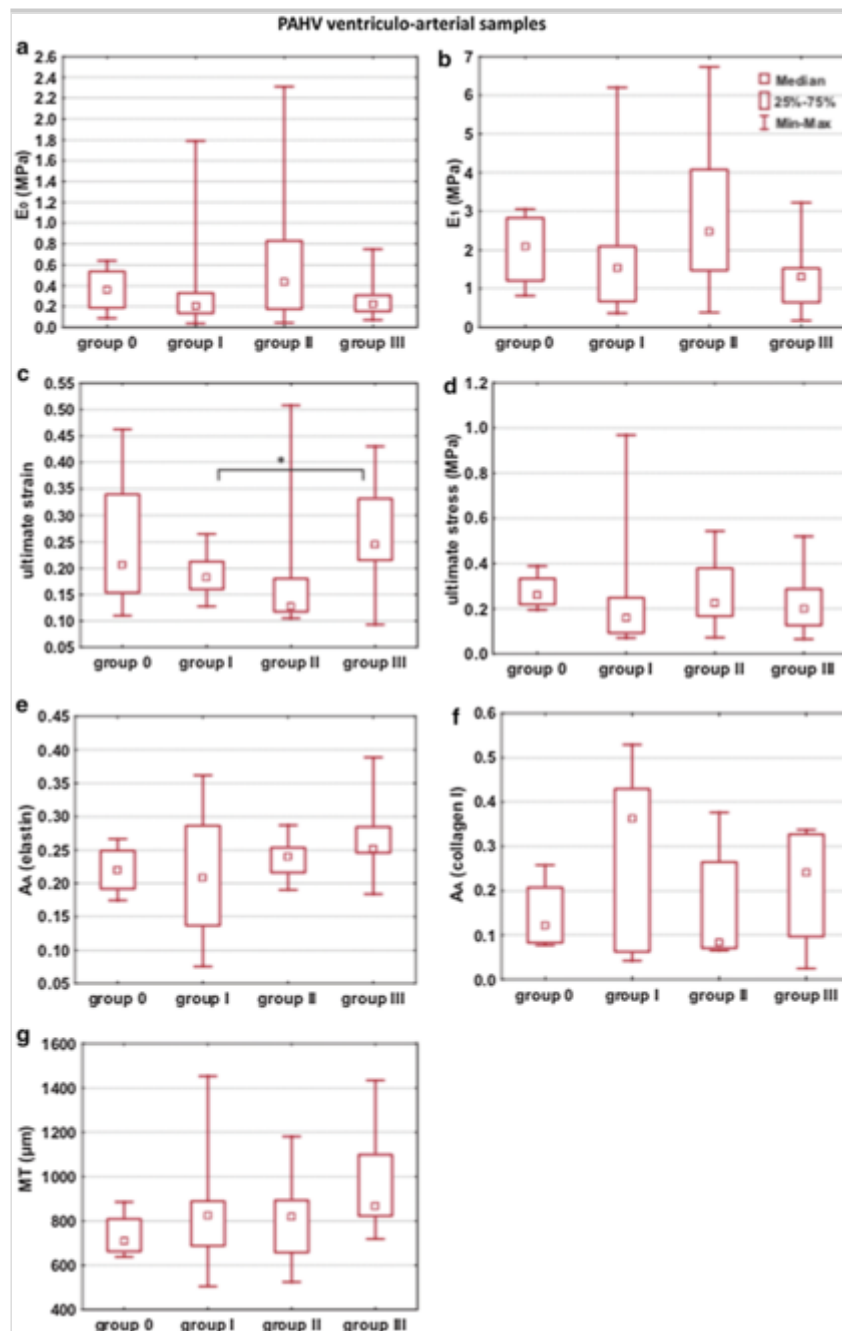
Comparison of mechanical and structural properties of aortic and pulmonary AHV according to the duration of cryopreservation

Differences between the groups 0–III (Kruscal-Wallis ANOVA) were found in the Young's moduli of elasticity in the small deformation region, ultimate strain and collagen amount for the PAHV arterial wall samples; in the Young's moduli of elasticity in the small deformation region, ultimate stress and collagen amount for the AAHV arterial wall samples. The pulmonary leaflets differed only in the ultimate stress between groups. The aortic leaflets differed in the ultimate strain

and the collagen amount between groups. Aortic ventriculo-arterial junctions differed only in the ultimate stress between groups. The differences are summarized in Table 6.

Table 6

The Kruscal–Wallis ANOVA = significant differences of parameters between time groups (group 0, I, II, III)

	E0	E1	Ultimate strain	Ultimate stress	A_A(elastin)	A_A(collagen I)	MT
Aortic AHV							
Arterial wall	$p < 0.01$	n.s.	n.s.	$p = 0.020$	n.s.	$p = 0.046$	n.s.
Leaflet	n.s.	n.s.	$p < 0.01$	n.s.	n.s.	$p < 0.01$	n.s.
V-A junction	n.s.	n.s.	n.s.	$p = 0.018$	n.s.	n.s.	n.s.
Pulmonary AHV							
Arterial wall	$p < 0.01$	n.s.	$p < 0.001$	n.s.	n.s.	$p = 0.014$	n.s.
Leaflet	n.s.	n.s.	n.s.	$p = 0.015$	n.s.	n.s.	n.s.
V-A junction	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

E_0 and E_1—Young's modulus of elasticity in the small and large deformation region, respectively

A_A, area fraction; MT, medium thickness; AHV, allograft heart valve; V-A junction, ventriculo-arterial junction; group 0, fresh, group I, cryopreserved for 0.1–4.9 years, group II, cryopreserved for 5–9.9 years, group III, cryopreserved for more than 10 years; n.s., means non-significant

Difference between each two storage time groups was tested by the Mann–Whitney U-test. The statistics is summarized in Table 7a, b.

Table 7

Mann–Whitney U-test = significant differences of parameters between two storage time groups (group 0–I, 0–II, 0–III, I–II, I–III, II–III) of (a) aortic allograft heart valve (AHV) samples and (b) pulmonary allograft heart valve (AHV) samples

	0–I	0–II	0–III	I–II	I–III	II–III

(a) Aortic AHV

Aortic wall	0–I	0–II	0–III	I–II	I–III	II–III
E_0	n.s.	n.s.	n.s.	$p = 0.030$	$p < 0.01$	$p < 0.01$
E_1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Ultimate strain	n.s.	$p = 0.031$	n.s.	n.s.	n.s.	n.s.
Ultimate stress	n.s.	n.s.	n.s.	n.s.	$p < 0.01$	$p = 0.015$
A_A(elastin)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
A_A(collagen)	n.s.	n.s.	n.s.	$p = 0.030$	n.s.	$p = 0.023$
MT	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Aortic leaflet						
E_0	n.s.	n.s.	n.s.	n.s.	$p = 0.044$	n.s.
E_1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Ultimate strain	n.s.	n.s.	$p = 0.025$	$p = 0.041$	n.s.	$p < 0.01$
Ultimate stress	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
A_A(elastin)	n.s.	n.s.	n.s.	n.s.	n.s.	$p = 0.014$
A_A(collagen)	n.s.	n.s.	$p = 0.020$	n.s.	$p = 0.031$	$p < 0.01$
MT	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Aortic ventriculo-arterial junction						
E_0	n.s.	n.s.	n.s.	n.s.	$p < 0.01$	n.s.
E_1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Ultimate strain	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Ultimate stress	$p = 0.016$	n.s.	n.s.	$p = 0.019$	n.s.	n.s.
A_A(elastin)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
A_A(collagen)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
MT	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
(b) Pulmonary AHV						

E_0 and E_1—Young's modulus of elasticity in the small and large deformation region, respectively
A_A, area fraction; MT, medium thickness; group 0, fresh, group I, cryopreserved for 0.1–4.9 years, group II, cryopreserved for 5–9.9 years, group III, cryopreserved for more than 10 years; n.s., means non-significant

Pulmonary artery wall

	0–I	0–II	0–III	I–II	I–III	II–III
E_0	n.s.	$p < 0.01$	n.s.	$p < 0.01$	n.s.	$p < 0.01$
E_1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Ultimate strain	n.s.	$p < 0.01$	n.s.	$p < 0.001$	n.s.	$p < 0.001$
Ultimate stress	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
A_A(elastin)	n.s.	n.s.	n.s.	$p = 0.039$	$p = 0.033$	n.s.
A_A(collagen)	n.s.	n.s.	n.s.	$p < 0.01$	n.s.	$p < 0.01$
MT	n.s.	n.s.	n.s.	n.s.	$p = 0.039$	n.s.
Pulmonary leaflet						
E_0	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
E_1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Ultimate strain	$p = 0.49$	n.s.	$p = 0.040$	n.s.	n.s.	n.s.
Ultimate stress	n.s.	n.s.	$p = 0.012$	n.s.	$p = 0.023$	$p = 0.015$
A_A(elastin)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
A_A(collagen)	$p < 0.01$	n.s.	n.s.	n.s.	n.s.	n.s.
MT	n.s.	n.s.	n.s.	$p = 0.022$	n.s.	n.s.
Pulmonary ventriculo-arterial junction						
E_0	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
E_1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Ultimate strain	n.s.	n.s.	n.s.	n.s.	$p = 0.042$	n.s.
Ultimate stress	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
A_A(elastin)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
A_A(collagen)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
MT	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

E_0 and E_1—Young's modulus of elasticity in the small and large deformation region, respectively

A_A, area fraction; MT, medium thickness; group 0, fresh, group I, cryopreserved for 0.1–4.9 years, group II, cryopreserved for 5–9.9 years, group III, cryopreserved for more than 10 years; n.s., means non-significant

AQ4

Aortic AHV

The Young's moduli of elasticity in the small as well as in the large deformation regions for all aortic AHV samples (arterial wall, leaflet, ventriculo-arterial junction) did not differ between fresh (group 0) and cryopreserved (group I, II, III) AAHV significantly, indicating that the stiffness was not influenced by cryopreservation. Changes in the Young's modulus of elasticity in the small deformation region showed that AAHV in the first 5 years of cryostorage were more rigid than the ones stored for the longer period.

The strain at the point of rupture differed only between groups 0–II for the arterial wall and 0–III for aortic leaflet samples. Median value of ultimate strain in the leaflet samples was smaller after 5 years of storage (group II) than that less than 5 years of storage (group I) but did not dropped under fresh aortic AHV initial values. Samples of the aortic leaflets cryostored longer than 10 years showed higher ultimate strain values than the group II samples.

The solidity of AAHV samples interpreted as the ability to sustain the highest load at the point of rupture (ultimate stress) was higher in all cryopreserved samples.

No differences in the elastin amount in all tissues were found in AAHV samples stored less than 10 years (group 0, I, II).

The type I collagen fibres amount for all AAHV samples did not differ between the fresh and cryopreserved tissue until 10 years of cryopreservation. The exception was the higher collagen amount in aortic wall in the group II when compared with group I.

There were no significant changes in the medium thickness of all AAHV samples.

Pulmonary AHV

The statistics showed differences in the Young's moduli of elasticity in the small deformation region between group 0–II, I–II and II–III for arterial wall samples. The stiffness of the pulmonary AHV was higher after 5 years of cryopreservation (group II) than those ones of other groups. No difference was observed in the Young's moduli of elasticity in the large deformation region. The pulmonary leaflet and V-A junction samples did not show any significant changes as well.

The ultimate strain values for wall samples was smaller in group II compared to group 0 and group I. Meaning that the samples of arterial wall in group II looked to be stiffer than the group 0 and group I. The leaflet samples differed in the

ultimate strain between group 0–I (near level of significance) and group 0–III. The V-A junction samples ultimate strain varied only between group I–III, where the value of group III was higher than that of group II.

No difference was observed in the ultimate stress for the pulmonary artery wall and V-A junction samples. The leaflet samples solidity was higher in group III compared with all other groups.

The area fraction of elastin in wall samples was higher in group II and sustained higher in group III compared to group I. The leaflet and V-A junction samples elastin amount did not differ between groups.

The area fraction of type I collagen fibres was smaller in group II and higher in the group III of the wall samples when compared with group 0 and group I. The amount of collagen was also higher in the pulmonary leaflet in group I when compared to group 0. The V-A junction samples did not differ between time groups.

There were no differences in the medium thickness in all tissue samples. The exceptions were: the smaller MT in the leaflet in group II when compared with the group I; and the smaller MT in the wall in group III when compared with group I.

Correlation between the duration of cryopreservation and mechanical and structural properties of AHV

The Spearman's rank correlation coefficients are summarized in the Supplement 2.

The aortic AHV wall and leaflet samples showed significant inverse relationship between the length of cryopreservation and the Young's moduli of elasticity (r_s $E_0 - 0.48$, r_s $E_1 - 0.30$ and r_s $E_0 - 0.30$, r_s $E_1 - 0.27$ respectively). The negative correlation was found also in E_0 for the aortic V-A junction samples ($r_s = -0.53$). Another inverse relationship was observed between the length of cryopreservation and the ultimate stress ($r_s = -0.31$) in the aortic wall.

The ultimate strain correlated medium positively with the collagen amount in the pulmonary leaflet, wall and V-A junction samples ($r_s = 0.44, 0.60, 0.44$, respectively) and with the collagen amount in the aortic leaflet ($r_s = 0.48$).

Discussion

The authors are aware of the complexity of the issue. Nevertheless, they do believe that the basic mechanical properties and morphology of semilunar AHV represent the most important characteristics of the AHV tissue from the surgical point of view. Time limit of AHV cryopreservation means one of the important factors limiting the AHV availability in tissue banks. The possibility to prolong the expiration AHV limits is of importance beyond doubt from the point of view of daily practice.

All the tissue specimens taken from the aortic as well as from pulmonary AHV exhibited stiffening due to increasing loading, i.e., the stress–strain curve was nonlinear. This behaviour is typical for soft biological tissue (Meyers et al. 2008). The initial loading in the region of small deformation was characterized by small Young's modulus of elasticity, while the further loading led to tissue stiffening and the linear region of large deformations was characterized by high Young's modulus of elasticity. In general, the stiffening can be explained by collagen crimping. The collagen is at normal non-stretch state in waving relaxed configuration and thus in the initial phase of loading contributes only marginally to tissue mechanical response. In this state contributes to the tissue mechanical response mostly the elastin with the Young's modulus of elasticity about 0.6 MPa (in ligamentum nuchae, (Vincent 1990). During the increasing mechanical loading, the collagen straightens the waving. After stretching, the collagen fibres with their high modulus of elasticity (1–2.5 GPa for collagen in rat-tail tendon, (Meyers et al. 2008) start to contribute significantly to the tissue mechanical response and thus the tissue becomes stiffer.

AQ5

The aortic and pulmonary leaflets had higher Young's moduli of elasticity and ultimate stress and oppositely smaller ultimate strain than the artery wall and ventriculo-arterial junction in both AHV. It could be explained by the fact, that the leaflets had much higher amount of collagen then the arterial wall samples.

Like many other mechanical studies, the design of the mechanical part of our study mimics the physiological loads to which the valves are subjected by transvalvular pressure in diastole but does not involve the shear stress of flexural forces exerted on the valve in its natural function. We suppose that the ultimate strain, ultimate stress and stiffness characterized by Young's moduli at the large deformation region (E_1) are the main mechanical variables that should be considered.

In aortic AHV, the physical deformation response of wall samples to stress did not changed significantly neither during the process of cryopreservation nor during the first 10 years of storage. The decline in ultimate strain values of

aortic leaflet in group II did not drop under initial value of fresh AHV samples. The pressure needed to rupture the tissue samples was approximately equal for all aortic AHV samples in groups 0, I and II. No significant changes in Young's modulus of elasticity at the large deformation region (E_1) were found in all tissue samples across the study groups indicating that the stress–strain curve gradient or in other words the stiffness of AHV was not markedly impaired during the process of cryopreservation and storage. The significant decrease in type I collagen fibres area fraction in the aortic AHV wall samples in group II is unclear as it is then followed by increase again in group III. Note, that the group II had the higher ratio female/male samples than the other groups. But for a deeper gender statistic we need more specimens. No significant changes were found in the area fraction of elastin and median thickness.

In the pulmonary AHV, the ultimate strain dropped in group II wall samples compared to group I, indicating that the pulmonary artery after 5 years of cryostorage was significantly less deformable at the point of the rupture. On the other hand, the ultimate stress in wall samples was equal during the first 10 years of cryostorage. The E_1 values did not change significantly as in the aortic AHV groups. The PAHV wall samples showed significant decrease in collagen area fraction after 5 years of cryostorage too, whereas the elastin fraction increased in the same group. The median thickness of leaflet samples was smaller in group II than group I but did not decline under the fresh PAHV median thick values.

The AHV cryostored longer than 10 years (group III) showed heterogenous results in many variables indicating that prolonged storage may influence the mechanical and structural characteristics to a greater extent.

The vast majority of mechanical and structural studies has been performed with porcine or ovine heart valves (Hlubocky et al. 2011; Stemper et al. 2007; Vesely 1998). The influence of cryopreservation on structural, chemical and immunoenzymatic properties of human aortic valve allografts were studied by Pfitzner et al. (2018). He came to the conclusion that the morphologies of fresh and cryopreserved aortic valve grafts were comparable, and no difference was demonstrated between short- and long-term storage of cryopreserved valves. Human heart valves are very precious study material. Sampling of fresh and cryopreserved AHV within their expiration period (less than 5 years) collides with both economic and ethical issues as they can be used for transplantation. Therefore, the number of samples in our control group (group 0) is limited. This project was granted by the courtesy of the National AHV Bank of the Department of Transplantations and Tissue Bank, Motol University Hospital, Prague, Czech Republic.

According to Vesely (1998) elastin serves as the tensioner of the collagen fibres during unloading. We have found no diminution in the elastin or collagen area fraction during cryopreservation. Stemper et al. (2007) states that the refrigeration of porcine aorta significantly reduced the mechanical attributes (primarily the ultimate stress and Young's modulus of elasticity). No such difference was observed in freezing at $-20\,°C$ or $-80\,°C$. The ultimate strain was not affected by storage technique. The cryopreservation process with cryoprotectant agent (10% DMSO) used in AHV however, differs significantly from common refrigerating and freezing described in his study design, therefore the results are not comparable. The mechanical characteristics of fresh and frozen human aorta (descending) were studied by Adham et al. (1996). According to his results, cryopreservation did not alter the stress–strain characteristics of the samples. The cryopreserved aorta had an elastic modulus almost identical to that of control (fresh) tissue. Similar results were observed also in decellularized rabbit carotid arteries (Fonck et al. 2008), indicating that cryopreservation does not affect the structure and mechanical properties of the arterial samples. Experimental work on the mitral sheep allografts deploying different model to characterize the tissue's mechanical behaviour (Hlubocky et al. 2011) also came to the conclusion that the tissue processing and cryopreservation do not alter the structural characteristics and elasticity.

One of the major limitations in the use of AHV is the disproportion between availability and request in many countries. The upward trend in the number of transplantations is in our settings caused by raising demand for pulmonary AHV whilst the number of transplanted aortic AHV remains at the same level (Fig. 10).

Fig. 10

The number of donor hearts and transplanted allograft heart valves (AHV) over a period of last 26 years in National AHV Bank of the Department of Transplantations and Tissue Bank, Motol University Hospital, Prague, Czech Republic shows the steady trend of donor hearts in contrast with increasing demand for AHV transplantation. The X-axis represents the years, the Y-axis the numbers, dashed lines represent the trends
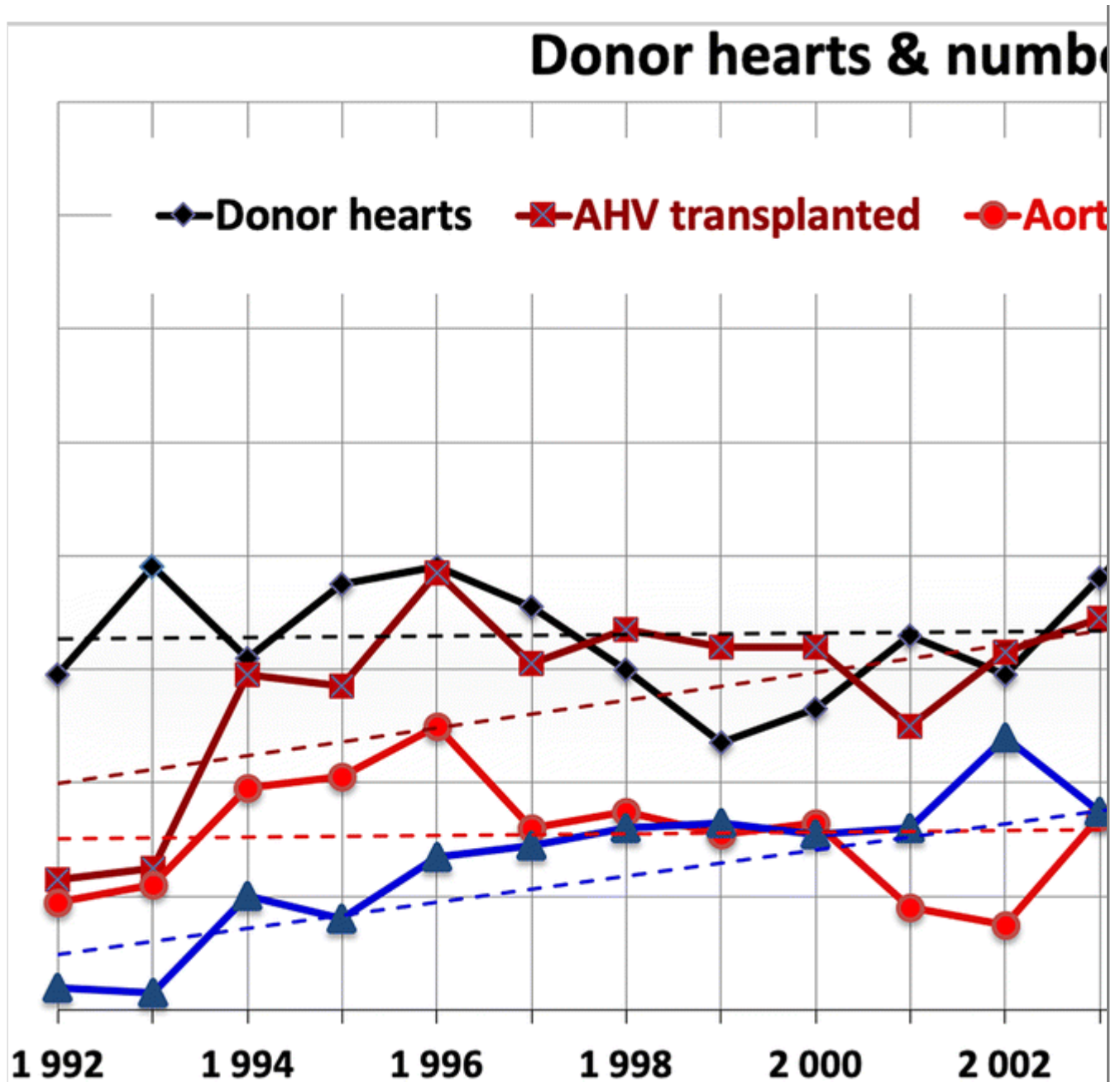
Other disadvantages include more demanding implantation technique than prosthetic valve replacement and degeneration and calcification of the graft. Our project does not address structural deterioration of implanted allografts that occur over time and is probably connected with the immune response. One of the research topics discussed in recent literature are decellularized AHV containing only connective tissue which may be repopulated with host cells and therefore reducing immunogenicity (Bibevski et al. 2017; Da Costa et al. 2006).

The endothelium plays a critical role in regulating valve mechanics and valve function and perhaps long-term durability (Simmons 2009). The role of the endothelium was not studied as it was proved that human valve allografts show severe endothelial destruction arising already in the initial steps of tissue processing at least in our Tissue Establishment—donor heart harvesting and antibiotic decontamination (Burkert et al. 2008).

This study has several limitations. The most important limitation was small number of fresh AHV due to their scarcity and high economical cost. A possible limitation of our protocol was not controlling for the gender as a factor that may influence heart valve mechanics. From our experience, as well as according to other heart valve bankers it is evident that female cardio-vascular tissue tends to be better preserved than the one from male donors of the same age. Balanced gender composition of groups or comparison of AHV from the same donor age and gender would reduce the risk of misinterpretation of the final results of evaluation. However, the number of samples for research purposes is limited. Future projects should involve more tissue establishments to get large numbers of the AHV, especially the aortic which remain in stock much longer than the pulmonary one and expire each year.

There are many other characteristics of histological and structural changes, which could be searched, but for our purposes we used just collagen and elastin fraction. It's obvious that the elastin and collagen content alone do not determine mechanics, their mutual organization plays a major role (Vesely 1998). There is also extensive discussion about the role of proteoglycans glycosaminoglycans (Eckert et al. 2013; Hopkins 2005) in connective tissue mechanics. Although histological quantification has some limitations (such as tissue shrinkage), it provides information about the spatial distribution of collagen I and elastin relatively to the other tissue components. The results of mechanical measurements carried out in the open air could be influenced by dehydration of the tissue. The dehydration could lead to a slight stiffening of the tissue during experimentation (Viidik 1979). This potential source of bias was, however, minimized by the short duration of the experiments (about 15 min) and by spraying the tissue with saline solution (1–2 mL of 0.9% solution of sodium chloride/min).

The results of our study offer a unique insight into human semilunar heart valves, aorta and pulmonary artery mechanics and histology. The mechanical data show that AHV cryostored longer than present expiration period have no reasonable defects. Their structure concerning the area fraction of type I collagen and elastin fibres does not change significantly. We conclude that the mechanical and structural properties are not deteriorated significantly neither during the process of cryopreservation (programmed cooling) nor during the first 10 years period of storage. These results will contribute to advocating the changes in cryopreserved AHV expiration period policy. Taking into account the group II cryostorage characteristics (median 5.26 for aortic and 7.48 years for pulmonary AHV), we do consider our data as validation for expanding the expiration period of AAHV to 6 years, and of PAHV to 8 years.

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Compliance with ethical standards

Conflict of interest   The authors declare that they have no conflict of interest.

Ethical approval   This article does not contain any studies with human participants or animals performed by any of the authors.

Electronic supplementary material

Below is the link to the electronic supplementary material.

Supplement 1: The complete primary data from the analyses (XLSX 57 kb)

Supplement 2: The Spearman's rank correlation coefficients showing correlation between the duration of cryopreservation and mechanical and structural properties of AHV (PDF 216 kb)

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