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Impact of current cryopreservation procedures on mechanical and functional properties of human aortic homografts

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Abstract We evaluated the impact of standard cryopreservation on mechanical and functional properties of human aortic homografts. From 14 human heart-valve donors, the thoracic descending aorta was obtained. Effects of cryopreservation on mechanical (elastic properties and breaking stress) and smooth muscle cell (SMC) and endothelium function were tested. Cryopreservation (cryo) did not significantly affect Young's modulus of elastin (fresh: 3.1 ± 1.0, cryo: 2.7 ± 0.9 × 10^5 Nm^{-2}), collagen recruitment pressure (fresh: 1.1 ± 0.3, cryo: 1.1 ± 0.4 × 10^4 Nm^{-2}), distensibility (fresh: 3.8 ± 1.8, cryo: 3.6 ± 1.6 × 10^5 $N^{-1}m^2$), or breaking stress (fresh: 2.4 ± 1.0, cryo: 2.2 ± 1.0 × 10^6 Nm^{-2}). Following explantation, no endothelium-dependent relaxation was found. SMC function and endothelium-independent relaxation were mainly intact after explantation but significantly decreased after cryopreservation. Aortic mechanical properties are not influenced by cryopreservation. Following explantation, almost no endothelial cell function is present, and SMC contractility is strongly affected after cryopreservation.

Keywords Aorta · Cardiovascular surgery · Endothelial function · Mechanical properties · Smooth muscle · Transplantation

Abbreviations *Br Stress* Breaking stress · *cryo* Cryopreservation · *D* Dynamic distensibility · *Pcol* Collagen recruitment pressure · *SMC* Smooth muscle cell · *TC* Tissue culture

Introduction

The use of fresh or freeze-dried arterial homografts was abandoned long ago because of difficulties in tissue procurement, long-term degeneration, and the development of improved prosthetic commercially available grafts [7, 9, 11, 15, 31, 33]. Recently, interest in the use of aortic homografts has reawakend. Excellent results in the use of homograft aortic valves and ascending aortas in endocarditis have encouraged vascular surgeons to use arterial homografts for the *in situ* repair of vascular infections of the great arteries [27, 42]. Indications comprise mycotic aneurysms, aorto-esophageal, aorto-bronchial and aorto-gastrointestinal fistulas and infected prosthetic grafts [1, 4, 12, 16, 17, 19, 26, 28, 32, 37, 38, 39, 40]. Bové et al. [8] were also encouraged by the use of nonvalved homografts of the thoracic aorta in operations for complex congenital cardiac diseases because of the convenience in handling and versatility in size and shape of aortic homografts. This procedure might also lead to a reduction in thrombotic, hemorrhagic, and infectious complications. Moreover, the de-

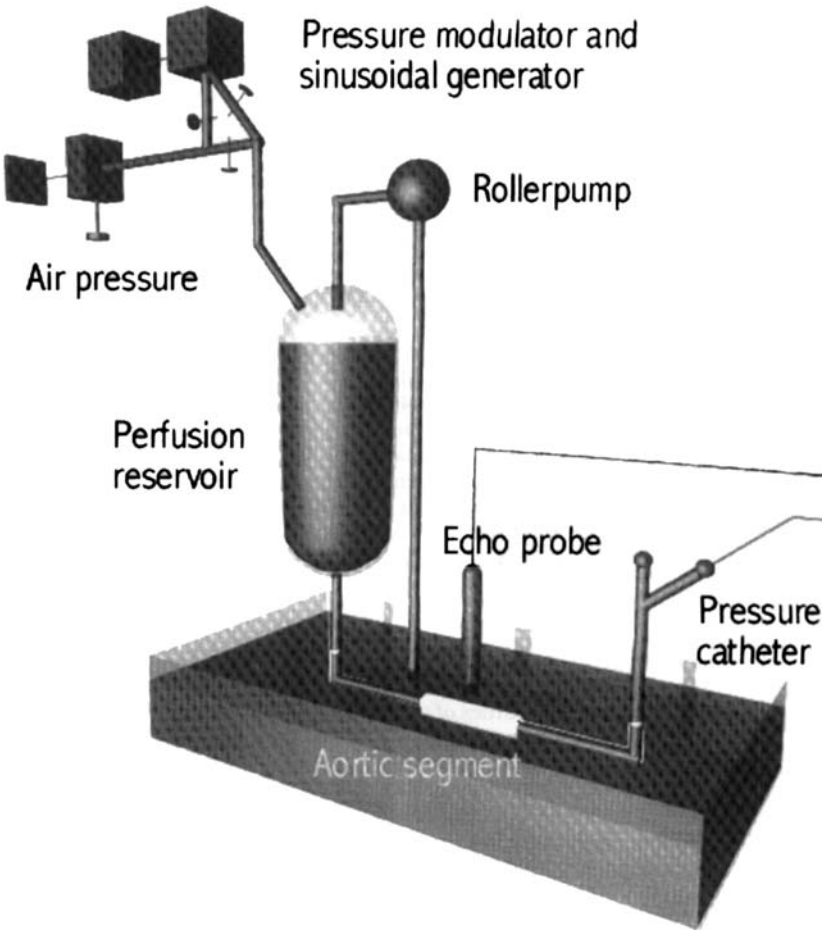

Fig. 1 Schematic drawing of the in-vitro experimental set-up for mechanical studies

velopment of tissue cryopreservation allowed the introduction of cryobanks that improved the clinically availability of homografts. Several studies have already focused on the effects of cryopreservation on either mechanical [2, 13, 35] or functional properties [3, 20, 21, 23, 24, 25, 29, 30, 41] of the arterial wall. However, to our knowledge no studies were performed concerning cryopreserved intact aortic segments harvested as part of a non-heart beating donor program. Also, the effect of cryopreservation on the breaking stress of intact human aortic segments has not yet been addressed [14]. Accordingly, the purpose of the present study was to evaluate the effects of cryopreservation on both the mechanical and functional properties of the human thoracic descending aortic homograft, harvested as part of a non-heart beating donor program.

Materials and methods

The thoracic descending aorta was explanted from 14 human non-heart beating heart valve donors (median age 44 years, range 15–59 years) without any known aortic disease. In the aortic segments of these 14 donors, mechanical and functional studies were performed before and after a standard cryopreservation procedure. Donor selection was made according to the European Standards for Tissue Banking, after written permission for experimental use of donor tissue was obtained from relatives.

Harvesting

During the aseptic harvesting procedure of the heart, including the pulmonary artery and the aortic arch, the aorta was ligated 1 cm distal from the left subclavian artery and fixed at its *in-vivo* length. Next, the descending aorta was carefully cleared from the surrounding tissue over a length of approximately 16 cm. The trachea and oesophagus were carefully kept intact to avoid contamination with infectious agents. The intercostal arteries were cut at a length of approximately 1 cm. Finally, the in-vivo length of the aortic segment was measured and the segment was explanted. Blood clots were removed by carefully rinsing the aortic segment with sterile NaCl 0.9% and 4° Celsius (C)-custordiol HTK (Histidine Tryptophan Ketoglutirate) solution according to Bretschneider. All tissues were harvested within 24 h after cardiac arrest.

Tissue preparation, decontamination and cryopreservation

From each donor the material was divided into 3 groups and stored for transport in sterile 4 °C custordiol HTK solution. Group 1 consisted of a small ring of approximately 2 mm and was used immediately for functional studies. The remaining tissue was divided into two equally sized segments of approximately 6.5 cm. A randomly chosen segment (group 2) was decontaminated for 5–6 h at 37 °C in a solution of the following antibiotics: ciprofloxacin 3 µg/ml, amikacin 1.2 µg/ml, metronidazol 1.2 µg/ml, vancomycin 1.2 µg/ml, flucitocin 30 µg/ml. Subsequently, the segment was divided into one subsegment for mechanical studies and into one ring for functional tests.

The other segment (group 3) was decontaminated for 5–6 h at 37 °C followed by submersion in tissue culture (TC)-199 medium with 10% dimethyl sulfoxide (DMSO) at 4 °C for 45 min and subsequent cryopreservation [34]. After 6 weeks storage at −197 °C, cryopreserved aortic grafts were slowly thawed by submersion of the grafts in a 40 °C-water bath. To prevent cellular damage, the cryoprotectant was removed according to standardised stepwise dilution in TC-199 medium [34]. The thawed segment was divided into one subsegment for mechanical studies and into one ring for functional tests.

Mechanical studies

Breaking stress and elastic properties of the intact fresh decontaminated (group 2) and cryopreserved (group 3) aortic subsegments were investigated in an *in-vitro* experimental set-up by loading the aortas with an increasing hydrostatic pressure (Fig. 1). The intercostal arteries of the aortic segments were ligated. Next, the segments were fixed to adjustable glass cannules, mounted in a basin with 0.9% NaCl at room temperature, connected to an expansion barrel filled with 0.9% NaCl, and attached to air pressure. A manual pressure controller (Fairchild, model 65A) produced static pressures until 3 bar. Dynamic pressure waves were created by an electronic pressure controller (Fairchild, model T5200) guided by a pressure generator (Krohn and Hitte, model 5700). A pressure catheter was placed inside the aortic lumen and externally coupled to a pressure transducer, which was adjusted to high pressures (Baxter, disposable PX-600F). After amplification and filtration (Bridge amplifier DPM-612A), a thermal pen recorder (WEKAgraph, model WK 250R), recorded the signal data. Aortic lumen diameter was continuously measured by means of a 5 MHz ultrasound echo-probe (Hewlett Packard) and recorded on videotape. The aortic segments were accommodated for 30 min at a pulse rate of 0.5 Hz with systolic/diastolic pressure-waves of 120/50 mm Hg to rule out any effect of SMC [10]. The experiment started with a dynamic pressure wave of 120/50 mm Hg at 0.5 Hz. Next, pressure was kept constant (static pressure) at approximately 10 mm Hg and the corresponding lumen diameter was measured. Static pressures were increased to an endpressure of 400 mmHg and kept constant for 20 min. These dynamic and static pressure-diameter measurements and the pressure loading for 20 min were repeated to a static endpressure of 800, 1200 and 1600 mm Hg respectively, until rupture occurred. External diameters of the aorta were measured either by means of two-dimensional echography at static pressures or by means of M-mode echography at dynamic pressure waves. After mechanical testing, the wet weight of the segment was determined (Sartorius, model H120) and the ruptured segments were photographed.

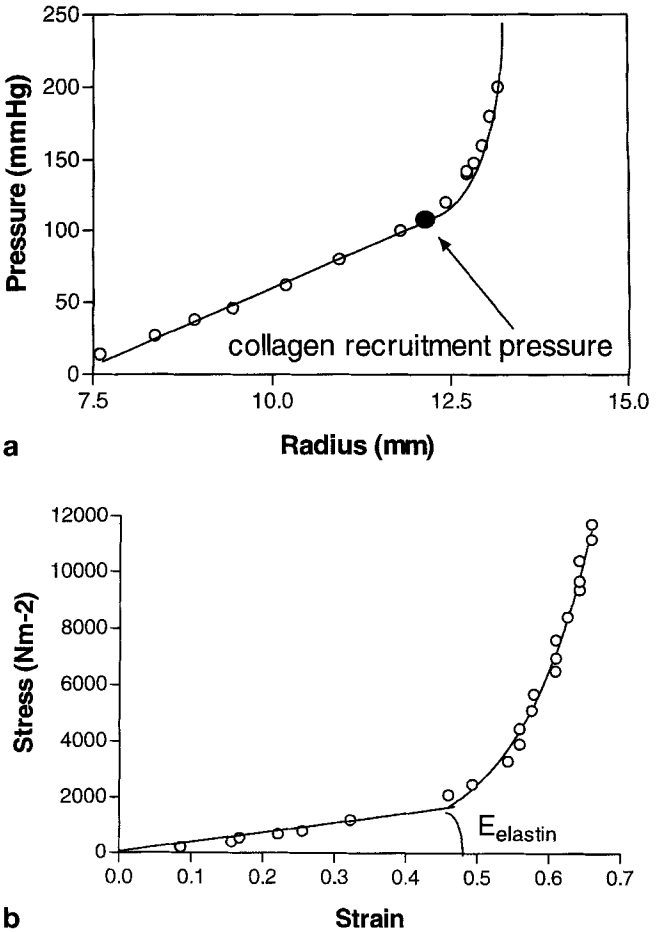

Fig. 2 **a** Typical example of the pressure-radius relationship of an aortic segment. The curve transients from a linear (elastin mediated) to an exponential (collagen mediated) course at a certain point of pressure (collagen recruitment pressure). **b** Typical aortic stress-strain curve. Linear fits have been performed in the low-pressure range to determine Young's modulus of elastin (*Eelastin*)

Calculations

External radii (R_e, mm) of the aortic segment and matching pressure data (P, Nm^{-2}) were analysed. Aortic wall volume (V, mm^3) was calculated from the weight of the aortic segment and the specific gravity of aortic tissue (ϱ = 1.066 $kg.m^{-3}$). Further, the internal radius (R_i) and midwall radius (R_{mid}) were calculated by means of the equations:

$$R_i = \sqrt{R_e^2 - \frac{V}{\pi \cdot L}} \qquad R_{mid} = \frac{R_e + R_i}{2}$$

In this relation, L is the in-vivo length of the aortic segment. Midwall radial wall stress (σ) was calculated using the following formulas, where R_{mid} is the midwall radius at the lowest pressure (50 mmHg) of the pressure wave:

$$\sigma = \frac{2 \cdot P \cdot (R_e \cdot R_i)^2}{R_e^2 - R_i^2} \cdot \frac{1}{R_{mid}^2}$$

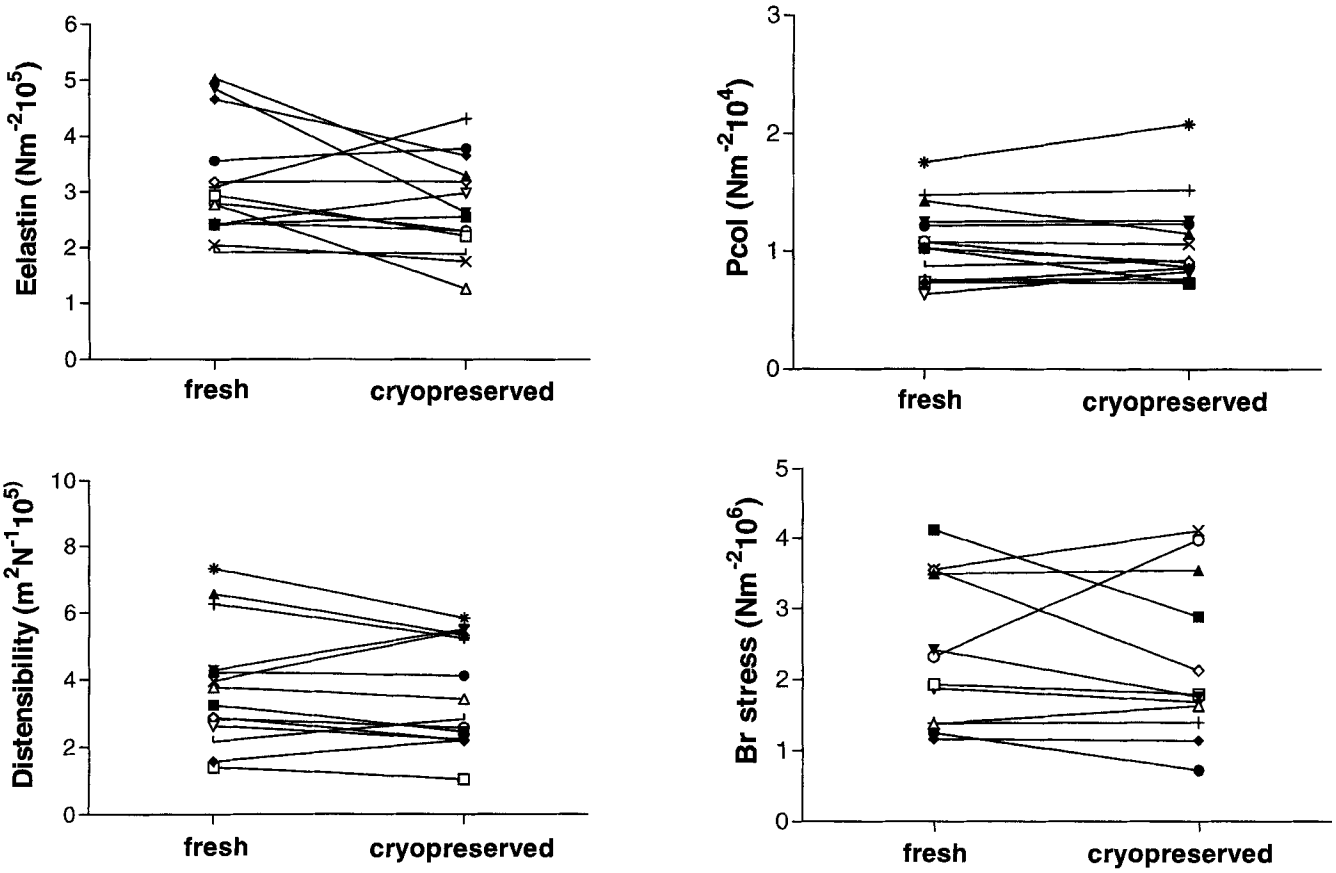

Fig. 3 Effects of cryopreservation on the aortic wall Young's modulus of elastin (*Eelastin*), collagen recruitment pressure (*Pcol*), distensibility at 0.5 Hz, and the breaking stress (*Br stress*)

Pressure-diameter and stress-strain curves (Fig. 2a and 2b) were constructed. These curves represent the non-linear relationship between the lumen diameter or derivative parameter strain ($R_{mid}/R_{mid\ at\ 40\ mmHg}$) and the intraluminal pressure or derivative parameter midwall radial wall stress. Such curves are composed of two parts. At low pressures, the resistance to stretch is mostly due to elastin fibres alone, and the pressure-diameter and stress-strain relationship are more or less linear functions. Beyond a certain point of pressure, which is called the collagen recruitment pressure (Pcol), the curve transients from linear to exponential, and the resistance to stress is determined mostly by the stiffness and organisation of the collagen skeleton (Fig. 2a). The slope of the linear part of the stress-strain curve is considered to be the Young's modulus of elastin (Eelastin, Fig. 2b) and represents static distensibility [5, 6]. Aortic rupture occurs when the wall stress exceeds the holding power or strength of the aortic wall. The wall stress at the point of rupture is called the breaking stress (Br stress). Dynamic distensibility (D) at 0.5 Hz was calculated using the following formula, where R_{min} is the midwall radius at the lowest pressure (50 mmHg) of the pressure wave:

$$D = \frac{2 \cdot \Delta R}{R_{min} \cdot \Delta P}$$

Functional studies

Contractility as a measure of SMC function and endothelium-dependent relaxation as a measure of endothelial cell function, were used as viability parameters of the aortic wall [36, 41]. Stimulated contraction was assessed by potassium chloride (KCl), causing membrane depolarisation, and by phenylephrine (PE), a α_1-adrenoceptor agonist that causes receptor dependent contraction. Acetylcholine (ACh) induced endothelium-dependent relaxation and endothelium-independent relaxation was evoked by the nitric oxide (NO)-donor sodium nitroprusside (SNP). These aortic wall characteristics were investigated in fresh aortic rings (group 1), fresh decontaminated aortic rings (group 2) and aortic rings which were both decontaminated and cryopreserved (group 3).

Aortic rings were mounted vertically between two glass hooks in a 20 ml organ bath containing oxygenated Tyrode's solution at a temperature of 37 °C and a pH of 7.4. The composition of the Tyrode's solution was as follows (mM): NaCl 136.0, KCl 2.5, $MgCl_2$ 1.1, $CaCl_2$ 1.8, NaH_2PO_4 0.3, $NaHCO_3$ 24.9 and glucose 5.5. The upper hook was connected to an isometric force transducer (Kyowa, model 120-T-10B, range 10 gram). After filtering and amplification (Bridge amplifier DPM-612A), the signal was recorded with a thermal pen recorder (WEKAgraph, model WK 250R).

Aortic rings were stretched to a baseline load of 2 g and allowed to stabilise for 30 min. The Tyrode's solution was replaced every 15 min. Subsequently, aortic rings were exposed to a high KCl concentration (120 mM) to induce membrane depolarisation and contraction. When the maximal response had developed the solution was replaced with normal Tyrode's solution. As soon as the baseline load of 2 g was reached, this procedure was repeated.

Table 1 Results (mean ± SD) of mechanical and functional experiments in human aortic homografts directly after explantation (*group 1*), after decontamination (*group 2*) and after cryopreservation (*group 3*). *Eelastin* Young's modulus of elastin, *Pcol* collagen recruitment pressure, *D* distensibility, *Br stress* breaking stress, *KCl* potassium chloride, *PE* phenylephrine, *ACh* acetylcholine, and *SNP* sodium nitroprusside. A positive response (+) in the functional tests indicates contraction, whereas a negative response (–) represents relaxation

					P-value		
		Group 1	Group 2	Group 3	(1–2)	(2–3)	(1–3)
Mechanical							
(10^5 Nm^{-2})	Eelastin (n = 14)		3.1 ± 1.0	2.7 ± 0.9		NS	
(10^4 Nm^{-2})	Pcol (n = 14)		1.1 ± 0.3	1.1 ± 0.4		NS	
(10^5 $N^{-1}m^2$)	D (n = 14)		3.8 ± 1.8	3.6 ± 1.6		NS	
(10^6 Nm^{-2})	Br stress (n = 12)		2.4 ± 1.0	2.2 ± 1.0		NS	
Functional							
(gram)	KCl (n = 14)	0.99 ± 1.14	0.57 ± 0.87	0.02 ± 0.06	0.02	0.03	0.006
(gram)	PE (n = 14)	0.95 ± 1.32	0.57 ± 0.72	0.04 ± 0.13	NS	0.02	0.02
(gram)	Ach (n = 14)	0.07 ± 0.14	0.05 ± 0.13	0 ± 0	NS	NS	NS
(gram)	SNP (n = 14)	–0.37 ± 0.49	–0.37 ± 0.43	–0.05 ± 0.15	NS	0.03	0.03

Then, rings were contracted with a α_1-adrenoceptor agonist, PE (100 μ M). When a steady state was reached, rings were exposed to ACh (10 μ M). Finally, the ability of SMC to relax in response to the endothelium derived relaxing factor NO was evaluated. Therefore, aortic rings were exposed to KCl (120 mm), washed with normal Tyrode's solution, treated with PE (100 μm), and lastly exposed to SNP (10 μm), respectively.

Statistical analysis

Results are represented as mean ± one standard deviation. Contraction and relaxation induced by KCl, PE, ACh or SNP in groups 1, 2 and 3 were expressed as absolute force of contraction (+) or relaxation (–) in grams. The paired Student's *t*-test was used for statistical analysis. A P-value < 0.05 was considered to be significant.

Results

Mechanical studies (Fig. 3)

The average length of the individual segments was 66 ± 6 mm. As shown in Fig. 3 and Table 1, cryopreservation did not significantly affect the slope of the first linear part of the stress-strain curve, called Young's modulus of elastin, the collagen recruitment pressure, and dynamic distensibility at 0.5 Hz. Cryopreservation did also not significantly reduce breaking stress in 12 out of 14 aortic segments. Two aortic segments were not analysed for breaking stress, due to technical difficulties.

Functional studies (Table 1 and Fig. 4)

SMC depolarization in response to KCl was significantly decreased after both decontamination and cryopreservation. The PE-induced SMC contraction reduced after decontamination and was significantly affected after cryopreservation. An almost negligible response to ACh was demonstrated in aortic specimens directly after explantation, which further decreased after decontamination and cryopreservation. Vasodilation was observed after addition of SNP in fresh aortic rings. Endothelium-independent relaxation was slightly attenuated after decontamination but significantly reduced after cryopreservation.

Discussion

Our study addressed the mechanical and functional properties of the human aorta with and without cryopreservation. Tissues were taken from 14 human heart-valve donors, and randomly divided into three groups. Two groups were decontaminated for 5–6 h and tested either unfrozen or after cryopreservation. One further group was functionally tested on the day of explantation. The present study showed that cryopreserved human aortic homografts have similar aortic stiffness and breaking stress properties as fresh human aortas, but SMC contractility and endothelial cell function were markedly attenuated. To our knowledge, there is a scarcity of data concerning breaking stress from complete segments of cryopreserved human aorta. Such data may have the potential to predict the efficacy of aortic homografts for surgical improvement of cardiovascular disease.

The maintenance of passive mechanical capacity in itself, despite diminished SMC contractility and endothelial cell function, allows the implantation of cryopreserved aortic homografts, as alternative grafts such as prosthetic grafts also possess no active vasomodulation capacity. Moreover, in cases where a prosthetic aortic graft is not desired because of aortic infections, the use

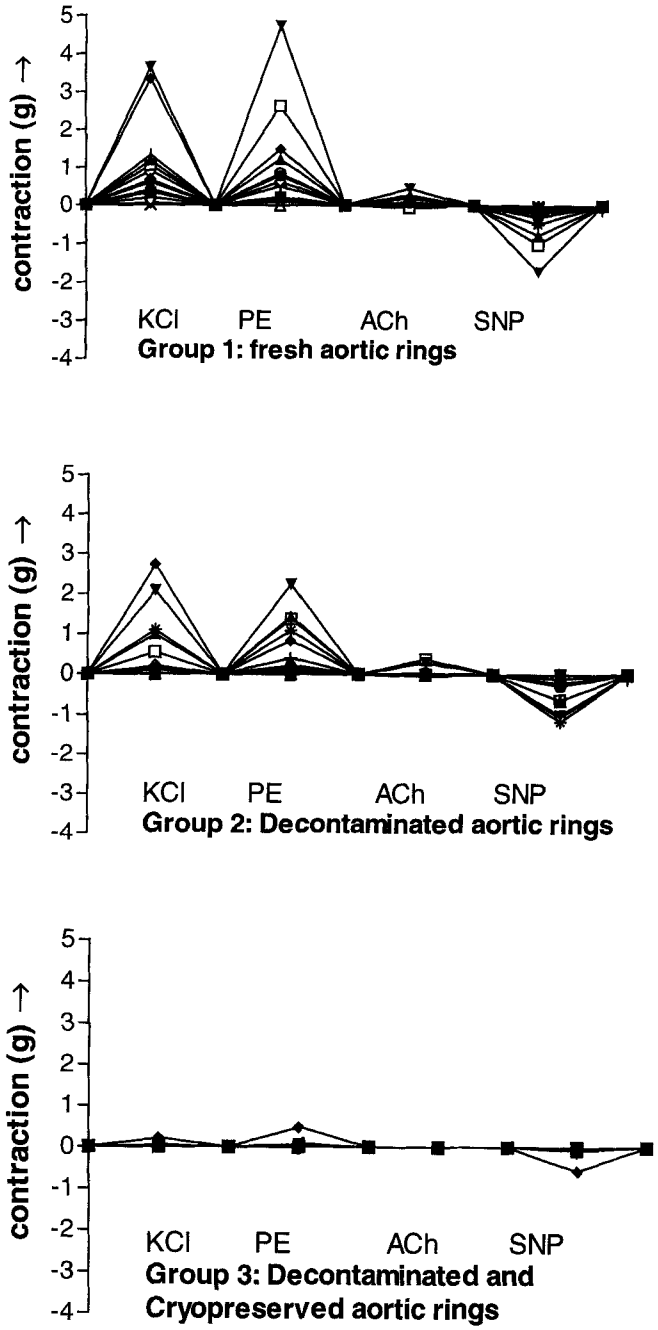

Fig. 4 Smooth muscle cell contractility in response to potassium chloride (*KCl*) and phenylephrine (*Phe*), and endothelial dependent (acetylcholine, *ACh*) and independent relaxation (sodium nitroprusside, *SNP*) in fresh (*group 1*), decontaminated (*group 2*) and cryopreserved human aortic homografts (*group 3*)

of aortic homografts appears justified [1, 4, 12, 16, 17, 19, 26, 28, 32, 37–40].

Recently we described the influence of aging and aortic stiffness on the breaking stress of the human aorta [14]. Age, distensibility and collagen recruitment pressure influenced the permanent deformation and rupture of the aorta following pressure overload. In the present study we found no significant change in the distensibility, Young's modulus of elastin and collagen recruitment pressure after cryopreservation. Therefore a shift of the wall stress distribution from the elastin to the collagen fibres is not expected to occur in the cryopreserved aortic wall.

Several studies have addressed breaking stress by using arterial strips rather than arterial segments [2, 13]. However, shape and integrity of the aortic wall might be better preserved using segments for breaking stress experiments. Indeed, we found higher breaking stress values than others using aortic strips (fresh $1.96 \pm 0.6 \times 10^6$ Nm^{-2}, cryopreserved $1.15 \pm 0.42 \times 10^6$ Nm^{-2}) [2, 13] (Table 1). It should be noted that neither in earlier studies nor in the present study a significant effect of cryopreservation on breaking stress was found.

The effect of cryopreservation on SMC and endothelium cell function has remained a matter of discrepancy. We found a general diminution in SMC contractility and endothelium-dependent and independent vasodilation after cryopreservation. Fresh aortic rings showed a negligible response after addition of ACh, which indicates that the endothelial layer was already damaged after explantation. The latter might be caused by manipulation of the thoracic descending aorta during the standard surgical explantation. During this complicated explantation procedure the trachea and oesophagus should remain intact for reasons of sterility. Moreover, the sensitive endothelium might be further damaged by the careful aortic rinsing procedure to remove blood clots.

In accordance with our results, studies showed nearly abolished SMC contractility in human internal mammary arteries [25], and diminished contractile and endothelium-dependent relaxant response of human coronary and internal mammary arteries after cryopreservation [23]. However, poorly preserved SMC function, but intact endothelial cell function in internal mammary arteries of dogs was found after cooling to −196 °C [3]. Pompilio et al. showed intact endothelial function in human internal mammary artery bypass grafts after cryopreservation [29]. Whilst this discrepancy might be due to species differences, it may also reflect different susceptibility of endothelial cells from different calibre arteries to the explantation, cryopreservation or thawing process. Furthermore, the reduction in SMC contractile force after cryopreservation might be explained by the prolonged (> 10 min) exposure to cryomedium (DMSO) before cryopreservation [22]. Further studies are required to further optimise this routinely used cryopreservation protocol.

In conclusion, our study demonstrates that cryopreservation of aortic homografts harvested from non-heart beating donors results in a conduit with comparable mechanical properties as the fresh human aorta,

whereas smooth muscle cell contractility and endothelial cell function are dramatically impaired.

The preservation of aortic mechanical properties following this standard cryopreservation procedure has given confidence to the Heart Valve Bank and the Bio Implant Services Foundation in The Netherlands to start explantation, cryopreservation, and implantation of the thoracic descending aorta. Currently, 17 cryopreserved thoracic descending aortic homografts were implanted over Europe. Transplantation indications in these patients comprised infectious aneurysms of the native aorta (3) or prosthetic graft (7), congenital heart disease (4) or other (3). Since Lehalle et al. reported early dilation and rupture of arterial cryopreserved homografts in three patients [18], accurate patient-follow up by means of computed tomography or magnetic resonance angiography to determine the individual fate of aortic homografts may be recommended.

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References

1. Abad C, Hurle A, Feijoo J, Gomez Marrero J, Abdallah A (1995) Total aortic arch replacement by a cryopreserved aortic homograft. Eur J Cardiothorac Surg 9: 531–533
2. Adham M, Gournier JP, Favre JP, De La Roche E, Ducerf C, Baulieux J, Barral X, Pouyet M (1996) Mechanical characteristics of fresh and frozen human descending thoracic aorta. J Surg Res 64: 32–34
3. Almassi GH, Farahbakhsh B, Wooldridge T, Rusch NJ, Olinger GN (1996) Endothelium and vascular smooth muscle function in internal mammary artery after cryopreservation. J Surg Res 60: 355–360
4. Alonso M, Caeiro S, Cachaldora J, Segura R (1997) Infected abdominal aortic aneurysm: in situ replacement with cryopreserved arterial homograft. J Cardiovasc Surg Torino 38: 371–375
5. Armentano RL, Barra JG, Levenson J, Simon A, Pichel RH (1995) Arterial wall mechanics in conscious dogs. Assessment of viscous, inertial, and elastic moduli to characterize aortic wall behavior. Circ Res 76: 468–478
6. Armentano RL, Levenson J, Barra JG, Fischer EI, Breitbart GJ, Pichel RH, Simon A (1991) Assessment of elastin and collagen contribution to aortic elasticity in conscious dogs. Am J Physiol 260: H1870–1877
7. Barner HB, DeWeese JA, Dale WA, Mahoney EB (1966) Aneurysmal degeneration of femoropopliteal arterial homografts. JAMA 196: 631–634
8. Bove T, Demanet H, Beyens T, Viart P, Dessy H, Deville A, Goldstein JP, Deuvaert FE (1996) Nonvalved homografts of thoracic aorta in operation for complex congenital cardiac disease. Ann Thorac Surg 62: 1347–1350
9. Brock L (1968) Long-term degenerative changes in aortic segment homografts, with particular reference to calcification. Thorax 23: 249–255
10. Cox RH (1975) Arterial wall mechanics and composition and the effects of smooth muscle activation. Am J Physiol 229: 807–812
11. Crawford ES, DeBakey ME, Morris GC, Garret E (1960) Evaluation of late failures after reconstructive operations for occlusive lesions of the aorta and iliac, femoral and popliteal arteries. Surgery 47: 79–104
12. Debras B, Enon B, Kanane O, Piard N, Guyetant S (1996) Successful management of an aortoesophageal fistula using a cryopreserved arterial allograft. Ann Vasc Surg 10: 292–296
13. Gournier JP, Adham M, Favre JP, Raba M, Bancel B, Lepetit JC, Barral X (1993) Cryopreserved arterial homografts: preliminary study. Ann Vasc Surg 7: 503–511
14. Groenink M, Langerak SE, VanBavel E, Van der Wall EE, Mulder JM, Van der Wal AC, Spaan JAE (1999) The influence of aging and aortic stiffness on permanent dilation and breaking stress of the thoracic descending aorta. Cardiovasc Res 43: 471–480
15. Key JA, Bigelow WG, Farber EP (1960) Assessment of the value of arterial grafting. Surgery 47: 74–78
16. Kieffer E, Bahnini A, Koskas F, Ruotolo C, Le Blevec D, Plissonnier D (1993) In situ allograft replacement of infected infrarenal aortic prosthetic grafts: results in forty-three patients. J Vasc Surg 17: 349–355
17. Koskas F, Plissonnier D, Bahnini A, Routolo C, Kieffer E (1996) In situ arterial allografting for aortoiliac graft infection: a 6-year experience. Cardiovasc Surg 4: 495–499
18. Lehalle B, Geschier C, Fieve G, Stoltz JF (1997) Early rupture and degeneration of cryopreserved arterial allografts. J Vasc Surg 25: 751–752
19. Mestres CA, Mulet J, Pomar JL (1995) Large-caliber cryopreserved arterial allografts in vascular reconstructive operations: early experience. Ann Thorac Surg 60: S105–107
20. Motomura N, Eishi K, Kitoh H, Kawazoe K, Kitoh Y, Kawashima Y, Oka T (1995) Cell viability of aortic allografts after long-term cryopreservation and clinical application to aortic root replacement in a patient with aortitis. Cardiovasc Surg 3: 231–234
21. Muller Schweinitzer E (1994) Applications for cryopreserved blood vessels in pharmacological research. Cryobiology 31: 57–62
22. Muller Schweinitzer E (1994) Arterial smooth muscle function after prolonged exposure to a medium containing dimethyl sulfoxide (Me2SO) and storage at –196 degrees C. Cryobiology 31: 330–335
23. Muller Schweinitzer E, Mihatsch MJ, Schilling M, Haefeli WE (1997) Functional recovery of human mesenteric and coronary arteries after cryopreservation at –196 degrees C in a serum-free medium. J Vasc Surg 25: 743–750
24. Muller Schweinitzer E, Stulz P, Striffeler H, Haefeli WE (1998) Functional activity and transmembrane signaling mechanisms after cryopreservation of human internal mammary arteries. J Vasc Surg 27: 528–537

25. Nataf P, Hadjiisky P, Lechat P, Mougenot N, Peuchmaurd M, Gouezo R, Gerota J, Cabrol C, Gandjbakhch I (1995) Effect of cold anoxia and cryopreservation on metabolic and contractile functions of human mammary artery. Cryobiology 32: 327–533
26. Nevelsteen A, Goffin Y, Lacroix H, Vandeginste N, Suy R (1995) Recurrent aortic infection: treatment by arterial homograft replacement. Cardiovasc Surg 3: 441–444
27. O'Brien MF, Stafford EG, Gardner MA, Pohlner PG, Tesar PJ, Cochrane AD, Mau TK, Gall KL, Smith SE (1995) Allograft aortic valve replacement: long-term follow-up. Ann Thorac Surg 60: S65–70
28. Pagano D, Guest P, Bonser RS (1996) Homograft replacement of thoraco-abdominal aorta for a leaking mycotic aneurysm. Eur J Cardiothorac Surg 10: 383–385
29. Pompilio G, Polvani GL, Antona C, Rossoni G, Guarino A, Porqueddu M, Buche M, Biglioli P, Sala A (1996) Retention of endothelium-dependent properties in human mammary arteries after cryopreservation. Ann Thorac Surg 61: 667–673
30. Salomon RN, Friedman GB, Callow AD, Payne DD, Libby P (1993) Cryopreserved aortic homografts contain viable smooth muscle cells capable of expressing transplantation antigens. J Thorac Cardiovasc Surg 106: 1173–1180
31. Sheranian LO, Edwards JE, Kirklin JW (1959) Late results in 110 patients with abdominal aortic aneurysm treated by resectional placement of aortic homograft. Surg Gynec Obst 109: 309–314
32. Subin K, Mainwaring RD, Lamberti JJ, Carter TL Jr, Billman G (1994) Management of mycotic aneurysm following repair of corrected transposition. J Card Surg 9: 55–60
33. Szilagyi DE, Rodriguez FJ, Smith RF, Elliott JP (1970) Late fate of arterial allografts. Observations 6 to 15 years after implantation. Arch Surg 101: 721–733
34. Thijssen HJM, Bos E, Konertz W, Suylen van RJ, By de TMMH (1992) Kryokonservierung humaner spenderherzklappen in der herzklappenbank in Rotterdam. Z Herz-, Thorax-, Gefäßchir 6: S49–55
35. Vesely I, Gonzalez Lavin L, Graf D, Boughner D (1990) Mechanical testing of cryopreserved aortic allografts. Comparison with xenografts and fresh tissue. J Thorac Cardiovasc Surg 99: 119–123
36. Vischjager M, Van Gulik TM, Pfaffendorf M, Van Swieten PA, Van Marle J, Kromhout JG, Klopper PJ, Jacobs MJ (1995) Morphology and function of dog arterial grafts preserved in UW-solution. Eur J Vasc Endovasc Surg 10: 431–439
37. Vogt P, Pasic M, von Segesser L, Carrel T, Turina M (1995) Cryopreserved aortic homograft for mycotic aneurysm. J Thorac Cardiovasc Surg 109: 589–591
38. Vogt PR, Pfammatter T, Schlumpf R, Genoni M, Kunzli A, Candinas D, Zund G, Turina M (1997) In situ repair of aortobronchial, aortoesophageal, and aortoenteric fistulae with cryopreserved aortic homografts. J Vasc Surg 26: 11–17
39. Vogt PR, von Segesser LK, Goffin Y, Niederhauser U, Genoni M, Kunzli A, Lachat M, Turina MI (1996) Eradication of aortic infections with the use of cryopreserved arterial homografts. Ann Thorac Surg 62: 640–645
40. Vogt PR, von Segesser LK, Goffin Y, Pasic M, Turina MI (1995) Cryopreserved arterial homografts for in situ reconstruction of mycotic aneurysms and prosthetic graft infection. Eur J Cardiothorac Surg 9: 502–506
41. Wassenaar C, Bax WA, van Suylen RJ, Vuzevski VD, Bos E (1997) Effects of cryopreservation on contractile properties of porcine isolated aortic valve leaflets and aortic wall. J Thorac Cardiovasc Surg 113: 165–172
42. Zwischenberger JB, Shalaby TZ, Conti VR (1989) Viable cryopreserved aortic homograft for aortic valve endocarditis and annular abscesses. Ann Thorac Surg 48: 365–369