

A Comparison of Mechanical Properties of Materials Used in Aortic Arch Reconstruction

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Background. Differences in the mechanical properties of aortic tissues and replacement materials can have unwanted hemodynamic effects leading to graft failure. The aim of this experimental study was to compare the mechanical properties of different graft-patch materials used in aortic arch reconstruction with those of healthy and dilated human ascending aortas (AAs).

Methods. Four square samples were taken from 30 healthy (n = 120) and 14 dilated (n = 56) AA rings and from 34 human pericardial sections (fresh [n = 68] and Carpentiers solution fixed [n = 68]). In addition, square samples from commercial bovine pericardium (n = 14) were also compared with woven Dacron grafts (n = 24) and tested biaxially. Stress-strain curves (0% to 30%) were generated using a biaxial tensile tester to quantify the anisotropic properties and stiffness of the materials at 37°C.

Results. We found significant differences in stiffness and anisotropy among all material types. Fresh and fixed human pericardia, bovine pericardium, and Dacron were 9.5, 7.1, 16.4, and 18.4 times stiffer than dilated AAs, which was 1.3 times stiffer than healthy AAs under physiologic stretch. Only dilated and healthy AAs showed an increase in anisotropic properties with increasing strain.

Conclusions. The significant differences in the mechanical properties among all materials we found are intended to increase the awareness of these differences in materials used in aortic reconstruction surgery. This finding suggests that improvements are needed in prosthetic material design to better mimic native tissue.

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Since the pioneering work in the 1950s of Dubost and colleagues [1], Swan and colleagues [2], Gross [3], and Lam and Aram [4], synthetic and biologic tissues have become indispensable in the treatment of aortic diseases. The number and complexity of thoracic aortic surgical interventions have risen and the patient age continues to increase. The decision for surgery is often a difficult choice between the risk of aortic rupture and the risks associated with surgery and its impact on concomitant diseases (valve disease, ischemic heart disease, peripheral vascular disease, heart failure).

The choice of a prosthetic material used in aortic surgery is highly dependent on the type of treatment and material available. Ideally the material should be impermeable, thromboresistant, compliant, biocompatible, durable, resistant to infection, easy to sterilize, easy to implant, readily available, cost-effective, and should match the mechanical properties of native aortic tissue [5, 6]. For example, Dacron and pericardium are easy to use, durable, and have manageable resistance to thrombosis formation when used in large caliber vessels. However, they also have distinctly different mechanical properties than the native aorta.

The differences in the mechanical properties between native aortic tissue and prosthetic material can alter the pressure and flow throughout the vasculature [7–10]. The associated pressure and flow changes with vascular prostheses can exacerbate peripheral vascular diseases and can also affect the function of the aortic valve and left ventricle.

The mismatch in the mechanical properties can also have a local effect around the attachments to the aorta. The difference in compliance or compliance mismatch is an important contributor to thrombosis and reduced graft patency [11]. This mismatch is believed to contribute to false aneurysm formation [12] and intimal hyperplasia because of local remodeling due to stress concentration [13, 14] or abnormal wall shear stress [15] at the junction between native tissues, vascular prosthetics, and (or) graft materials.

Despite these important effects, very little data are available on the difference in the mechanical properties of materials commonly used in aortic arch reconstruction surgery. Increasing the awareness of these mechanical differences may help in the surgical decision-making process.

Material and Methods

Sample Collection and Preparation

All human tissue specimens were obtained under the guidelines of the Canadian Tri-Council Policy Statement on ethical research [16]. After resection, all tissues were

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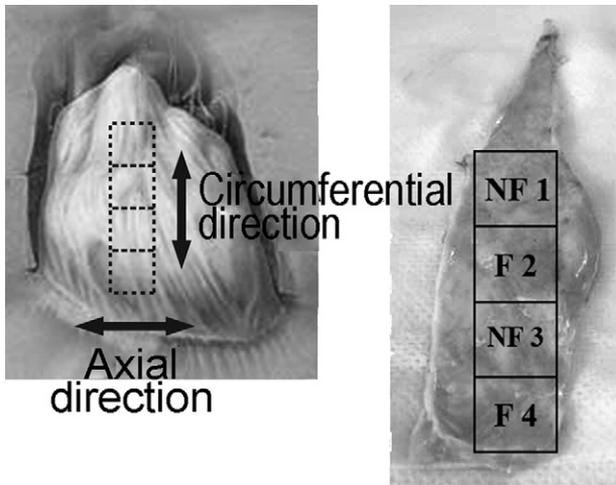


Fig 1. Locations from which human pericardium samples were taken. (F = fixed; NF = nonfixed.)

stored in sterile physiologic saline solution (0.9% sodium chloride) and tested within 24 hours.

ASCENDING AORTA. Healthy human ascending aortas (AAs) were collected at the Maisonneuve-Rosemont Hospital and the Toronto General Hospital. Autopsy cases (20 men, 10 women) from noncardiac causes of death, with aortic tissue that appeared healthy on gross examination, were taken as healthy aortas. Four samples around the circumference of each AA were taken to account for regional variations in the mechanical properties of the tissue [17, 18]. The average age of the healthy AA patients was 53.3 ± 15.3 years old.

Dilated AAs (bicuspid valve patients) were collected at the Montreal Heart Institute from patients under aortic replacement surgery because of a dilated AA (12 men, 2 women). Four samples around the circumference of each AA were taken to account for regional variations in the mechanical properties of the tissue [17, 18]. The average age of this cohort was 61.6 ± 10.3 years old.

Four samples per AA ring were taken in order to get an average value of the mechanical properties of the whole sample. Each square sample was 15×15 mm and marked using a red dye in order to distinguish the circumferential direction from the axial direction for testing purposes. For the dilated cases, we took the samples in the middle of the dilation and in the healthy tissue 0.5 cm above the sinotubular junction.

HUMAN PERICARDIUM. Sections of fresh human pericardium tissue (fibrous layer with parietal membrane attached), measuring roughly 100×20 mm in size, were harvested from coronary artery bypass graft surgery at the Montreal Heart Institute (30 men, 4 women; 65.1 ± 10.3 years old). Sections of tissue were cut into four square smaller samples of 15×15 mm along the circumferential direction (apex to base) defined in Figure 1. A total of 34 sections were collected for mechanical testing. The naming of the orientation was chosen to match the common

insertion orientation in the aorta. The circumferential direction (apex to base) on each sample was carefully identified using a red dye. These four samples were split semirandomly into two groups: two kept in the native state (nonfixed: NF), and the other two chemically treated with Carpentier's solution (0.625% glutaraldehyde) for 10 minutes (fixed: F) for a total of 68 fresh and 68 fixed pericardium square samples. A pilot study had confirmed that there was no significant variation in pericardium mechanical properties throughout the samples.

BOVINE PERICARDIUM. Commercially available Peri-Guard bovine pericardium (Synovis Surgical Innovations Inc, St. Paul, MN) was obtained and tested. The bovine pericardium was chemically sterilized by the manufacturer using glutaraldehyde, ethanol, and propylene oxide and was stored in this solution until use. A total of 14 square samples of 15×15 mm were obtained. We have carefully oriented each sample according to the orientation of the fibers in order to distinguish the two testing directions. Tissue fiber direction was defined as the circumferential direction and marked using a red dye.

DACRON GRAFT. Gelweave Dacron grafts (Sulzer Vascutek), also available commercially, were tested. Three gelatin impregnated woven vascular grafts were cut into square samples (15×15 mm uncrimped), for a total of 24 samples. Samples were prepared in a dry state. The circumferential direction was carefully identified using a red dye in the same way as the aortic rings.

Thickness Measurements

Thickness of all samples was measured at 5 points on the material surface using a digital thickness counter equipped with constant force transducer in order to apply a constant force for a better repeatability (Mitutoyo: Litematic VL-50A, Mitutoyo Corp, Kanagawa, Japan). The Dacron thickness was obtained by the manufacturers.

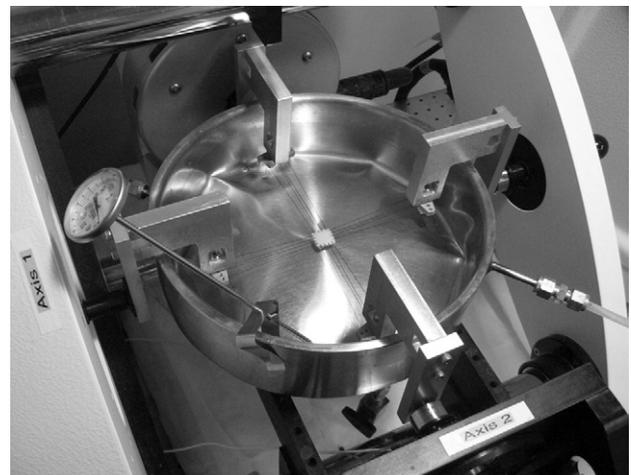


Fig 2. EnduraTEC Electro Force 3200 Biaxial Tensile Tester.

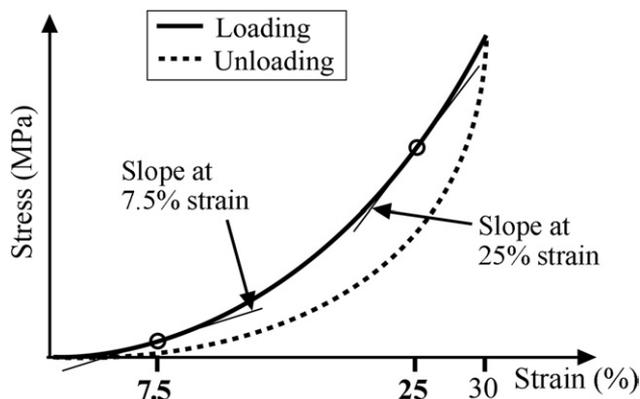


Fig 3. Typical loading-unloading curve shape illustrating both stiffness values at a low and a high strain region. (— = loading; --- = unloading.)

Mechanical Testing

ENDURATEC SYSTEM. Equibiaxial tensile testing was performed using the EnduraTEC Electro Force 3200 Biaxial Tensile Tester (Bose, Eden Prairie, MN) (see Fig 2). The system is equipped with two load cells and two displacement transducers, one for each axis. The load cells and displacement transducers were connected to computer software, WinTest (Bose, Eden Prairie, MN), which displayed and saved displacement and force data of each axis.

BIAXIAL TESTING OF SAMPLES. All samples were attached to the grip of the tensile tester using sutures. Two sutures were placed on each side of the square sample using silk surgical thread 4-0 with polytetrafluoroethylene pledgets (Ethicon, Somerville, NJ) to prevent tissue from tearing at the suture points. During tensile testing, tissue samples were immersed in a bath of Krebs-Ringer buffered solution (Sigma-Aldrich, St Louis, MO) and kept at 37°C. The tissue was placed in the bath for 15 minutes with no applied strain. After this time a tension force of 0.05 N was added in both directions to make sure the tissue was under the same stress conditions in both directions. This loading was taken as the zero stress point. A standard equibiaxial test consisted of 13 cycles: 10 preconditioning cycles to 30% strain, then three experimental runs to 30% strain. The percent strain was based on the average relaxed excised dimensions measure between sutures. All tissue materials were tested at a strain rate of 0.1 mm/second.

Data Analysis

ENGINEERING STRESS-STRAIN CURVES. The force and displacement data collected from the biaxial tensile tester was processed by a MATLAB (The Mathworks Inc, Natick, MA) program to calculate engineering stress and strain data using the following equations:

$$\varepsilon = \frac{x}{d_u} \quad (1)$$

$$\sigma = \frac{F}{A_u} \quad (2)$$

where ε is the engineering strain, x is the displacement (mm), and d_u the unloaded distance between the sutures (mm). σ is the engineering stress (MPa), F is the measured force (N), and A_u is the unloaded area (mm²) calculated from the unloaded distance between the sutures and the unloaded average tissue thickness.

STIFFNESS. As the stress-strain response of biologic tissues is nonlinear, it was necessary to define strain values from which stiffness would be obtained for statistical comparison. From the stress-strain curves the stiffness was obtained at a low (7.5%) and a high strain value (25%) of the loading curve (Fig 3).

ANISOTROPY. All tensile testing was performed using a biaxial tensile tester and therefore it was possible to compute the difference in directional response of the materials, or in engineering terms, the degree of anisotropy. The anisotropic index is the difference in stiffness between the directions (circumferential and axial) divided by the average stiffness, a similar equation to that of Lee and colleagues [19]:

$$AI = \frac{E_c E_a}{E_{avg}}, \quad \text{where } E_{avg} = \frac{E_c + E_a}{2}, \quad (3)$$

where E_c and E_a are stiffness in the circumferential and axial directions, respectively.

Statistical Analysis

The statistical analyses were conducted using the Prism v5.00 software (GraphPad Software Inc, San Diego, CA). Differences were considered significant for a p value less than 0.05. Stiffness, anisotropy, and thickness of each graft material were compared to healthy and dilated AAs using a one-way analysis of variance (ANOVA) and the Tukey post-test [20]. Degree of anisotropy was quantified using a one sample t test testing if the mean was different from zero [20]. All data are presented as the mean \pm standard deviation.

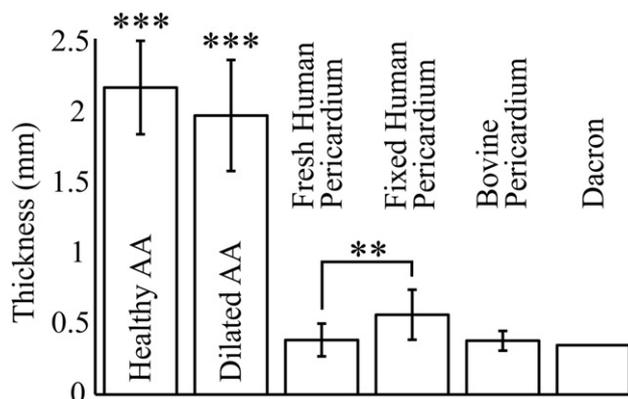


Fig 4. Thickness values for all materials tested. No error bars for Dacron material because the manufacturer value was used; (AA = ascending aortas; *** p value < 0.001, one-way ANOVA, Tukey post-test; ** p value < 0.01, one-way ANOVA, Tukey post-test.).

Results

There was a significant variation in the age of the tissue groups (p value = 0.0013, one-way ANOVA). However, we found no significant variation between patient's age for dilated AAs and pericardial tissue (p value > 0.05, Tukey post-test). This result must be kept in mind because it has been shown that mechanical properties of human aortic tissue have a dependency on age [21], and that dependency could also appear for pericardial tissue. Therefore, having the same age between patients from dilated AA cohort and pericardial tissue cohort increases the comparison validity between these two groups. However, there was a significant difference between the healthy AAs and pericardial tissue (p value < 0.001, Tukey post-test). Although older, the aneurysm patient age was not significantly different than the healthy AA.

After excising the ascending aorta from patients (dilated AA) and autopsy cadavers (healthy AA) the ex vivo

diameter was measured. Dilated AAs had a diameter of 36.1 ± 3.8 , which was significantly different (p value < 0.001, one-way ANOVA, Tukey post-test) from healthy AAs with a diameter of 23.8 ± 3.9 . An in vivo diameter of 52.5 ± 6.5 for dilated AAs was also measured from a computed tomographic scan, which was significantly different from the ex vivo diameters (p value < 0.001, one-way ANOVA, Tukey post-test).

Thickness

Figure 4 shows the thickness variations between materials tested. Human aortic tissues were significantly thicker than all other materials and between each other (p value < 0.001, one-way ANOVA, Tukey post-test). There was also a significant difference in the thickness of fresh and fixed human pericardium (p value < 0.01, one-way ANOVA, Tukey post-test). Fresh and fixed human pericardium, bovine pericardium, and Dacron are at least 74% thinner than healthy and dilated AAs.

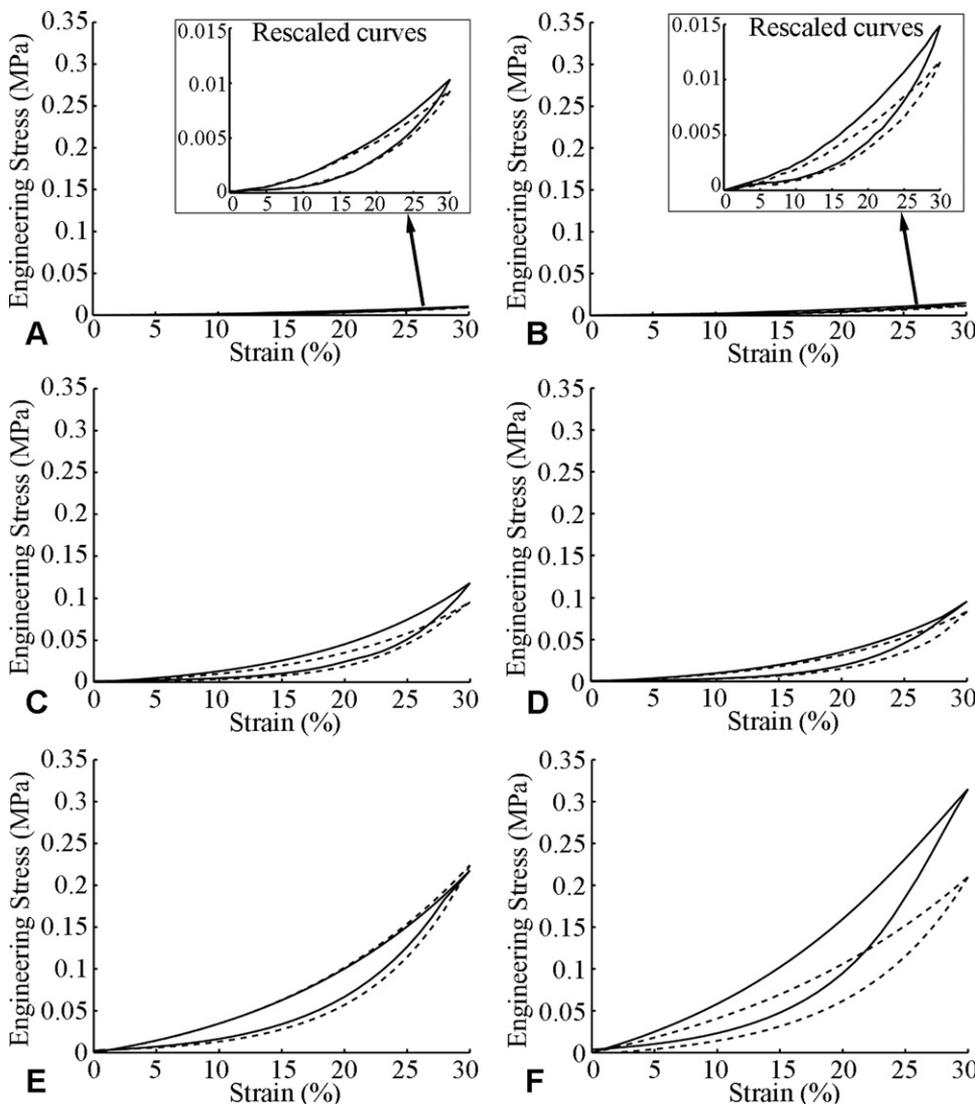


Fig 5. Average loading-unloading curves; dashed and full lines represent circumferential and axial directions, respectively. (A) Healthy ascending aortas [AA]; (B) dilated AA; (C) fresh human pericardium; (D) fixed human pericardium; (E) bovine pericardium; (F) Dacron.

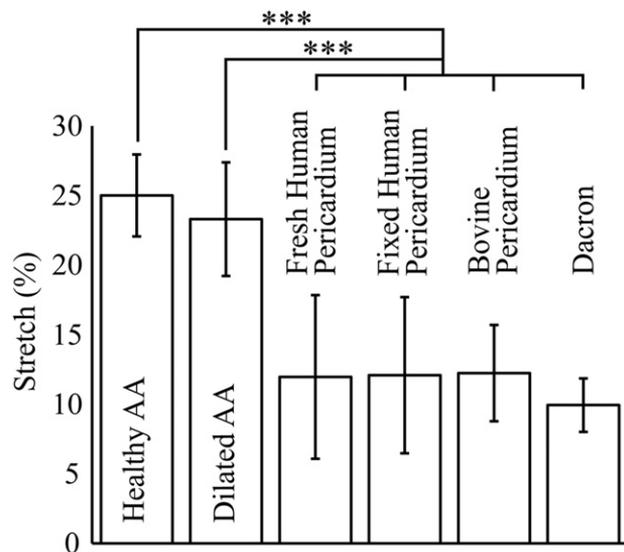


Fig 6. Stretch variations for a specific force giving a 25% stretch to healthy ascending aortas (AA). (***) p value < 0.001; one-way ANOVA, Tukey post-test).

Loading-Unloading Curves

Figure 5 shows the averaged loading and unloading curves for all tissue types in both directions (axial and circumferential). Upon loading, all materials tested become increasingly stiffer with increased strain (stretch). The viscosity in the material causes the unloading curve to be lower than the loading curve. This type of response is termed viscoelastic.

Figure 6 is an illustration of the same data showing the variation in stretch each material would have for the force that would produce a stretch of 25% on healthy AAs. This helps to illustrate how much stretch (or compliance) is lost when this given force is applied to the other types of materials. We found a significant decrease in stretch for fresh and fixed human pericardia, bovine pericardium, and Dacron in comparison with healthy and dilated AAs (p value < 0.001, one-way ANOVA, Tukey post-test).

Stiffness

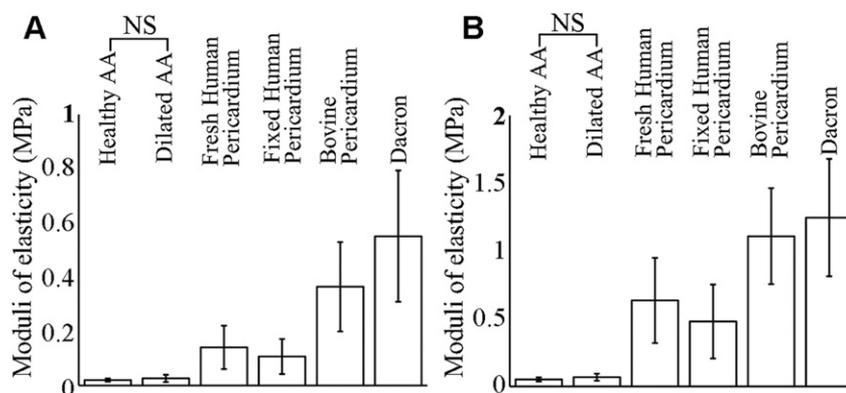
The incremental elastic modulus was used to compare the stiffness of each sample at 7.5% and 25% strain. Here we present the average stiffness of both directions in order to get the bulk stiffness value of each material. There were significant differences in the stiffness between all material types at low strain (7.5%; p value < 0.001, one-way ANOVA, Tukey post-test) and high strain values (25%; p value < 0.01, one-way ANOVA, Tukey post-test) except between healthy and dilated AAs for both strain values (see Fig 7A; B, respectively). Fresh and fixed human pericardia, bovine pericardium, and Dacron were, respectively, 5.4, 4.1, 13.9, and 21.0 times stiffer than dilated AAs, which were 1.3 times stiffer than healthy AAs at low strain. At high strain, fresh and fixed human pericardia, bovine pericardium, and Dacron were, respectively, 9.5, 7.1, 16.4, and 18.4 times stiffer than dilated AAs, which were again 1.3 times stiffer than healthy AAs.

Anisotropy

There were significant variations in the stiffness of the circumferential and axial directions suggesting a directional dependency of all materials. The anisotropic index (AI), calculated at the low (7.5%) and high strain values (25%) for all material types, is shown in Figure 8. For all materials the average AI was positive, meaning the circumferential direction is stiffer than the axial direction. When comparing only two samples, either a paired or unpaired t test was used. For pericardial samples, apex to base was defined as the circumferential direction.

At low strains (Fig 8A), healthy AAs and bovine pericardium show no significant directional dependency (isotropic) with an AI not significantly different from zero. Dilated AAs, fresh and fixed human pericardia, and Dacron have an AI significantly different from zero (represented by a dagger; p value < 0.05, one sample t test) showing a significant directional dependency (anisotropic) at low strain. Dacron and fixed human pericardium were significantly more anisotropic than healthy AAs (p value < 0.001, and p value < 0.01, respectively; one-way ANOVA, Tukey post-test). Dacron was also significantly more anisotropic than bovine pericardium (p value < 0.05, respectively; one-way ANOVA, Tukey post-test).

Fig 7. Stiffness at low and high strain values: (A) Low strain, 7.5%; (B) high strain, 2.5%. (AA = ascending aortas; NS = not significant; p value > 0.05).



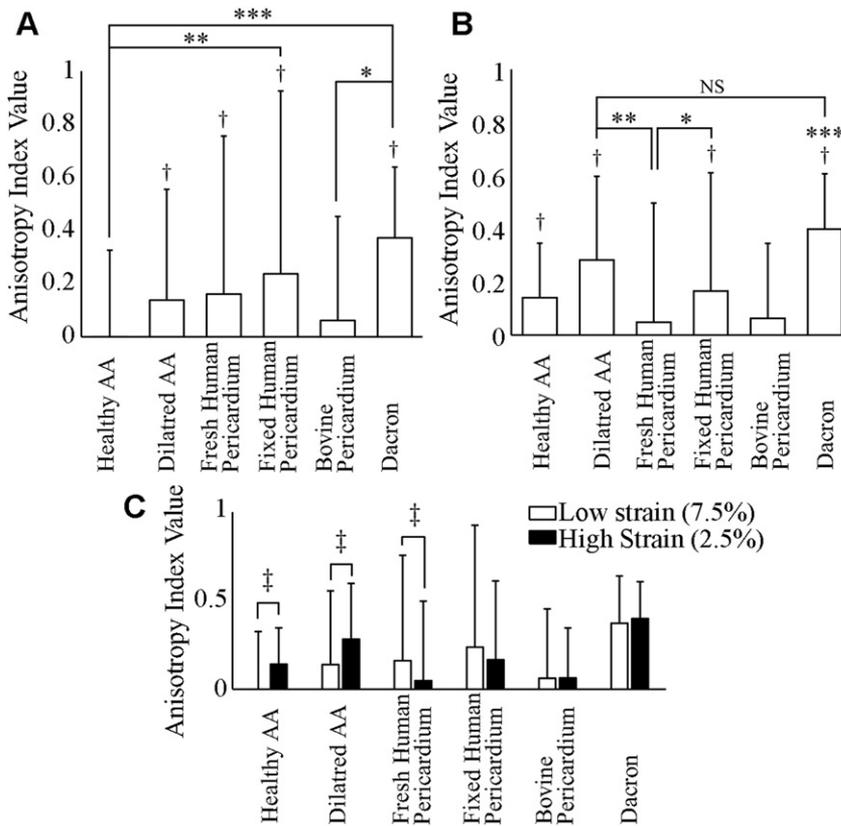


Fig 8. Index of anisotropy for all graft-patch materials and native ascending aortas (AA). (A) Low strain, 7.5%; (B) high strain, 7.5%; (C) grouped low and high strain values. († p value < 0.05, one sample t test, mean different from zero; *** p value < 0.001, one-way analysis of variance [ANOVA], Tukey post-test; ** p value < 0.01, one-way ANOVA, Tukey post-test; * p value < 0.05, one-way ANOVA, Tukey post-test; ‡ p value < 0.001, paired t test; NS = not significant, p value > 0.05).

At high strains (Fig 8B), healthy and dilated AAs, fixed pericardium, and Dacron materials show significant directional dependency. Dacron is significantly more anisotropic than all other material types (p value < 0.001; one-way ANOVA, Tukey post-test) except with dilated AAs. Fixing human pericardium increases the degree of anisotropy (p value < 0.05, one-way ANOVA, Tukey post-test). There was a change in AI between low and high strain values for healthy and dilated AAs and fresh human pericardium (p value < 0.001; paired t test) (see Fig 8C). All other materials had similar AI values at low and high strain.

Comment

In this paper we present the mechanical characteristics of common materials used in the reconstruction of the ascending aorta tissue and compare these properties with healthy and dilated AAs. By presenting the mechanics of these materials, we hope to make surgeons more aware of the significant differences in mechanical properties and the potential effect they may have on treatment outcome.

We show that both healthy and dilated AAs to be significantly less stiff than any of the replacement materials (Fig 7). Healthy AAs were obtained at autopsy and dilated AAs collected during surgery for comparison with the replacement materials. All the materials become stiffer with increasing stretch (Fig 5). At physiologic strain [22], our results show that Dacron is ~25 and ~18 times

stiffer than healthy and dilated AAs (Fig 7A; B). No other studies have directly compared the mechanical properties of Dacron with the AA. Although closer in stiffness to AA tissue than Dacron, all types of pericardia are still significantly stiffer. We found that human pericardium and bovine pericardium to be ~11 and ~22 times stiffer than the healthy AA and ~8 and ~16 times stiffer than the dilated AA. Our stiffness results for bovine pericardium are similar to those reported by Lee and colleagues [23]. Interestingly, fixing human pericardium in Carpentiers solution slightly reduces the stiffness of the tissue. Further testing (data not shown) has proven this effect is due to a swelling of the tissue in solution and not a fundamental change in the tissue structure.

To put these results in context, we looked at the amount each material would stretch at the force needed to stretch healthy AAs 25% in the circumferential direction (Fig 6). Dilated AAs removed from tricuspid valve patients showed a reduction in stretch to 23.3%. Dacron will only stretch 9.9%. Human pericardia (fresh and fixed) and bovine are best matched, but still will reduce the local stretch to around 12%.

When large amounts of aortic tissue are replaced, as with Dacron grafts, this reduced elasticity limits the redistribution of energy from systole to diastole [24]. Studies in dogs have demonstrated that an inelastic graft placed in the aorta increases myocardial oxygen consumption during exercise [8, 25]. A local response at the suture lines is also believed to occur. In smaller caliber vessels, the compliance mismatch between the prosthetic

material and vessel is believed to cause intimal hyperplasia at the anastomosis site [11, 13, 15]. It must be noted that suturing alone can cause intimal hyperplasia and the sutures and suturing technique have an effect on the local compliance [26].

In this paper we used biaxial tensile testing to better characterize all materials using a standardized testing technique. This technique has the advantage to measure the mechanical behavior in two perpendicular directions. All samples were carefully oriented and tested in the same configuration in order to be able to distinguish any differences between the circumferential and axial response. We used the anisotropic index to quantify the difference in stiffness between the directions. At low strains, the healthy AA is relatively isotropic (no differences in direction stiffness), while the samples taken from dilated tricuspid valve patients were anisotropic at the same strain. With increased strain, both the healthy and dilated AAs showed a significant increase in anisotropy. Anisotropy has been previously observed in aortic tissue [27, 28]. Sato and colleagues saw a similar change in the degree of anisotropy from low to high strains in the aorta of dogs [29].

None of the replacement materials demonstrated an increase in anisotropy with strain. Dacron was the most anisotropic material with the circumferential stiffness being 1.5 times greater than the axial at both low and high strain. This behavior comes from the way the material is woven [30, 31]. There was very little change in the anisotropy in the Peri-Guard bovine pericardium with the circumferential direction only marginally stiffer than the axial direction. The effect of fixation on bovine pericardium is well-documented and has shown that the fixed tissue is relatively isotropic [23]. The anisotropy of the human pericardium samples actually decreased with increased strain going from 1.2 times the axial value to only 1.05 times. Only one study has looked at the biaxial mechanical properties of fresh human pericardium. In this study, Lee and colleagues [32] found that the tissue was nearly isotropic under a tension of 40 g/cm giving an average strain value of 10% and 11% in the axial and circumferential directions, respectively.

Ideally, the material used in the reconstruction of the ascending aorta should match the stiffness and anisotropy of the remaining aortic tissue. Practically, knowledge of the anisotropy is important when placing patch materials. If oriented improperly, the stiffness mismatch can be increased. For example, when using human pericardium, the base to apex edge of the pericardial patch would be best sutured circumferentially across the aorta. Peri-Guard bovine pericardium is relatively isotropic, so no special orientation is required.

Despite the durability of currently available biologic and synthetic materials used in aortic surgery there exist significant differences in mechanical properties and behavior in comparison with human ascending aortic tissue. Making surgeons aware of these mechanical differences can aid in surgical decision making and hopefully inspire the design of new materials or techniques which better match the native aortic mechanical properties.

We have performed biaxial tensile testing with 30% deformation cycles at a strain rate of 0.1 mm/s. The deformation was based on displacement of the stretcher. This limits our results to the engineering stress-strain.

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