Supplementary Material

Tensile mechanical testing, analysis and modeling

Planar biaxial mechanical testing was conducted as before for skin tissue(2). Aortic specimens were thawed at 4°C overnight prior to testing. Samples were trimmed to approximately 1 cm^2 and mounted in a four-motor biaxial testing apparatus (ElectroForce Systems, TA Instruments, Springfield, MO, USA) using four hooks attached to each side with 6.0 braided silk suture. Five dots were made on the luminal surface of each specimen using a surgical marker for camera tracking. Samples were hydrated for the duration of testing with 37°C 1X phosphate-buffered saline. Two 22.5 N load cells were pre-loaded to 0.01 N to remove residual slack in the sutures before testing. Motor control captured global specimen movements during testing.

Specimens were conditioned for 10 cycles per protocol at a rate of 0.3 mm/s at 40% global strain; data were collected at sampling frequency of 200 Hz on the 10^{th} cycle. Five protocols with differing tension ratios per axis were run: 1:1 – equibiaxial loading of each axis to 40% strain; 1:0.75 – one axis pulled to 40% strain and the other to only 75% of that; 1:0.5; 0.5:1; and 0.75:1.

Modeling and analysis of data were conducted as before(2), and maximum tangential stiffness (kPa), the maximum slope of the stress-strain curve, was computed along the circumferential direction, the same direction as histopathology analysis.

MRI acquisitions

MRI exams were conducted on MAGNETOM 1.5T Aera, Avanto or Espree (n=32) and 3T Skyra (n=15) scanners (Siemens Medical Systems, Erlangen, Germany).

4D flow MRI data were acquired in a sagittal volume encompassing the thoracic aorta. Respiration gating was performed using a 16 mm-acceptance window size navigator placed on the lung-liver interface. Parallel imaging (GRAPPA) along the phase encoding direction (y) was used with a reduction factor R=2 (24 reference lines) to accelerate the acquisition. Other sequence parameters were as follows: repetition time = 4.8±0.1 ms; echo time = 2.4±0.1 ms; acquisition matrix = 160-192x80-116; isotropic pixel in-plane spacing = 2.2±0.2 [1.7-2.9] mm; slice thickness = 2.7±0.3 [2.2-3.5] mm; 2 k-space segments per cardiac time frame; temporal resolution = 38.8±1.2 [36.0-41.6] ms; receiver bandwidth = 445-460 Hz/pixel. A flip angle of 7° was used for healthy volunteers, while it was set to 15° in BAV patients who received injection of a gadolinium-based contrast agent (0.03 mol/kg Ablavar, Lantheus Medical Imaging, N. Billerica, MA, USA; or 0.1-0.2 mmol/kg Gadavist or Magnevist, Bayer, Leverkusen, Germany; or 0.1-0.2 mmol/kg Multihance, Bracco Diagnostics Inc., Township, NJ, USA). Finally, an encoding sensitivity Venc = 150 cm/s was used in controls, and Venc varied from 150 to 400 cm/s in BAV patients depending on the presence and severity of aortic valve stenosis.

Assessment of left ventricular (LV) function and aortic dimensions

LV end-systolic (ESV) and end-diastolic (EDV) volumes were measured with conventional contouring of electrocardiogram (ECG)-gated cine balanced steady state free precession short-axis images, while including the papillary muscles and chamber trabecula, using QMass v7.2 (Medis, Leiden, The Netherlands). Stroke volume, SV = EDV-ESV and ejection fraction, EF = SV/EDV were calculated. Aortic diameter measurements were obtained from contrast-enhanced MR angiography images using the open-source, free DICOM medical image viewer Horos™ (2015, http://www.horosproject.org/; based on OsiriX). Sinuses of Valsalva diameter was the maximal value among the 3 sinus-to-sinus measurements, including the external walls. Diameter at all other locations was the maximal value over 2 orthogonal measurements in a reformatted plane orthonormal to the aorta, including the external walls(1).

4D flow MRI data preprocessing

For each 4D flow dataset, preprocessing was first applied using a previously described Matlab program (MathWorks, Natick, MA, USA)(3), including eddy current correction, background noise suppression and velocity aliasing unwrapping. A 3D angiogram (PC-MRA) was computed by multiplying absolute velocity by magnitude images and averaging over all cardiac phases(3), to segment the aortic volume (Mimics, Materialize, Leuven, Belgium) and subsequently mask the flow velocities.

Supplementary References

1. Trinh B, Dubin I, Rahman O, Ferreira Botelho MP, Naro N, Carr JC, et al. Aortic Volumetry at Contrast-Enhanced Magnetic Resonance Angiography. Invest Radiol. 2017 Apr;52(4):216–22.

2. Rosin NL, Agabalyan N, Olsen K, Martufi G, Gabriel V, Biernaskie J, et al. Collagen structural alterations contribute to stiffening of tissue after split-thickness skin grafting. Wound Repair Regen. 2016;24(2):263–74.

3. Schnell S, Entezari P, Mahadewia RJ, Malaisrie SC, McCarthy PM, Collins JD, et al. Improved Semiautomated 4D Flow MRI Analysis in the Aorta in Patients With Congenital Aortic Valve Anomalies Versus Tricuspid Aortic Valves. J Comput Assist Tomogr. 2016;40(1):102–8.

Supplementary Tables

Table S1. Location of resected aortic tissue samples in the 27 BAV patients, according to longitudinal zone and circumferential quadrant. For each, n denotes the total number of samples available over all patients.

Longitudinal zone Circumferential quadrant	AA_{prox} (26 patients)	AA_{dist} (5 patients)
Anterior, n	23	2
Posterior, n	21	2
Greater curvature, n	25	5
Lesser curvature, n	12	3

AA_{prox} and AA_{dist}: proximal and distal ascending aorta