Bioprinting of Decellularized Porcine Cardiac Tissue for Large-Scale Aortic Models

Beu P. Oropeza1, Jason R. Adams2, Michael E. Furth1, Jack Chessa2 and Thomas Boland1*

1Biomedical Device, Delivery and Diagnostic Laboratory, Metallurgical, Materials and Biomedical Engineering Department, The University of Texas at El Paso, El Paso, TX, United States, 2Aerospace and Mechanical Engineering, The University of Texas at El Paso, El Paso, TX, United States

Bioprinting is an emerging technique used to layer extrudable materials and cells into simple constructs to engineer tissue or arrive at in vitro organ models. Although many examples of bioprinted tissues exist, many lack the biochemical complexity found in the native extracellular matrix. Therefore, the resulting tissues may be less competent than native tissues—this can be especially problematic for tissues that need strong mechanical properties, such as cardiac or those found in the great vessels. Decellularization of native tissues combined with processing for bioprinting may improve the cellular environment for proliferation, biochemical signaling, and improved mechanical characteristics for better outcomes. Whole porcine hearts were decellularized using a series of detergents, followed by lyophilization and mechanical grinding in order to produce a fine powder. Temperature-controlled enzymatic digestion was done to allow for the resuspension of the decellularized extracellular matrix into a pre-gel solution. Using a commercial extrusion bioprinter with a temperature-controlled printhead, a 1:1 scale model of a human ascending aorta and dog bone shaped structures were printed into a reservoir of alginate and xanthium gum then allowed to crosslink at 37°C. The bioengineered aortic construct was monitored for cell adhesion, survival, and proliferation through fluorescent microscopy. The dog bone structure was subjected to tensile mechanical testing in order to determine structural and mechanical patterns for comparison to native tissue structures. The stability of the engineered structure was maintained throughout the printing process, allowing for a final structure that upheld the dimensions of the original Computer-Aided Design model. The decellularized ECM (Ɛ = 920 kPa) exhibited almost three times greater elasticity than the porcine cardiac tissue (Ɛ = 330 kPa). Similarly, the porcine cardiac tissue displayed two times the deformation than that of the printed decellularized ECM. Cell proliferation and attachment were observed during the in vitro cell survivability assessment of human aortic smooth muscle cells within the extracellular matrix, along with no morphological abnormalities to the cell structure. These observations allow us to report the ability to bioprint mechanically stable, cell-laden structures that serve as a bridge in the current knowledge gap, which could lead to future work involving complex, large-scale tissue models.

Keywords: decellularization, tissue engineering and regenerative medicine, bioprinting, biomechanics, biomaterials, extracellular matrix
INTRODUCTION

In the United States alone, 40,000 children are born with a congenital heart problem each year (Jenkins et al., 2007). The current procedures used to treat aortic defects involve synthetic materials, usually textiles, as grafts that replace aortic sections or stents that give additional support to the aortic wall (Kleigman, 2011). Each of these options results in numerous surgical interventions throughout a child’s developing years, leading to extended hospital stays, and potential trauma (Fawzy et al., 2004; Walhout et al., 2004; Carr et al., 2005). Additionally, it has been found that children who suffer from congenital heart defects can be subject to developmental and language delays (Limperopoulos et al., 2002; Easson et al., 2019; Fourdain et al., 2019). Bioprinting, an emerging technique in the biomedical engineering field, has the potential to solve the problems that arise from repetitive surgical intervention. Bioengineers are able to extrusion print using materials specifically characterized for the engineered tissue. The layered biomaterials and cells form precise structures modeled from native biological tissues that can grow along with the child (Murphy and Atala, 2014; Zhang et al., 2016; Rider et al., 2018).

Although many tissues have been engineered using bioprinting, maintaining the biochemical complexity and structural integrity necessary to retain native function has posed a problem in the past. The decellularization of native tissues in order to acquire the extracellular matrix (ECM) as a whole or as components has allowed researchers to bridge the gap in biochemical mimicry. It has been found that ECM helps control tissue-specific functions using interactions with the surrounding cells through a dynamic bidirectional system extending past the cell membrane to the level of gene expression (Bissell et al., 1982; Bornstein et al., 1982; Nelson and Bissell, 2006). This relationship between the cell and its biochemical environment is crucial for the proper development and function of tissues. By decellularizing native tissues and preparing them for bioprinting, researchers can create functional substitutes that more closely mimic the native ECM.

![Figure 1](image_url)

**FIGURE 1** | (A) Fresh whole porcine heart. (B) Cut sections of porcine cardiac tissue prior to decellularization. (C) Cut sections of cardiac tissue post chemical decellularization. (D) Lyophilized decellularized ECM. (E) Powdered decellularized ECM. (F) Final gelled form of the enzymatically digested decellularized ECM. (G–I) Enzymatically digested decellularized ECM at different pH after a 1 h incubation at 37°C.
surroundings stimulates proliferation, cell growth and, through
the presence of laminin and fibrin, increases cell adhesion
properties (Ott et al., 2008; Badylak et al., 2009; Park and
Woo, 2012; Wolf et al., 2012).

Equal importance must be given to the mechanical properties
of the engineered tissues. As with all living things, these structures
are meant to exist in a dynamic environment, oftentimes with
relatively large forces acting upon them (Fung, 1933). Removing
the tissue from its native environment and processing it for
decellularization leads to alterations in the ECM’s physical,
structural, and mechanical characteristics (Gilbert et al., 2006).
This study places particular interest in defining the ECM’s
characteristics prior to decellularization as well as after the
bioprinting process is complete. Using samples of porcine
cardiac tissue as a control, we compare it to printed dECM
and assess the physiological and biochemical makeup, surface
topography, and mechanical characteristics.

MATERIALS AND METHODS

Decellularization of Porcine Hearts
Fresh whole porcine hearts were acquired from Nebraska
Scientific (Omaha, NE) and cut down to sections not
exceeding 2 mm³. The tissues were then placed in 2.5 L of a
1% SDS solution for 48 h, stirred continuously. The solution
was replaced at 24 h, and it was ensured no agglomerations had
formed. The cardiac tissue was then treated with 1% Triton X-
100 for 30 min followed by 3 days of PBS washes, where the buffer
was replaced every day, and the tissues are stirred continuously.
To sterilize the decellularized tissue, it was treated with 1% peracetic acid for 4 h and washed five times with sterile PBS
and sterile distilled water. All decellularization steps were done at
room temperature. The strained tissue was then frozen at -80°C
overnight and lyophilized. All decellularization techniques
followed were taken from Pati et al. with minimal modification (Pati et al., 2014).

Cell Culture
Human Aortic Smooth Muscle Cells were grown in vascular
growth media (ATCC PCS-100-030) plus smooth muscle kit
(ATCC PCS-100-042) under normal environmental culture
conditions at 37°C with 5% CO₂ in a fully aseptic
environment. Before printing, cell membranes were stained
using PKH26 red fluorescent live cell membrane stain (Sigma
MINI26-1KT).

Bioprinting
The lyophilized dECM was digested and re-suspended in order to
form a printable gel material. Lyophilized dECM was
mechanically pulverized using burr grinding methods, then 3 g
of dECM were digested with 30 mg of pepsin from porcine gastric
mucosa (Sigma P7125-100G, ≥3,200 units/mg protein) in 100 ml
of 0.5 M acetic acid for 48 h at room temperature with continuous
stirring (Pati et al., 2014). Centrifugation at 500 x g for 10 min
prior to pH adjustment was done to allow separation of any
particles that were not solubilized; this allowed for a homogenous
texture when printing. Ice cold 10M NaOH was used to adjust the
pH of the dECM solution to the physiological range; while the
dECM was kept below 10°C to avoid premature crosslinking
of the solution. A Bio X bioprinter (Cellink, Boston, MA) equipped
with a cooling printhead and an 18-gauge blunt needle was used
for all printed samples. Printhead temperature was maintained at
14°C to avoid premature crosslinking of the bioink, print bed
temperature was kept at 30°C to help begin the crosslinking process prior to the 1-hour incubation period to achieve full gelation. A print stabilization solution of 1% Xanthium Gum was used to ensure the stability of the print prior to gelation, a modification of the technique described in Noor et al. (2019). For the cell-laden bio printed structure a cell suspension of $1 \times 10^{12}$ cells/mL was mixed with the solubilized, pH-adjusted dECM immediately prior to bioprinting. Printhead and print bed temperatures were maintained at 14 and 30°C, respectively.

Autodesk Fusion 360 was used for Computer-Aided Design (CAD). The tensile testing shape was drawn to ASTM D412- Die A for thermoplastic elastomers, with minor modifications due to the biological nature of the samples. Dimensions for the aorta construct were taken from previous literature (Walther et al., 1985; Jarvisalo et al., 2001; Skilton et al., 2005; Taketazu et al., 2017).

Histological Staining

The tissue samples were processed using a Thermo Scientific Spin Tissue Processor Microm STP-120. Tissues were fixed using 4% formalin at 4°C overnight, followed by dehydration. Samples were dehydrated in increasing ethanol concentrations (starting with 70, 95, and 100%), followed by immersion in xylene two times and paraffin infiltration. Paraffin-embedded tissues were sectioned at 4–6 μm using a Shandon Finesse™ E/ME microtome. All samples were deparaffinized in three xylene washes then rehydrated in decreasing ethanol concentrations (100, 95, 70, and 50%), with each wash lasting 3 min. Hematoxylin and eosin (H&E) staining was done post-deparaffinization. Following rehydration, the samples were exposed to hematoxylin solution for 2 min, then rinsed with running water for 1-minute, distilled water for 30 s, and 95% alcohol for an additional 30 s. Immediately after, the samples were exposed to eosin solution for 1 min, then washed with increasing amounts of alcohol (95% x2, 100% x2) for 2 min each, then two xylene washes each for 2 min. Trichrome staining was done using Masson’s Trichrome Staining Kit; all kit procedures were precisely followed. All samples were mounted using Cytoseal™60 (Thermo Scientific REF#8310-4).

Microscopy

For scanning electron microscopy imaging, samples were fixed using 4% formalin and dehydrated in increasing ethanol concentrations prior to lyophilization. All specimens were sputter-coated for 20 s and viewed using a Hitachi S-4800-II SEM. A Nikon AZ100 inverted light microscope was used for histological imaging.

Mechanical Testing

Uniaxial tensile testing was done at room temperature in order to determine the mechanical properties of native tissue and decellularized printed tissue. One group of samples was taken from a fresh whole porcine heart; rectangles were cut vertically along the sagittal plane. Each sample’s thickness was determined by measurements using a digital caliper. The second sample group consisted of printed decellularized ECM made to ASTM standard D638—Type I and also measured using a digital caliper. All specimens were patted dry, fixed to grips, and placed in the uniaxial electromechanical tensile testing machine (MTESTQuattro), where they were subjected to a strain rate of 5 mm min⁻¹ at room temperature until rupture. Forces were measured by a 200 lb. load cell and elongation by the internal sensor as well as by displacement of the reference grid.

Tensile Data Analysis

The stress-strain relationships for the samples tested were obtained using the Mooney-Rivlin Model for incompressible hyperelastic materials (Peattie et al., 1996; Rubin and Krempl, 2010; Ruiz de Galarreta et al., 2016). Where:

\[
Tensile\ stress\ \sigma = \frac{F}{A_0} \quad (1)
\]

\[
Stretch\ ratio\ \lambda = \frac{\Delta L}{L_0} - 1 \quad (2)
\]

Because the mechanical testing conducted was a uniaxial tensile test:

\[
\sigma_{1,1} - \sigma_{3,3} = 2C_1\left(\lambda^2 - \frac{1}{\lambda}\right) - 2C_2\left(\frac{1}{\lambda^2} - \lambda\right) \quad (3)
\]

And:

\[
\sigma_{2,2} - \sigma_{3,3} = 0 \quad (4)
\]

In this case, the model can be further simplified under the assumption of simple tension making:

\[
\sigma_{2,2} = \sigma_{3,3} = 0 \quad (5)
\]

Therefore:

\[
\sigma_{1,1} = \left(2C_1 + 2C_2\right)\left(\lambda^2 - \frac{1}{\lambda}\right) \quad (6)
\]

This data was then plotted to find the predicted stress-stretch ratio diagrams. Young’s modulus was derived from the slope at the linear region of the graph. Poisson’s ratio was determined by taking the transversal elongation and dividing by axial compression.

RESULTS

Decellularization of Porcine Hearts

Chemical decellularization of the cardiac tissue resulted in visual, physical changes to the structure and state of the tissue sections (Figures 1A–F). When subjected to temperatures of 37°C post enzymatic digestion the decellularized ECM showed three different stages of crosslinking. At a pH of 3 it was a low viscosity fluid, transitioned to being highly viscous at a pH of 5, and finally crosslinked successfully at a pH of 7 (Figures 1G–I). Hematoxylin and eosin staining of the cardiac tissue pre-decellularization confirmed normal morphology of the cellular structures, no nuclear irregularity or clumping of the chromatin to indicate cancerous areas. Staining with Masson’s Trichrome showed collagenous areas throughout the section; a normal
extracellular matrix was visible and necessary for future experiments’ success. Once chemical decellularization, enzymatic digestion, and gelation of the decellularized ECM was completed, the stains were repeated to determine the presence of cellular matter and collagen. Staining showed a lack of cellular structures and nuclear matter; collagen fibrils are uniformly seen throughout the decellularized sample (see Figure 2). Randomly oriented collagen fibrils can be seen in the decellularized ECM’s dense network, whereas the printed decellularized ECM’s topography was similar to that of the porcine cardiac tissue (see Figure 3).

**Mechanical Characteristics**

Video stills of the tensile testing allowed for precise comparison of the change in length \((\Delta L)\) and change in width \((\Delta H)\) (Figure 4) in addition to the information gathered for the stress-strain ratio curves. Due to the samples’ large deformations when undergoing forces, a Mooney-Rivlin model was used to find the samples’ effective stresses (Figure 5). The response of the cardiac tissue (Figures 5A, C) indicates an overall higher tensile strength and greater plasticity than that of the printed decellularized ECM (Figures 5B, D). Young’s Modulus and Poisson’s Ratio for the individual samples are shown in Table 1. The printed decellularized ECM \((\varepsilon = 920 \text{ kPa})\) exhibited 2.7 times greater elasticity than the porcine cardiac tissue \((\varepsilon = 330 \text{ kPa})\). Similarly, the porcine cardiac tissue displayed 1.8 times the deformation \((\psi = 3.93)\) prior to failure than the printed decellularized ECM \((\psi = 2.13)\). Neither value showed a significant \((p < 0.05)\) difference between the two sample types.

**Bioprinting**

Due to the highly viscous nature of the bioink used a larger gauge needle was selected for bioprinting. The dog bone and cylindrical structures were printed without deformation issues or bleeding of the bioink into the support bath (Figure 6). The printed aortic structures maintained their original shape and dimensions post crosslinking, as did the tensile testing samples. Confocal imaging of the bioprinted HASMC indicates normal cell attachment and morphology 24 h post-print, with no necrotic areas as seen in Figure 6D.

**DISCUSSION**

The study examined mechanical characteristics and cellular compatibility of bioprinted decellularized ECM. Successful decellularization is an essential first step in the process; cellular remnants in the bioink cause necrotic pockets, which lead to contamination and complete failure of the construct. Additionally, as the project’s end goal is implantation into a body, rejection is a considerable concern; complete decellularization ensures the possibility of rejection is lowered. Hematoxylin and Eosin staining of the tissues pre-decellularization show normal physiology with clear musculature orientation; once the tissue has undergone decellularization, a drastic change to the physiological properties occurs, with little to no cells present in the sample. Trichrome staining exhibited large quantities of collagen in both samples. Once decellularized, the fibers are no longer uniformly oriented; instead, they are found disbursed throughout the sample, seemingly at random. This can also be seen in the SEM images. Fibrillar collagen and additional elastic fibers comprise approximately 50% of the aorta’s dry weight, making it the primary defining element in the mechanical properties of...
FIGURE 4 | Video stills of porcine cardiac tissue (A) and decellularized ECM (B) undergoing tensile testing. Red arrows indicate initial failure point.

FIGURE 5 | Stress vs. stretch ratio graphs of the raw testing data from porcine cardiac tissue (A) and printed decellularized ECM (B). Mooney-Reiflin fit lines for the testing data curves of cardiac tissue (C) and the printed decellularized ECM (D).
the large vessel (Harkness et al., 1955; Borst et al., 1983; Jana et al., 2019). Fiber orientation is responsible for the strength of the vessel wall; therefore, the non-uniform orientation of the fibers diminishes the tensile strength of the tissue (Wagenseil and Mecham, 2009).

Similarly, the digestion of the dECM must be carefully monitored. Enzymatic activity is temperature, pH, and concentration sensitive, as is protein structural stability; the lower pH of 3 and 5 lack the environmental factors necessary to promote the electrostatic and hydrophobic interactions between the collagen molecules that facilitate proper thermal crosslinking (Theocharis et al., 2019). At physiological pH (7), not only are the crosslinking capabilities optimized, but the environment for cellular adhesion, proliferation, and signaling is ideal.

Currently, SDS treatment for decellularization, such as what was used, is considered the preferred method for preserving the structural components of elastin and collagen (Grauss et al., 2003; Liao et al., 2007). These previous studies have been done on whole tissue sections and found minor, non-significant differences in the tangent modulus (Zou and Zhang, 2012). Alternatively, bioprinting of the matrix requires more aggressive treatments to the tissue, leading to significant structural and chemical alterations to the matrix. Fresh cardiac tissue samples were compared to the bioprinted samples for uniaxial tensile testing. Though both exhibited hyperplastic properties, the printed dECM samples saw a heightened elasticity, 2.7X that of the cardiac tissue. This elasticity of 920 kPa is 1.8X that of a native infant ascending aorta at normal physiological pressure (60 mmHg) and closer to the elasticity seen under the high stress conditions of higher blood pressure (967.45 kPa) (Ligere et al., 2012).

A lack of stiffness in a vessel is usually associated with structural immaturity, which is remedied by progressive blood pressure increases during fetal development, or aging of the vessel (Dobrin, 1978; Lang et al., 1994; Akira and Yoshiyuki, 2006). In this case, the tested samples contained no cells, thus lacking the

### TABLE 1 | Mechanical Characteristics.

| Sample | Young’s modulus (E) kPa | Poisson’s ratio (ν) |
|--------|-------------------------|--------------------|
| CS1    | 233                     | 3.17               |
| CS2    | 288                     | 6.93               |
| CS3    | 470                     | 1.69               |
| ES1    | 1,284                   | 1.61               |
| ES2    | 1,134                   | 0.74               |
| ES3    | 831                     | 3.00               |
| ES4    | 576                     | 3.83               |
| ES5    | 770                     | 1.46               |

CS1–3 refers to the porcine cardiac tissue samples 1–3. ES1–5 refers to the printed decellularized extracellular matrix samples 1–5.
additional stability given by the smooth muscle fibers and the biomechanical morphological changes that come from the environmental and biochemical stimuli the cells receive.

Additionally, elasticity of the aortic tissue is greatly affected by surgical interventions. Depending on the type of anastomosis that the physician chooses, the moduli can range from 683.29–1,232.79 kPa for an extended end-to-end anastomosis or 902.39–1,261.35 kPa for an end-to-end anastomosis (Ligere et al., 2012). As the elastic moduli of the dECM falls within the parameters that are seen for post-operative aortic tissues, we anticipate the addition of cells to the construct will bring the overall elasticity down to levels near those of the native aorta.

In a step towards making full aortic constructs, we incorporated human cells by co-printing and culturing ASMC in the dECM. This allowed us to determine cellular viability and adhesion capabilities of the cells within the matrix. 24 h post-printing, the ASMC exhibited normal morphology, and no necrotic areas were visible. The biocompatibility of the dECM post-printing is promising, pointing to the ability of the cells to grow within the matrix for extended periods of time. Overall, the study found an increase in hyperelasticity of the dECM and an ability for ASMC to thrive in vitro post-printing, indicating our ability to bioengineer an immature large-scale aortic model, furthering the capabilities of vascular engineering.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

BO—design of experiments, bioprinting, data analysis, drafting of manuscript. JA—data analysis. MF—imaging. JC—conceptual design, mechanical testing design, mechanical data interpretation. TB—conceptual design, experimental design, editing of manuscript.

FUNDING

This material is based upon work supported by the National Science Foundation (NSF) Graduate Research Fellowship under Grant No. 1744621. This research and BO was also funded by the National Institute of General Medical Sciences (NIGMS) of the National Institutes of Health (NIH) under linked Award Numbers RL5GM118969, TL4GM118971, and UL1GM118970. The content of this study is solely the responsibility of the authors and does not necessarily represent the official views of the NIH, NSF, and UTEP.

REFERENCES

Akira, M., and Yoshiyuki, S. (2006). Placental Circulation, Fetal Growth, and Stiffness of the Abdominal Aorta in Newborn Infants. J. Pediatr. 148, 49–53. doi:10.1016/j.jpeds.2005.06.044
Badylak, S. F., Freytes, D. O., and Gilbert, T. W. (2009). Extracellular Matrix as a Biological Scaffold Material: Structure and Function. Acta Biomater. 5, 1–13. doi:10.1016/j.actbio.2008.09.013
Bissell, M. J., Hall, H. G., and Parry, G. (1982). How Does the Extracellular Matrix Direct Gene Expression? J. Theor. Biol. 99, 31–68. doi:10.1016/0022-5193(82)90388-5
Borst, H., Walterbusch, G., and Schaps, D. (1983). Extensive Aortic Replacement Using “Elephant Trunk” Prosthesis. Thorax. Cardiovasc. Surg. 31, 37–40. doi:10.1055/s-2007-1020290
Carr, J. A., Amato, J. J., and Higgins, R. S. D. (2005). Long-Term Results of Surgical Coarctectomy in the Adolescent and Young Adult with 18-Year Follow-Up. Ann. Thorac. Surg. 79, 1950–1956. doi:10.1016/j.athoracsur.2005.01.004
Dobrin, P. (1978). Mechanical Properties of Arteries. Physiol. Rev. 58, 397–460. doi:10.1152/physrev.1978.58.2.397
Eason, K., Dahan-Oliel, N., Rohlicek, C., Sahakian, S., Brossard-Racine, M., Mazer, B., et al. (2019). A Comparison of Developmental Outcomes of Adolescent Neonatal Intensive Care Unit Survivors Born with a Congenital Heart Defect or Born Preterm. J. Pediatr. 207, 34–41. e2. doi:10.1016/j.jpeds.2018.11.002
Fawzy, M. E., Awad, M., Hassan, W., Al Kadhi, Y., Shoukri, M., and Fadley, F. (2004). Long-term Outcome (Up to 15 years) of Balloon Angioplasty of Discrete Native Coarctation of the Aorta in Adolescents and Adults. J. Am. Coll. Cardiol. 43, 1062–1067. doi:10.1016/j.jacc.2003.10.040
Fourdaiin, S., St-Denis, A., Harvey, J., Birca, A., Carmant, L., Gallagher, A., et al. (2019). Language Development in Children with Congenital Heart Disease Aged 12–24 Months. Eur. J. Paediatr. Neurol. 23, 491–499. doi:10.1016/j.ejpn.2019.03.002
Fung, Y. (1933). Mechanics of Living Tissues. 2nd Edn. Berlin/Heidelberg, Germany: Springer.
Gilbert, T. W., Sellaro, T. L., and Badylak, S. F. (2006). Decellularization of Tissues and Organs. Biomaterials 27, 3675–3683. doi:10.1016/j.biomaterials.2006.02.014
Grauss, R. W., Hazekamp, M. G., Van Vliet, S., Gittenberger-De Groot, A. C., and Gittenberger-De Groot, A. C. (2003). Decellularization of Rat Aortic Valve Allografts Reduces Leaflet Destruction and Extracellular Matrix Remodeling. J. Thorac. Cardiovasc. Surg. 126, 2003–2010. doi:10.1016/s0022-5229(03)00956-5
Harkness, M., Harkness, R., and McDonald, D. (1955). The Collagen and Elastin Pathobiology of the Endothelial Cell, P and S Biomedical Sciences Symposia, 6, 215–232.
Jarana, S., Hu, M., Shen, M., and Kassiri, Z. (2019). Extracellular Matrix, Regional Heterogeneity of the Aorta, and Aortic Anerysmus. Exp. Mol. Med. 51. doi:10. 1038/s41227-019-0286-3
Järvisalo, M., Jarvi, L., Nanto-Salonen, K., Irjala, K., Rönnemaa, T., Hartila, J. J., et al. (2001). Increased Aortic Intima-media Thickness: a Marker of Preclinical Atherosclerosis in High-Risk Children. Circulation 104, 2943–2947. doi:10. 1161/01.cir.90.100522
Jenkins, K. J., Correa, A., Feinstein, J. A., Botto, L., Britt, E. A., Daniels, S. R., et al. (2007). Noninherited Risk Factors and Congenital Cardiovascular Defects: Current Knowledge - A Scientific Statement from the American Heart Association Council on Cardiovascular Disease in the Young. Circulation 115, 2995–3014. doi:10.1161/circulationaha.106.183216
Kleigman, R. M. (2011). Nelson Textbook of Pediatrics. Amsterdam, Netherlands: Elsevier.
Lang, R. M., Cholley, B. P., Korcarz, C., Marcus, R. H., and Shroff, S. G. (1994). Measurement of Regional Elastic Properties of the Human Aorta: A New Application of Transesophageal Echocardiography with Automated Border Detection and Calibrated Subclavian Pulse Tracings. Circulation 90, 1875–1882. doi:10.1161/01.cir.90.4.1875
