Published in final edited form as:

J Mech Behav Biomed Mater. 2016 June ; 59: 71–77. doi:10.1016/j.jmbbm.2015.11.041.

Stem cell therapy restores viscoelastic properties of myocardium in rat model of hypertension

Andres Rubiano¹, Yanfei Qi², Dominic Guzzo², Kyle Rowe¹, Carl Pepine², and Chelsey Simmons^{1,2,3}

¹Department of Mechanical and Aerospace Engineering, College of Engineering

²Division of Cardiovascular Medicine, Department of Medicine, College of Medicine

³Department of Biomedical Engineering, College of Engineering

Abstract

Extensive remodeling of the myocardium is seen in a variety of cardiovascular diseases, including systemic hypertension. Stem cell therapy has been proposed to improve the clinical outcomes of hypertension, and we hypothesized that changes in mechanical properties of the myocardium would accompany the progression of disease and the results of treatment conditions. Using spontaneously hypertensive rats (SHR) as a model of hypertension, we treated 13-week-old hypertensive rats with a single injection of adipose-derived stem cells (ADSC) isolated from a normotensive control. We indented the isolated ventricles of control, untreated sham-injected SHR, and ADSC-treated SHR hearts with a custom cantilever-based system and fit the resulting data to a standard linear solid model. SHR animals had higher blood pressure (198.4 ± 25.9 mmHg) and lower ejection fraction ($69.9 \pm 4.2\%$) than age-matched control animals (109.0 ± 1.6 mmHg, $88.2 \pm 1.3\%$), and increased viscoelastic properties accompanied these clinical changes (Right ventricle effective stiffness, SHR: 21.97 ± 5.10 kPa, Control: 13.14 ± 3.48 kPa). ADSC-treated animals saw improvement in clinical parameters compared to the untreated SHR group, which was also accompanied by a significant restoration of viscoelastic properties of the myocardium (ADSC-treated SHR: 9.77 ± 6.96 kPa).

Graphical Abstract

Corresponding author: Chelsey Simmons, css@ufl.edu, 352-392-4365, PO Box 116250, Gainesville, FL 32611.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

Tissue mechanics; spontaneously hypertensive rat; cell therapy; cardiac material properties

Introduction

High blood pressure or hypertension (HTN) affects approximately 80 million American adults over age 20, and in 30–40% of those individuals, HTN remains untreated or uncontrolled and their risk of stroke and heart attack remains high.¹ In patients with hypertensive heart disease and in animal models of HTN, hypertrophic thickening of the myocardium and fibrosis are seen along with high blood pressure, though the relationship between the progression of HTN and stiffening of myocardium is poorly understood. Since stem cell therapies have been found to reduce post-myocardial infarction fibrosis generally^{2,3} and stiffness specifically,⁴ we hypothesized that stem cells may improve function in hypertensive heart disease through a remodeling mechanism as well.

Spontaneously hypertensive rats (SHR) are a murine model of HTN that develop high blood pressure and express hallmark signs of cardiovascular disease. These pathological findings correlate well with mechanical properties of the myocardial tissue itself; hearts from spontaneously hypertensive rats (SHR) with developed HTN have twice the elastic modulus of pre-hypertensive SHR hearts.⁵ Using methods similar to those proposed for human stem cell therapies, we isolated adipose-derived stem cells (ADSC) from normotensive rats and administered to SHR by injection to the jugular vein to investigate effects on myocardial properties and overall cardiovascular improvement. Since prior work with simple small molecules shows a short-lived treatment effect, we chose a cell-based therapy to encourage a long-term effect.

Common techniques to characterize material properties of the myocardium include macroscale compression and tension of centimeter-scale tissue samples, nano-scale indentation by atomic force microscopy or nano-indenter instruments, and “incremental elastic modulus” calculations based on intracavitary pressure-volume data. However, the wide variety in indentation methods and material models obfuscate comparisons among the data (Fig. 1). Reported data for the elastic modulus of myocardium using these various methods and models spans three orders of magnitude, overlapping with commonly accepted values for brain tissue (~1 kPa) through collagenous bone (>100 kPa). Furthermore, the typical emphasis on left ventricle properties may ignore important changes occurring in the right ventricle and pulmonary system.

To characterize the material properties of our myocardial samples, we developed a custom indentation system capable of characterizing transverse slices of intact left and right ventricle in multiple locations.⁶ We then fit the indentation data to the common standard linear solid viscoelastic material model, which allows us to calculate an instantaneous elastic modulus for comparison to existing data in the literature, as well as a steady-state elastic modulus and viscosity. The steady-state elastic modulus is independent of the indentation strain-rate, suggesting that comparisons among these moduli data from different researchers would be more reliable. Furthermore, using this viscoelastic model, we can quantify changes

in viscosity that may inform studies of impaired relaxation and diastolic dysfunction also observed in hypertensive patients.^{7,8}

Here, we have used our custom indentation system to explore the effects of stem cell therapy in restoring normal viscoelastic properties to hypertensive rat myocardium. By examining the left and right ventricles separately, we have also identified unexpected remodeling in the right heart. The right ventricle appears to be very responsive to ADSC treatment. Our indentation system combined with a standard linear solid viscoelastic model demonstrates a useful way to investigate changes in tissue viscoelastic properties for disease and treatment models.

Material and methods

Force-displacement quantification with custom indenter

We modified a tool designed to measure friction forces⁶ to create a custom Multi-Scale Indenter (MSI, Figure 2). The cantilever-based probe is displaced vertically using a software-controlled piezoelectric stage. The probe is brought into contact with the tissue sample following a time-dependent displacement profile set by the user in custom LabVIEW code. The reacting normal force of the tissue bends the titanium cantilever, and the relative displacement of the cantilever tip is measured by capacitive probe. The stiffness of the cantilever, calibrated by contact with glass, is utilized by the LabVIEW code to determine the normal load throughout the indentation cycle.

Stem cell therapy for SHR model

To investigate the therapeutic potential of adipose-derived stem cells (ADSC) in a rat model of hypertension, ADSC from normal rats were injected into SHR. ADSCs are reasonable to obtain and isolate, rich in mesenchymal cells, and been used in scar models previously (Reviewed in Madonna et al.²). First, ADSC were isolated from inguinal adipose tissue of a normotensive Wistar-Kyoto (WKY) rat and characterized by flow cytometry using common markers of stemness (CD44+, CD90+, CD34– and CD45–). Their adipocyte differentiation ability was confirmed by Oil Red “O” staining. Thirteen week-old SHR animals were randomly assigned to control and ADSC-treated groups, and ADSC (5×10^6 cells/animal) were intravenously administered through jugular vein into treatment group. Untreated control group was administered a sham injection of serum-free media. Three weeks after treatment, left ventricular function was assessed using echocardiography, and direct BP was measured using a Millar catheter. Four hearts from rats from each of the three groups (SHR, ADSC, and age-matched controls) were examined between one and four months after treatment. Rats were euthanized, hearts excised, and ~3 mm-thick transverse heart slices were mounted onto optical glass with small amount of tissue glue (Fig. 2C). We confirmed that glue did not affect measurements compared to hearts that were not glued down. Four indentations were conducted for each heart slice.

Indentation of heart slices

Excised heart slices are placed on the optical glass of MSI and moistened with three drops of room temperature culture media (Dulbecco's Modified Eagle Medium with 4.5 g/L glucose &

L-glutamine without sodium pyruvate). Unless otherwise indicated, left ventricle (LV) and right ventricle (RV) walls are indented with a 1.75 mm-diameter flat probe at two locations each to a final depth of 300 μm at a rate of 100 $\mu\text{m}/\text{min}$. Stress relaxation was observed by holding the indentation depth constant for five mins. Data were recorded at 10 Hz throughout each indentation and exported to a CSV-formatted spreadsheet at the end of each experiment.

Theory and calculations

Contact mechanics model for Hertz JKR flat punch

Instead of further sectioning and potentially damaging the heart slice for platen-based compression tests, a 1.75 mm aluminum cylindrical probe tip in the MSI was used to indent the intact transverse heart slice in multiple locations. Force-displacement data from the original indentation (Fig. 3A, bottom curve) is converted into Elastic Modulus as a function of time²³ (Fig. 3A, top curve) using the Hertz-JKR contact model for flat circular surfaces²⁴:

$$E(t) = \frac{F(t) \cdot (1 - \nu^2)}{2 \cdot R \cdot \delta(t)} \quad (1)$$

where E = elastic modulus, F = force calculated by MSI through LabVIEW, ν = Poisson's Ratio, R = flat punch radius, and δ = indentation depth. Poisson's Ratio for myocardial tissue was measured by compressing cylindrical 3 mm tall by 1.5 mm diameter tissue samples using MSI and calculated as

$$\nu = -\varepsilon_{\text{transverse}} / \varepsilon_{\text{axial}} \quad (2)$$

Where $\varepsilon_{\text{transverse}}$ = radial strain, or change in radius over original radius and $\varepsilon_{\text{axial}}$ = axial strain, or change in height over original height. Calculated Poisson's Ratio ranged from 0.36–0.38 for each sample, regardless of disease progression or ventricle location, so the average value of $\nu = 0.37$ was used for all calculations.

Standard linear solid model of viscoelasticity

The standard linear solid (SLS) is a common viscoelastic mechanical network model, composed of representative springs (linear elasticity) and a dashpot (viscosity).^{23,25} This simple combination of linear elastic and viscous components (Fig. 3b) has a straightforward physical interpretation, making this model particularly useful for interdisciplinary investigations with collaborators from broad backgrounds. The strain rate-dependent and “liquid-like” properties of the material are represented by viscosity, η , and $E_{ss} + E_a$ is the effective modulus of the tissue during initial compression. As the dashpot η relaxes, E_a trends to zero and E_{ss} can be interpreted as the steady-state or equilibrium modulus. To determine these parameters, we fit the relaxation portion of the calculated $E(t)$ versus time plot to the following equation²³:

$$E_t(t) = E_{ss} + E_a \cdot e^{-t/\tau} \quad (3)$$

Where E_t = total effective elastic modulus as a function of time, E_{ss} = steady state elastic modulus after complete relaxation, E_a = apparent strain-rate-dependent contribution to initial elastic modulus, and $\tau = \eta/E_a$ with η = viscosity. Fitting data to the relaxation portion of the curve as with the SLS model (see fitted line in Figure 3A) and our approach makes the data less dependent on the depth of the original indentation and rate of indentation compared to other common viscoelastic models and fitting strategies.^{24,26,27}

Results and Discussion

Viscoelastic properties of SHR myocardium return to normal after single ADSC injection

Hypertensive RV were approximately 70% stiffer than normotensive hearts and twice as viscous (Figure 5, Table 1). One injection with ADSC recovered SHR heart stiffness and viscosity values to near WKY control values. Welch's t-test revealed significant differences between SHR RV and other RV populations but no significant difference between normotensive WKY and ADSC-treated samples. Welch's t-test is a conservative test appropriate to our data that has large, mismatched variance; however, analysis of variance (ANOVA) was run for consistency with similar data reported elsewhere and yielded significant differences among the conditions for both steady-state modulus and viscosity ($p < 0.002$). Reported elastic moduli values correspond to the tissue's elastic component at equilibrium after stress relaxation is achieved.

Elastic moduli calculated for left ventricle WKY and SHR hearts are similar to those quantified in other studies.⁵ However, unlike other studies that often do not clarify location of indentation or only consider left ventricle, we specifically characterized left and right ventricles independently, identifying a preferential increase in right ventricle modulus in systemic hypertension. We also demonstrated subsequent recovery to near-normal values with ADSC treatment. Since we are the first to our knowledge to use a viscoelastic model for isolated rat myocardium, we are thus the first to report an increase in viscosity with hypertension. SHR hearts had approximately double the viscosity of control and ADSC-treated animals (Table 1). Viscosity is the rate of strain of a material for a given applied stress, and thus an increase in viscosity implies that more force is required to move tissue the same distance over the same time period. Since diastolic dysfunction occurs in both SHR and hypertensive patients,^{7,28} an increase in viscosity may represent this reduction in relaxation observed clinically. By extension, reduction of viscosity after ADSC treatment may suggest restoration of diastolic function, though we have not carefully studied this phenomenon here.

Commonly reported properties for most rheological and some viscoelastic characterization refer to the complex modulus or "dynamic stiffness", also referred to as the storage (G') and loss (G'') modulus.²⁹ By using Poisson's Ratio, G' and G'' coefficients can be approximated as 1/3 the Steady State Elastic Modulus (E_{ss}) and the Apparent Elastic Modulus (E_a) for similar strain rates, respectively. However, the SLS model enables standardization for quantifying tissue properties by including a strain rate-independent term (E_{ss}) while storage and loss moduli are inherently strain rate-dependent.

Viscoelastic properties of myocardium trend with clinical parameters

Traditional clinical parameters of cardiac output were also quantified, and improvement in clinical parameters followed similar trend as viscoelastic properties (Table 2). Collectively, our data demonstrate that ADSC arrested the progression of hypertension and associated left ventricular dysfunction in the hypertensive animals. This trend in clinical parameters was mirrored by our indentation data: viscoelastic effective stiffness goes up as BP and ventricular mass go up and ejection fraction goes down (SHR), and viscoelastic effective stiffness returns to near-normal levels as clinical parameters improve (ADSC). While a drop in BP with ADSC and associated unloading treatment could be responsible for some of the reduced viscoelastic properties seen in our data, the time between treatment and characterization suggests a more direct effect on myocardial tissue. Further investigation of this distinction will be pursued in the future.

Elastic modulus of left and right ventricle vary in normal and disease states

We observed a significant increase in RV modulus in SHR animals over WKY control animals and also over the SHR LV modulus ($p = 0.035$; Table 1, Figure 4). ADSC treatment reduced modulus to values similar to control hearts (statistically insignificant differences). This difference between LV and RV viscoelastic properties has not been carefully characterized in the literature, and we find RV stiffness demonstrates counterintuitive changes that would not have been detected with LV characterization only. While many mechanical studies have been conducted on hearts from normal and disease-model animals, very few have looked at the difference between left and right ventricle properties. Macroscale characterization systems often do not have the spatial resolution to ascertain this data, and other studies report only left ventricle data. For example, one compression study on the left ventricle of hearts from 8 week old Goldblatt II hypertensive animals found similar values to control animals of the same age (6.61 ± 0.63 kPa and 7.14 ± 0.71 kPa, respectively).¹⁵ Another tensile stretching experiment of isolated papillary muscle measured SHR values 2–4 times stiffer than WKY controls, but the order of magnitude was inconsistent with our data (~ 200 – 900 kPa) perhaps due to high strain rate and papillary preparation rather than myocardium.^{22,30}

Our custom indentation system allows us to spatially characterize the ventricle wall in a way that has not been previously reported. For the first time, we find dramatic mechanical stiffening of the RV in the SHR model of hypertension, in keeping with previous reports of RV and pulmonary pathologies in SHR. Increased pulmonary artery pressure has been observed in SHR³¹ along with reports of RV remodeling, volume increase and reduced systolic function.^{32,33} As with human patients, the RV and pulmonary effects of systemic hypertension deserve closer inspection, as highlighted by our finding of substantial LV and RV differences in elastic modulus.

Conclusion

Stem cell therapy is being investigated for a wide range of cardiovascular diseases, and our results indicate that viscoelastic properties of relevant tissues correlate well with outcomes of cell therapy treatments. We have demonstrated an indentation system and viscoelastic

model for analysis that yields quantitative mechanical parameters; these viscoelastic mechanical parameters trend with clinical parameters of heart function such as blood pressure and ejection fraction. We also found that the abnormally stiff right ventricle of hypertensive rat hearts remodels to near normal values after a single injection of adipose-derived stem cells. In addition to demonstrating the effect of stem cell therapy on hypertensive rat hearts, the millimeter-scale spatial resolution we can achieve with our system has highlighted the need for the cardiovascular community to focus on both the left and right heart chambers in systemic hypertension.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors gratefully acknowledge support from the NIH-NHLBI-funded Cardiovascular Cell Therapy Research Network Skills Development Program (5UM1HL087366), the University of Florida's NIH-funded Clinical and Translational Science Institute (UL1 TR000064), and other NIH projects (R01 HL056921, HL033610). A.R. acknowledges fellowship support from the Institute for Cell and Tissue Science and Engineering at the University of Florida.

References

1. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, et al. Heart disease and stroke statistics--2015 update: a report from the American Heart Association. *Circulation*. 2015 Jan 27; 131(4):e29–322. [PubMed: 25520374]
2. Madonna R, Geng Y-J, De Caterina R. Adipose tissue-derived stem cells: characterization and potential for cardiovascular repair. *Arteriosclerosis, Thrombosis, and Vascular Biology* Lippincott Williams & Wilkins. 2009 Nov; 29(11):1723–9.
3. Ammar HI, Sequiera GL, Nashed MB, Ammar RI, Gabr HM, Elsayed HE, et al. Comparison of adipose tissue- and bone marrow- derived mesenchymal stem cells for alleviating doxorubicin-induced cardiac dysfunction in diabetic rats. *Stem Cell Res Ther*. 2015; 6(1):148. [PubMed: 26296856]
4. Berry MF, Engler AJ, Woo YJ, Pirolli TJ, Bish LT, Jayasankar V, et al. Mesenchymal stem cell injection after myocardial infarction improves myocardial compliance. *Am J Physiol Heart Circ Physiol* [Internet]. 2006 Jun; 290(6):H2196–203.
5. Conrad CH, Brooks WW, Hayes JA, Sen S, Robinson KG, Bing OH. Myocardial fibrosis and stiffness with hypertrophy and heart failure in the spontaneously hypertensive rat. *Circulation* [Internet]. 1995 Jan 1; 91(1):161–70.
6. Krick BA, Vail JR, Persson BNJ, Sawyer WG. Optical In Situ Micro Tribometer for Analysis of Real Contact Area for Contact Mechanics, Adhesion, and Sliding Experiments. *Tribol Lett* [Internet]. 2011 Oct 30; 45(1):185–94.
7. Palmiero P, Zito A, Maiello M, Cameli M, Modesti PA, Muiesan ML, et al. Left ventricular diastolic function in hypertension: methodological considerations and clinical implications. *J Clin Med Res*. 2015 Mar; 7(3):137–44. [PubMed: 25584097]
8. Szelényi Z, Fazakas Á, Szénási G, Tegze N, Fekete B, Molvarec A, et al. The mechanism of reduced longitudinal left ventricular systolic function in hypertensive patients with normal ejection fraction. *J Hypertens*. 2015 Sep; 33(9):1962–9. [PubMed: 26154942]
9. Ogneva IV, Lebedev DV, Sherkman BS. Transversal Stiffness and Young's Modulus of Single Fibers from Rat Soleus Muscle Probed by Atomic Force Microscopy. *Biophysical Journal*. 2010 Feb; 98(3):418–24. [PubMed: 20141755]

10. McCain ML, Parker KK. Mechanotransduction: the role of mechanical stress, myocyte shape, and cytoskeletal architecture on cardiac function. *Pflugers Arch* [Internet]. 2011 Jul; 462(1):89–104. [PubMed: 21499986]
11. Ott HC, Matthiesen TS, Goh S-K, Black LD, Kren SM, Netoff TI, et al. Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. *Nat Med*. 2008 Jan 13; 14(2):213–21. [PubMed: 18193059]
12. Levental I, Georges PC, Janmey PA. Soft biological materials and their impact on cell function. *Soft Matter*. 2007; 3(3):299–306.
13. Heller LJ, Mohrman DE, Prohaska JR. Decreased passive stiffness of cardiac myocytes and cardiac tissue from copper-deficient rat hearts. *Am J Physiol Heart Circ Physiol*. 2000 Jun; 278(6):H1840–7. [PubMed: 10843880]
14. Bhana B, Iyer RK, Chen WLK, Zhao R, Sider KL, Likhithpanichkul M, et al. Influence of substrate stiffness on the phenotype of heart cells. *Biotechnol Bioeng*. 2010 Apr 15; 105(6):1148–60. [PubMed: 20014437]
15. Kissling G, Gassenmaier T, Wendt-Gallitelli MF, Jacob R. Pressure-volume relations, elastic modulus, and contractile behaviour of the hypertrophied left ventricle of rats with Goldblatt II hypertension. *Pflugers Arch*. 1977 Oct; 369(3):213–21. [PubMed: 142962]
16. Niederhoffer N, Kieffer P, Desplanches D, Lartaud-Idjouadiene I, Sornay MH, Atkinson J. Physical exercise, aortic blood pressure, and aortic wall elasticity and composition in rats. *Hypertension*. 2000 Apr; 35(4):919–24. [PubMed: 10775562]
17. Zhao, Fuzhang; Chen, JSJ.; Blagg, AG.; Berretta, R.; Margulies, KB. NEBC-03. IEEE; 2003. An integrated method to determine the stress-strain relationship of beating heart; p. 273-4.
18. Higuchi M, Miyagi K, Kayo S. Acceleration of stiffness in underperfused diabetic rat hearts by glyburide, a KATP channel blocker, and its prevention by levromakalim and insulin. *Cardiovascular Res*. 1997; 35:303–14.
19. Janz RF, Kubert BR, Mirsky I, Korecky B, Taichman GC. Effect of age on passive elastic stiffness of rat heart muscle. *Biophysj*. 1976 Apr; 16(4):281–90.
20. Lacolley P, Labat C, Pujol A, Delcayre C, Benetos A. Increased Carotid Wall Elastic Modulus and Fibronectin in Aldosterone-Salt-Treated Rats Effects of Eplerenone. *Circulation*. 2002
21. Lacolley P, Safar ME, Lucet B, Ledudal K, Labat C, Benetos A. Prevention of aortic and cardiac fibrosis by spironolactone in old normotensive rats. *Journal of the American College of Cardiology*. 2001 Feb; 37(2):662–7. [PubMed: 11216994]
22. Youcef G, Olivier A, L'Huillier CPJ, Labat C, Fay R, Tabcheh L, et al. Simultaneous characterization of metabolic, cardiac, vascular and renal phenotypes of lean and obese SHHF rats. *PLoS ONE*. 2014; 9(5):e96452. [PubMed: 24831821]
23. Moore SW, Keller RE, Koehl MA. The dorsal involuting marginal zone stiffens anisotropically during its convergent extension in the gastrula of *Xenopus laevis*. *Development*. 1995 Oct; 121(10):3131–40. [PubMed: 7588048]
24. Hayes WC, Keer LM, Herrmann G, Mockros LF. A mathematical analysis for indentation tests of articular cartilage. *Journal of Biomechanics*. 1972 Sep; 5(5):541–51. [PubMed: 4667277]
25. Koehl M. Biomechanical approaches to morphogenesis. *Semin Dev Biol*. 1990:367–78.
26. Rosenbluth MJ, Lam WA, Fletcher DA. Force microscopy of nonadherent cells: a comparison of leukemia cell deformability. *Biophysj*. 2006 Apr 15; 90(8):2994–3003.
27. Li QS, Lee GYH, Ong CN, Lim CT. AFM indentation study of breast cancer cells. *Biochem Biophys Res Commun*. 2008 Oct 3; 374(4):609–13. [PubMed: 18656442]
28. Hasenfuss G. Animal models of human cardiovascular disease, heart failure and hypertrophy. *Cardiovascular research*. 1998 Jul 1; 39(1):60–76. [PubMed: 9764190]
29. Tidball JG. Energy Stored and Dissipated in Skeletal-Muscle Basement-Membranes During Sinusoidal Oscillations. *Biophysj*. 1986 Dec; 50(6):1127–38.
30. Merryman WD, Bieniek PD, Guilak F, Sacks MS. Viscoelastic properties of the aortic valve interstitial cell. *J Biomech Eng*. 2009 Apr.131(4):041005. [PubMed: 19275434]
31. Aharinejad S, Schraufnagel DE, Böck P, MacKay CA, Larson EK, Miksovsky A, et al. Spontaneously hypertensive rats develop pulmonary hypertension and hypertrophy of pulmonary

venous sphincters. Am J Pathol American Society for Investigative Pathology. 1996 Jan; 148(1): 281–90.

32. Al-Shafei AIM, Wise RG, Grace AA, Carpenter TA, Hall LD, Huang CLH. MRI analysis of right ventricular function in normal and spontaneously hypertensive rats. Magnetic Resonance Imaging [Internet]. 2001 Dec; 19(10):1297–304.
33. Guazzi M, Castelvécchio S, Bandera F, Menicanti L. Right Ventricular Pulmonary Hypertension. Curr Heart Fail Rep. 2012 Aug 3; 9(4):303–8. [PubMed: 22864855]

- custom indentation system used for viscoelastic characterization of heart tissue
- Right ventricle of hypertensive hearts stiffer than age-matched controls
- Adipose-derived stem cell treatment restores mechanical properties to normal
- Changes in tissue mechanical properties trend with clinical parameters

Figure 1.

Characterization methods for mechanical properties of myocardium vary widely, as do resulting values for elastic modulus. Results from atomic force microscopy (AFM) indentation,^{4,9,10} tensile testing,^{5,11–13} compression testing,^{14,15} and analytical and computational models based on imaging data^{16–22} span nearly four orders of magnitude for purportedly similar myocardial tissue.

Figure 2.

Set up and schematic of Multiscale Indenter (MSI). As depicted in (d), piezoelectric stage (1) actuates cantilever (2), and deflection of probe (4) is detected by capacitive sensor (3). (c) Photograph of representative heart slice from SHR rat is ~12 mm in diameter.

Figure 3.

(a) Representative normal load (bottom dashed line) and elastic modulus (top solid line) profiles as a function of time. Linear elastic behavior is displayed during indentation after initial toe region, and viscous relaxation is seen as indentation depth is held constant. An exponential fit (thick dashed line on top trace) is used to obtain viscoelastic properties. (b) Schematic representation of SLS model. Springs (E_{ss} and E_a) represent linear elastic elements that sum as the effective elastic modulus of tissue, and the dashpot (η) is the viscous element that relaxes in a time-dependent manner.

Figure 4.

Results of myocardium characterization show increase in viscoelastic parameters for SHR right ventricles (RV). These parameters are recovered to normal levels with ADSC treatment. P-values indicate significant findings from conservative Welch's t-test of populations.

Table 1

Viscoelastic properties of control (WKY, Wistar-Kyoto), hypertensive (SHR), and ADSC-treated SHR heart tissue. Matching superscripts represent significantly different populations as determined by Welch's t-test ($p < 0.05$). As a reference, viscosity of syrup is ~ 100 Pa·s and tar is ~ 100 MPa·s.

<i>Mean $\pm$ SD</i>	Steady-State Elastic Modulus (kPa)		Viscosity (MPa·s)	
	LV	RV	LV	RV
WKY – Normotensive	14.96 ± 4.52	13.14 ± 3.48^a	1.44 ± 0.62^c	2.38 ± 1.46
SHR – Untreated	11.94 ± 4.25	$21.97 \pm 5.10^{a,b}$	3.40 ± 1.11^c	4.59 ± 1.50^d
ADSC – Treated SHR	6.52 ± 4.8	9.77 ± 6.96^b	2.52 ± 0.85	1.92 ± 0.35^d

Table 2

Clinical Parameters of control (WKY, Wister-Kyoto), hypertensive (SHR), and ADSC-treated heart tissue. Basal systolic blood pressure (BP) for SHR at 13 weeks of age (before treatment) was 191.6 ± 5.6 mmHg as measured by a tail-cuff method.

<i>Mean $\pm$ SD</i>	Basal Systolic BP [mmHg]	Ventricular Mass [g/kg]	Ejection Fraction [%]
WKY – Normotensive	109.0 ± 1.6	2.9 ± 0.1	88.2 ± 1.3
SHR – Untreated	198.4 ± 25.9	3.3 ± 0.2	69.9 ± 4.2
ADSC – Treated SHR	142.5 ± 25.8	2.7 ± 0.3	80.0 ± 3.4