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A Novel Approach to Assess the In Situ Versus Ex Vivo Mechanical Behaviors of the Coronary Artery

Ex vivo mechanical testing has provided tremendous insight toward prediction of the in vivo mechanical behavior and local mechanical environment of the arterial wall; however, the role of perivascular support on the local mechanical behavior of arteries is not well understood. Here, we present a novel approach for quantifying the impact of the perivascular support on arterial mechanics using intravascular ultrasound (IVUS) on cadaveric porcine hearts. We performed pressure-diameter tests ($n=5$) on the left anterior descending coronary arteries (LADCA) in situ while embedded in their native perivascular environment using IVUS imaging and after removal of the perivascular support of the artery. We then performed standard cylindrical biaxial testing on these vessels ex vivo and compared the results. Removal of the perivascular support resulted in an upward shift of the pressure-diameter curve. Ex vivo testing, however, showed significantly lower circumferential compliance compared to the in situ configuration. On a second set of arteries, local axial stretch ratios were quantified ($n=5$) along the length of the arteries. The average in situ axial stretch ratio was 1.28 ± 0.16 ; however, local axial stretch ratios showed significant variability, ranging from 1.01 to 1.70. Taken together, the data suggest that both the perivascular loading and the axial tethering have an important role in arterial mechanics. Combining nondestructive testing using IVUS with traditional ex vivo cylindrical biaxial testing yields a more comprehensive assessment of the mechanical behavior of arteries. [DOI: 10.1115/1.4035262]

Keywords: vascular mechanics, finite elasticity, arterial stiffness, intravascular ultrasound

Introduction

The contribution of perivascular support on vessel mechanics and its role in mechanobiologically mediated mechanisms remains poorly understood; however, the role of perivascular support is thought to play an important role in arterial mechanics [1–4]. Arteries may be invested in surrounding fascia of varying densities (e.g., aorta and carotid arteries), or they may be invested by musculature (e.g., coronary and peripheral arteries). Much of our understanding of arterial mechanics arises by identifying predictive, often microstructurally motivated constitutive models based on data from ex vivo mechanical testing [5–8]. Although such an approach is experimentally tractable and provides accurate predictions of the deformations and stresses experienced during ex vivo testing, given that the in vivo boundary conditions (i.e., the applied load on the outer wall of the artery due to perivascular loading) may be significantly different than ex vivo testing,

translating such models requires accurate prediction of the mechanical role of the perivascular support.

The coronary artery is heavily embedded in perivascular support that largely consists of adipose tissue and cardiac muscle and the support tissue type varies significantly with anatomical locations (Fig. 1(a)). The boundary of the perivascular support is not well defined, making ex vivo assessment of the mechanical contribution difficult. Since coronary arteries are embedded into the cardiac muscle and influenced by the pericardial fat [9,10], it is not surprising that there is wide variability in the investment of coronary arteries with portions of the artery routinely becoming intramuscular. For instance, the intramyocardial bridged coronary artery segments are generally free of atherosclerotic lesions that are found in nonbridged segments [10]. This may be due to off-loading of pathologic stresses to more proximal portions of the coronary artery, whereas softer perivascular adipose tissue has been suggested as the simulator for atherosclerosis [11–14]. It would therefore be important to understand and quantify the amount of mechanical loading that perivascular support contributes to the overall mechanical response of coronary arteries.

The purpose of this study is to demonstrate a novel approach to help understand the role of coronary perivascular support on the

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Fig. 1 (a) A representative cross section of a porcine coronary artery showing the extensive perivascular support surrounding the artery. The artery is subjected to nonuniform perivascular support loading due to different materials primarily adipose tissue and cardiac muscles. This is evident from this image in which the V-shaped stiff cardiac muscle (at the adipose–cardiac muscle interface) appears to be compressing the bottom portion of the artery. (b) and (c) show the method of measuring the local axial stretches that use fiducial markers. (b) The markers are adhered to the artery prior to being dissected out of the heart through a thin incision in the perivascular support. The distances between the i th pair of markers in this configuration are measured as ℓ_i . (c) The artery is then completely dissected free, and the distances are measured again as L_i . The local axial stretch ratios can then be calculated from the ratios of these two measurements.

mechanical response of coronary arteries. We characterized the pressure-diameter response of the coronary artery in three configurations by sequentially isolating the same artery from its perivascular environment through means of mechanical dissection. These configurations consisted of (I) the in situ configuration in which the artery was left undisturbed from its perivascular environment in an excised heart, (II) the partially dissected configuration in which the perivascular support was removed around the artery but the artery was kept axially fixed to the heart, and (III) the ex vivo configuration in which the artery was completely isolated from the heart and subjected to cylindrical biaxial loading on a mechanical testing system. We also investigated the influence of perivascular support on the axial stretch of the artery by measuring the axial stretch ratios between the in situ and traction-free configurations and the ex vivo and traction-free configurations.

Materials and Methods

Tissue Harvest and Preparation. Twelve whole porcine hearts of similar size and weight were freshly harvested at a local slaughterhouse (Holifield Farms, Covington, GA) from 2-yr-old female American Yorkshire farm pigs weighing 150–200 kg. The animals were exsanguinated by severing of their carotid artery. Once the heart was excised and removed from the body, the coronary vasculature was immediately flushed with cold Ca^{+2} and Mg^{+2} free Dulbecco's phosphate-buffered saline (DPBS, Lonza Group, Basel, Switzerland) through the aortic sinus and the surfaces of the hearts were rinsed with DPBS. The sizes and weights of the hearts were not recorded, but previous published work using hearts harvested from the same slaughterhouse from pigs of similar size weighed on average 0.76 ± 0.10 kg [15]. The hearts were transported on ice to the laboratory. Five hearts were used for the pressure-diameter measurements, and an additional five were used for the local axial stretch measurements. The remaining two hearts were used for histology. Approximately 6 h elapsed between harvesting the hearts and starting the experiments. The hearts were stored in 4°C chilled Ca^{+2} and Mg^{+2} free DPBS hydrated environment during this time.

Measurement of the Local Axial Stretch Ratios. The top surface of the left anterior descending coronary artery (LADCA) was carefully exposed with a thin incision in the perivascular support.

Small spherical plastic fiducial markers (1 mm in diameter) were adhered to the surface of the artery with cyanoacrylate-based glue applied through the tip of a 27-gauge needle. Six to eight markers were applied along the centerline of the artery spaced 5–10 mm apart starting immediately distal of the bifurcation of the left anterior descending (LAD)–circumflex artery (Fig. 1(b)). Spacing between the markers was minimized as much as possible in tortuous sections in order to reduce the effect of curvature on the linear distance measurements. The distances between each pair of markers were measured manually using a digital caliper (VWR Inc., Radnor, PA). Precision of the measurement technique was assessed by performing 20 blind measurements on a set of

Fig. 2 Representative portion of the experimental setup for measuring the pressure-diameter response of the coronary artery in the in situ and partially dissected configurations. Two pressure transducers, P1 and P2, are cannulated and secured between the coronary artery segment of interest. Pressure is provided by a syringe pump (not shown). The IVUS transducer is delivered into the arterial lumen through a one-way port (not shown) and rests in the middle of the dissected segment. A series of ligatures and vascular clips are used to stop outflow from peripheral branches. During testing, the heart is fully submerged in a DPBS bath maintained at physiological temperature (not shown).

Fig. 3 Measurements of the local axial stretch ratios along the length of the coronary arteries ($n=5$). Distance is measured from the bifurcation of the LAD–circumflex coronary artery. Each marker type represents a single coronary artery. Linear regression of the data (black line) yields poor correlation between local stretch ratio and location along the artery ($R^2 = 0.05$).

markers, and we found the variability to be within ± 0.1 mm. The artery was then dissected free of adherent perivascular support, excised from the heart, and placed in a Ca^{+2} and Mg^{+2} free DPBS bath at 37°C for 3 h to achieve a passive geometric configuration [16]. The traction-free distances between the same pairs of markers were measured again after the incubation. The local in situ axial stretch ratio is defined as $\lambda_{z,i}^* = \ell_i/L_i$, where ℓ_i and L_i are the distances between the i th pair of adjacent markers in the in situ and traction-free configurations, respectively, see Figs. 1(b) and 1(c).

Pressure-Diameter Responses of the In Situ and Partially Dissected Configurations. Pressure-diameter experiments were performed on the LADCA in the in situ and partially dissected configurations. For the in situ configuration, lumen diameter was measured using intravascular ultrasound (IVUS) (CORE Mobile, Volcano, San Diego, CA) while being pressurized under a quasi-static ramp rate (2 mm Hg/s). IVUS is a radially projecting catheter-based ultrasound imaging modality that can be inserted into the lumen to provide transverse images of the artery. The LADCA was first cannulated with polycarbonate-barbed fittings between its bifurcation from the circumflex artery to approximately two-thirds along the length of LADCA (Fig. 2). Pressure transducers P1 and P2 (PT-F/PM-4, Living Systems Instruments, Burlington, VT) were connected to the distal and proximal end of the artery through a three-way port. The proximal port was then connected to a 1000 mL syringe filled with Ca^{+2} and Mg^{+2} free DPBS and mounted to a computer-controlled syringe pump (Ph.D. 2000, Harvard Apparatus, Holliston, MA), which was used to pressurize the artery. The distal port was then connected to a one-way access sheath allowing the IVUS transducer (Visions PV.018 20 MHz, Volcano, San Diego, CA) to be inserted into the arterial lumen without releasing pressure. The remaining open port on both three-way ports was connected to each other through a manual valve. The port opening and tubing were sufficiently wide enough to accommodate the IVUS transducer wire while not obstructing the pressure transducer's ability to measure pressure. The IVUS transducer was secured approximately equal distances between the two pressure transducers at an axial location in which the artery was noticeably encased in perivascular support. This placement was performed visually if possible and by running a finger along the artery until the tip of the transducer was felt.

The flow path was primed with Ca^{+2} and Mg^{+2} free DPBS prior to the start of the experiment. A series of vascular clips and ligatures were used to stop outflows from peripheral branches. Prior to testing, the heart was gradually warmed and maintained at 37°C by fully submerging it in a circulating Ca^{+2} and Mg^{+2} free DPBS bath. The level of the bath was maintained at approximately 2 cm above the heart surface; the swallow depth is to minimize any effects due to hydrostatic pressure. The artery was first preconditioned through six pressurization cycles from 0 to 90 to 0 mm Hg; this was followed by another four cycles during which the lumen was imaged using IVUS. The perivascular support around the portion of the artery with the IVUS catheter was then carefully exposed using sharp dissection, with care taken not to damage the arterial adventitia. The exposure left approximately 1.5 cm length of the LADCA, measured with digital calipers, partially exposed but still attached at the end (Fig. 2). The pressure-diameter measurements were then repeated for this configuration (partially dissected). IVUS images were collected at 23 fps.

Measurement of the Ex Vivo Mechanical Responses.

Following the measurements of the partially dissected configuration, the perivascular support around the full length of the cannulated LADCA segment was dissected away, and the exposed peripheral branches were ligated using 6-0 silk sutures. Preparation of the coronary arteries for testing followed previous approaches by Pandit et al. [17]. Approximately 3 cm of the artery was then cut from the heart and mounted cylindrically on a custom cylindrical biaxial mechanical testing device adapted from that reported in Zaucha et al. [18] and subjected to biaxial loading conditions. Silk suture ligatures were used to secure the artery to the cannula, and the final unloaded distance measured between the cannula sutures was approximately 2 cm. The artery underwent cyclic pressurization cycles from 0 to 90 to 0 mm Hg at fixed axial lengths, while the outer diameters and axial forces were measured and recorded. Based on established methodology, the in vivo axial stretch is defined as the stretch at which the axial force remains nearly constant with increasing lumen pressure [19,20]. Following this methodology, the loaded axial stretch ratio λ_z was increased at increments of 0.05 until the axial force remained constant across the pressure cycle. The loaded axial stretch ratio is defined as $\lambda_z = l/L$, where l is the overall length of the artery under axial load, and L is the overall traction-free length. The arterial wall was assumed to be incompressible. We did not find any intramyocardial segments during dissection; thus, the results of this study apply only to the coronary arteries embedded in adipose tissue perivascular support. However, the overall mechanical response of the artery may be affected by the contributions of other tissues beyond the adjacent adipose, such as cardiac muscles and the visceral pericardium.

IVUS Image Segmentation. A semi-automated routine was developed to segment the lumen boundary using the IVUS DICOM images in MATLAB (MathWorks, Natick, MA). To expedite the processing, images were sampled every 20 frames. For each image, 15 equally spaced points were manually selected around the lumen boundary using an overlaid radial grid as reference. These points were fitted to an ellipse using a least-squares regression elliptical fitting algorithm. Using the parameters of the fitted ellipse, the area of the arterial lumen was calculated, which was used to determine a nominal diameter, see Ref. [21] for detailed description of this approach. In general, the lumen shape was circular. However, at low pressures less than 20 mm Hg, the arterial walls appeared to collapse around the IVUS transducer, resulting in the inability to accurately delineate the lumen boundary (Supplemental Fig. S1 is available under the “Supplemental Materials” tab for this paper on the ASME Digital Collection). As such, IVUS measurements taken at pressures less than 20 mm Hg were not used in the results. The IVUS images were synchronized to the lumen pressure using the local time stamps provided by the

Fig. 4 Mean axial stretch ratios calculated using the local stretch measurements for each artery (error bars are mean $\pm$ SD). Stretch ratios exhibit significant artery-to-artery variability (* $P < 0.05$ for artery 2 compared to arteries 1 and 4; # $P < 0.01$ for artery 5 compared to arteries 1, 3, and 4).

internal clock of the respective computers. The clocks IVUS was synchronized prior to starting the experiments. Linear interpolation was used to normalize the data length for plotting and statistically analysis.

Statistical Analysis. Multiple independent means were analyzed for statistical significance using one-way ANOVA and Tukey's post hoc test, assuming normality. Paired Student's t -test was used to analyze statistical significance between two means. Statistical significance was defined as $P < 0.05$. Statistical

analysis, postprocessing, and plotting were performed using commercial software EXCEL (Microsoft, Redmond, WA) and MATLAB.

Histology. Representative histological images were taken of the coronary artery in the in situ and ex vivo configurations to visualize the boundary between the perivascular support and the arterial wall. For the in situ configuration, a block of tissue measuring 1 cm on each side, centered around the artery and surrounded by perivascular support, was cut out of the heart with a scalpel. For the ex vivo configuration, the artery was dissected using our standard technique. The tissues were fixed in 10% formalin, processed, and embedded in paraffin, and sliced 5 μ m thick. The sections were then stained with hematoxylin and eosin (H&E), and the images are captured under 10 $\times$ bright-field magnification.

Results

Measurement of the Local Axial Stretch Ratios. The local axial stretch ratios exhibited large variations along the length of the arteries ranging from 1.01 to 1.70 (Fig. 3). Linear regression of the data revealed poor correlation between local stretch ratios and axial locations ($R^2 = 0.05$). The overall mean axial stretch ratio calculated using all the measurements was $\lambda_{z,i}^* = 1.28 \pm 0.16$ (mean $\pm$ SD). Averaging the local stretch ratios for each artery did reveal some significant differences between the arteries (Fig. 4). The lowest averaged axial stretch ratios by artery were 1.13 ± 0.10 and 1.22 ± 0.11 (mean $\pm$ SD) for arteries 5 and 2, respectively.

Pressure-Diameter Responses of the In Situ and Partially Dissected Configurations. The LADCA exhibited increased lumen dilation when partially dissected compared to its in situ configuration, suggesting that the perivascular support restricted

Fig. 5 Comparison of the pressure-diameter response between the in situ ($n = 5$; solid line circle markers) and partially dissected ($n = 5$; dashed line square markers) configurations. A significant increase in diameter was observed in the dissected arteries for pressures between 20 and 60 mm Hg (* $P < 0.05$; error bars are mean $\pm$ SD). Note that diameter data are not available for less than 20 mm Hg due to the arterial wall collapsing around the IVUS transducer.

Fig. 6 Comparison of compliance-pressure response of ex vivo configuration (dashed line triangle markers) to the in situ (solid line) and partially dissected (dotted line) configurations (error bars are mean $\pm$ SD). The partially dissected configuration exhibits higher overall compliance compared to in situ. The ex vivo configuration exhibits the least compliance, demonstrating a critical role of the perivascular support on the mechanical behavior.

the distension (Fig. 5) at lower pressures. The increase in lumen diameter was significantly greater for pressures from 20 to 60 mm Hg, and this data support a critical role of the extravascular matrix in limiting coronary artery compliance during the diastolic portion of the cardiac cycle [22]. The partially dissected configuration also exhibited a greater nonlinear pressure-diameter response curve compared to the in situ configuration. Figure 6 shows the incremental compliance C calculated from the pressure-diameter response at increments of 5 mm Hg using the following equation:

$$C = \frac{\Delta d}{d_o \Delta P} 100\%$$

Measurement of the Ex Vivo Mechanical Response. Representative histological images of the in situ configurations show that the artery is embedded in adipose tissue, whereas in the ex vivo configuration the perivascular tissue was removed while leaving the arterial wall intact (Fig. 7). The estimated in vivo axial stretch ratio determined by the axial force response from the biaxial mechanical testing was $\bar{\lambda}_z = 1.32 \pm 0.08$, which was found to be statistically similar ($P = 0.20$, one-tailed t -test) to the overall

Fig. 8 Comparison of the pressure-diameter response of ex vivo configuration (dashed line triangle markers) to the in situ (solid line) and partially dissected (dotted line) configurations (error bars are mean $\pm$ SD). The ex vivo pressure-diameter response exhibits appreciable differences, namely, lower compliance between 20 and 80 mm Hg. The ex vivo response exhibits the typical strain-stiffening response at higher pressure.

mean axial stretch ratio determined from the local axial stretch measurements of $\bar{\lambda}_z^* = 1.28 \pm 0.16$. Examining the ex vivo pressure-diameter response revealed appreciable differences compared to the other two configurations (Fig. 8). Since ex vivo measurements typically measure the outer diameter, for comparative purposes against the IVUS images, measurements of the outer diameter were converted to lumen diameter by invoking the incompressibility assumption. Both the in situ and the partially dissected configurations appeared more compliant than the ex vivo. Whereas the in situ and partially dissected configurations did not exhibit significant stiffening even at 80 mm Hg, the ex vivo response began to significantly stiffen at 40–50 mm Hg.

Discussion

The contribution of the perivascular support to arterial function has recently been demonstrated in a variety of platforms [23–25]. However, the mechanical contribution of perivascular support likely plays an important role in the local mechanical environment across the arterial wall, as well as arterial wall remodeling after intervention. The biomechanical properties of coronary arteries have been well characterized, both in humans and large animal

Fig. 7 Representative histological images of the (a) in situ and (b) the ex vivo configurations. Note that the perivascular support in the in situ configuration largely consists of adipocytes. The ex vivo configuration image demonstrates proper removal of the perivascular support, while leaving the arterial wall intact. The lumen is denoted by the letter L, and the black scale bar represents 400 μm .

models [26–28]. However, a critical gap remains in translating the constitutive model that captures the ex vivo biomechanical response of arteries to the in vivo stresses and strains, when the artery is embedded in perivascular support and tethered both by perivascular support and branches.

We also found large variability in the local axial stretch ratios along the length of the artery. Interestingly, when the local stretches were averaged together across all the arteries, the mean value was comparable to the estimated in vivo axial stretch ratio determined through ex vivo testing. There may be several interesting consequences of this finding. In finite deformation, there is a strong coupling effect between circumferential and longitudinal stretches. Thus, the different local axial stretches may serve to modulate the local circumferential stress, which may be due to differences in the local geometry (e.g., wall thickness and diameter) or the stiffness of the local perivascular support that integrates the adipose tissue, cardiac muscle, and the visceral pericardium. This normal compensatory relationship may be disrupted under restrictive cardiomyopathies (e.g., pericarditis).

Removal of the perivascular support resulted in an upward shift in the pressure versus diameter curve, which was statistically significant from 20 to 60 mm Hg. Thus, the perivascular support reduced arterial deformation via an applied load at the external surface of the artery. The perivascular support may be thought to impose an external pressure, which effectively reduces the transmural pressure, thereby reducing the deformation. Results from the ex vivo testing, however, showed a significantly different mechanical response, compared to the in situ results. The ex vivo mechanical response showed a significantly lower compliance, compared to the in situ and partially dissected configurations (Fig. 8). These data suggest that both the perivascular loading and the axial tethering have a significant effect limiting arterial deformation in these experiments.

The results of the study should be considered within the context of its limitations. First, it was performed in healthy porcine hearts, and the results may not be translatable to other species or diseased states. We also do not have in vivo data to pair with the in situ and ex vivo measurements. Thus, it is not clear how these coronary arteries (after being divested of their perivascular support) would behave under native pulsatile pressures. However, the differences observed between the same artery measured in the in situ perivascular support, partially dissected support, and ex vivo on the biaxial system strongly suggest a critical role of the perivascular support tissue on the deformation and local mechanical environment of coronary arteries. Second, even if a cardioplegia solution was administered to these porcine hearts at the slaughterhouse, it is unlikely that this would prevent rigor mortis during transportation and testing. Thus, it is not feasible to control for this ex vivo, and this limitation could overestimate the contribution of the perivascular support. In vivo IVUS testing of these arteries (before and after dissection from matrix) would be required to definitively test this, but this was beyond the scope of this current project. As such, we caution against applying the results of the in situ configuration to a physiological understanding.

Excitingly, this technique may be applied for studying myocardial bridge segments, where the coronary artery segments run completely into the myocardium. Thus, their perivascular environment is significantly stiffer than arteries embedded in adipose tissue. Further, this technique may be used to quantify the impact of perivascular support on arterial mechanics in different vascular beds as well as in diseased conditions and could be modified to incorporate alternative imaging modalities, such as optical coherence tomography. This would yield higher resolution images that would make segmentation more automated and accurate [29].

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