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Short Communication

Stretch calculated from grip distance accurately approximates mid-specimen stretch in large elastic arteries in uniaxial tensile tests

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ABSTRACT

The mechanical properties of vascular tissues affect hemodynamics and can alter disease progression. The uniaxial tensile test is a simple and effective method for determining the stress–strain relationship in arterial tissue ex vivo. To enable calculation of strain, stretch can be measured directly with image tracking of markers on the tissue or indirectly from the distance between the grips used to hold the specimen. While the imaging technique is generally considered more accurate, it also requires more analysis, and the grip distance method is more widely used. The purpose of this study is to compare the stretch of the testing specimen calculated from the grip distance method to that obtained from the imaging method for canine descending aortas and large proximal pulmonary arteries. Our results showed a significant difference in stretch between the two methods; however, this difference was consistently less than 2%. Therefore, the grip distance method is an accurate approximation of the stretch in large elastic arteries in the uniaxial tensile test.

1. Introduction

The mechanical properties of large proximal arteries are of great interest because these arteries strongly impact blood flow in the circulation and heart function (Milnor, 1975; Stevens et al., 2012; Wang and Chesler, 2011; Weinberg et al., 2004). Understanding the changes in arterial mechanical properties during cardiovascular disease progression is useful for prognoses and monitoring therapeutic efficacy. Several ex vivo mechanical tests have been used to characterize the mechanical properties of large elastic arteries including uniaxial and biaxial tests, and pressure inflation-force test (Holzapfel, 2006; Kao et al., 2011; Lammers et al., 2008; Sommer et al., 2010; Golob et al., 2015). The uniaxial test is performed with an apparatus that applies a tensile force to a rectangular strip in only one direction. As a result, this test does not mimic the physiological state of the artery, which is loaded three-dimensionally, and cannot fully characterize the

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anisotropic properties of the artery (Tian and Chesler, 2012; Holzapfel and Ogden, 2009). Despite these limitations, the uniaxial test is often preferred to the other, more physiologically relevant tests due to its simplicity and feasibility when tissue size is limited (Tian and Chesler, 2012; Holzapfel, 2006).

The question of how to accurately measure strain or stretch is important for uniaxial testing of arterial tissue. Many uniaxial test studies use the grip-to-grip distance before and during loading for the stretch calculation (Butler et al., 1984; Lammers et al., 2008; Tian et al., 2011). Conversely, some studies mark regions of the specimen with visible markers (e.g., ink) prior to testing and obtain the stretch at the mid-section of the specimen by tracking the distances between these markers during the test (Butler et al., 1984; Holzapfel et al., 2004; Holzapfel, 2006; Woo et al., 1983; Zernicke et al., 1984). This latter method, the imaging method, is useful for avoiding or reducing the clamped boundary effect associated with the uniaxial test. However, it requires a significant amount of equipment, software, and calculations, while the determination of mean strain from grip distance is relatively simple. Unfortunately, no experimental studies have verified whether the calculation of strain based on grip distance is an accurate approximation compared to marker motion in the mid-section of tissue specimens from large elastic arteries.

The purpose of this study was to examine if the stretch calculated from grip distance during uniaxial testing on specimens from large elastic arteries is an accurate approximation of the stretch in the middle of the specimen.

2. Methods

2.1. *Materials*

All experimental studies were performed after approval by the Institutional Animal Care and Use Committee of the University of Wisconsin-Madison and Northwestern University. The large proximal pulmonary arteries and descending aortas were harvested after the euthanasia of the animals from 4 adult canines at Northwestern University and 16 adult canines at the University of Wisconsin-Madison, and stored in PBS at 4 °C before dissection and testing.

2.2. *Mechanical testing*

The aorta and the large proximal pulmonary arteries were prepared for a uniaxial tensile test, less than 24 h after the euthanasia of animals, with modified experimental protocol of previous studies (Lammers et al., 2008; Tian et al., 2011). Any connective tissue visible on the arteries was removed from the outer wall. This was done carefully and completely to avoid any alterations in the mechanical properties of the vasculature. The pulmonary vasculature was cut with a razor blade into three sections: left pulmonary artery (LPA), right pulmonary artery (RPA), and main pulmonary artery (MPA). Rectangular sections were cut from LPA, RPA, MPA, and aorta in the circumferential direction for testing. Longitudinal rectangular section was also cut from the artery if sufficient longitudinal length remained after the circumferential section was removed. Table 1 summarizes the tissues that were available for testing. Prior to the uniaxial tensile test, the width and thickness of each artery specimen were measured from scaled digital image, and each specimen was marked with black adhesive (Loctite 380 Black Max; Henkel, CT, USA) on the arterial intimal surface for tracking during the testing and for local stretch calculations. Several small markers (>4) were made on the tissue surface within the middle region along the midline of the specimen in the loading direction (Fig. 1). All markers on the specimen, with a distance of 1–3 mm between two consecutive ones, were kept as small as possible (0.5–1 mm in diameter) to avoid alteration of the mechanical properties, and they were also kept close to the midline to ensure that they were aligned with the direction of uniaxial loading. The specimen was then clamped by self-aligning grips with sandpaper on each end to avoid specimen slippage. The length between the two grips was measured with digital calipers (with a resolution of 0.5 μm) prior to the test as the reference length (L_0). All tests were done with an Instron 5548 MicroTester tensile testing system (Instron; Norwood, MA, USA), equipped with a 10 N load cell. The MicroTester has a resolution of 0.125 μm and the load cell has a resolution of 10 mN. The tissue specimen was immersed in PBS at 37 °C in an environmental chamber throughout the test. Each specimen was first extended by 30% of the initial length which in general won't break the tissue (Lammers et al., 2008) and at a strain rate of 20% gauge length/s which is close to the physiological range (Ingram et al., 1970). The specimen was returned to its initial state before the next test.

Table 1 – Summary of the number and geometry of specimens prepared for testing according to type of tissue and direction of specimen. AO, aorta; MPA, main pulmonary artery; LPA, left pulmonary artery; RPA, right pulmonary artery; N, number of specimens; *L*, gage length of specimen during the uniaxial test; *W*, specimen width; N1, the number of specimens within group-1 ($L/W \geq 4$), and the rest specimens are categorized as Group-2. A threshold of 4 for *L*/*W* was chosen based on the literature (see text).

Tissue	Circumferential				Longitudinal			
	N	N1	*L* (mm)	L/W	N	N1	*L* (mm)	L/W
AO	19	7	14.5±3.6	3.9±1.0	19	14	22.7±5.0	4.4±1.1
MPA	17	16	18.6±2.8	5.7±1.4	N/A			
LPA	17	5	10.6±2.1	3.5±0.9	5	0	10.3±2.2	2.8±0.7
RPA	17	9	12.7±1.6	4.1±0.9	12	2	11.1±2.7	3.2±1.0