AN EXPERIMENTAL METHOD FOR MEASURING MECHANICAL PROPERTIES OF RAT PULMONARY ARTERIES VERIFIED WITH LATEX

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INTRODUCTION
Pulmonary hypertension is an important factor in determining post-operative morbidity and mortality in children with congenital heart disease. The additional right-side afterload and the relative inability of the right ventricle to cope with chronic volume and pressure loading can also lead to catastrophic failure of the right heart if left untreated. The etiology of the disease is still not completely understood, although two general distinctions are made: primary and secondary. Primary pulmonary hypertension is a diagnosis of exclusion, where no single causative parameter has been identified; secondary pulmonary hypertension can occur due to a number of reasons, including the congenital heart defect itself. Regardless of the underlying manifestation, pulmonary hypertension in children continues to be a significant clinical diagnosis, especially for children born at altitudes appreciably above sea level.

Clinical studies [1] have shown that prostaglandin analogs lower pulmonary vascular resistance and therefore appear promising in treating pulmonary hypertension. However, very little is known about the biomechanics and hemodynamics of pediatric pulmonary hypertension. Such information would allow development of novel non-invasive methods to evaluate pulmonary hypertension; provide fundamental information about the dynamics of this highly reactive vasculature; facilitate development of sophisticated numerical models incorporating blood flow and arterial wall characteristics, which in turn can be used to study disease progression, reaction to clinical interventions, long-term changes, etc.; provide information on key metrics that may be better markers of clinical outcome than pulmonary vascular resistance. In this regard, the availability of animal models of inflammatory and restrictive pulmonary hypertension presents an opportunity to study the biomechanics of pulmonary hypertension. The Sprague-Dawley rat model, with pulmonary hypertension induced through hypoxia or injection of monocrotaline, in particular, has been extensively used for pulmonary hypertension studies. However, the extremely small size of these arteries presents unique experimental issues for mechanical testing. This paper represents our initial efforts in the development and validation of the experimental apparatus to mount and test the main pulmonary arteries from the rat.

TEST PROCEDURE
Our experimental setup is a bubble-inflation technique similar to many of those described in the literature [2–6], and more recently in [7]. References [4] and [7] suggest the suitability of the bubble inflation test for membrane-like soft tissues. Qualitatively, the inability of the rat’s pulmonary artery to support out-of-plane shear is indicative of membrane-like behavior. As with the system described in [7], our fixture is designed for small specimens. The stainless steel fixture has an aperture of 2.78 mm. In advance of testing tissue, latex is used to test the performance of the fixture and experimental technique. The latex sample is stained in a square array with OsO4 vapor [8] using a copper grid designed for a transmission electron microscope (TEM) with a 74 µm pitch for the mask; the pattern is used to define the curved surface of the anisotropic tissue.

The experiment is designed so that each component that contributes to the data set has computer control (Figure 1). Through a computer program, the motion controller can move the bellows with feedback from the pressure transducer, so that pressure can be applied and recorded in well-controlled increments (±13.8 Pa). Upon reaching the predetermined pressure setting, the multi-channel video control card allows images to be collected from camera 1 at 30° intervals, controlled by the computer-driven rotating stage, from 0° to 150°, then switches to collect an image along the pole direction from camera 2. The specimen-containing fixture sits in a reservoir of de-ionized water for the latex test, or in a buffered saline solution for testing the pulmonary arteries. De-ionized water or buffered saline solution fills the bellows and tubing that comprise the pressure system, so that neither surface of the specimen is in contact with air during testing.

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EXPERIMENT
To test the system, measurements were made on commercially obtained latex. A disk of latex, nominally 4 mm in diameter by 0.075 mm thick, was inserted and patterned in the biaxial fixture, and attached to the pressurizing system. All air was removed from the biaxial fixture, and as much as possible from the tubes, bellows, and pressure transducer. Because the pressures experienced by the pulmonary artery are relatively low, the test pressures remained correspondingly low. The latex sheet was pressurized in 690 Pa increments, starting from 0 Pa up to $1.72 \times 10^4$ Pa. Images were collected at each pressure increment from both cameras. Due to the isotropic behavior of latex, camera 1 collected only one image, rather than multiple images at various angles of rotation. For this experiment only, as the latex is isotropic and the stretch ratio is $<<2$, it was assumed that the deformation was spherical and could be determined from the bubble height $h$.

RESULTS
We measure only two variables: pressure, $P$, and the height of the bubble, $h$. From $h$ and $C_0$, the initial length across the sample (2.78 mm), we can calculate the radius of curvature of the bubble, $r$, from the geometry. The results of the measurements are shown on a plot of true stress versus true strain in Figure 2, along with a curve showing the expected result from the theory of large elastic deformations. Using the strain energy function, the biaxial true stress is

$$\sigma = \mu (\lambda^2 - 1/\lambda^4),$$

where $\mu = 3.21 \times 10^5$ Pa, based on a fit to the current data. The standard deviation of the biaxial true stress about the model line is $5.8 \times 10^3$ Pa. The biaxial true strain is obtained from the biaxial stretch using $\varepsilon = \ln(\lambda)$.

The measurement data show no bias, as they are essentially randomly scattered around the theoretical curve. The low value of final strain, 13.2%, at the highest pressure measured, $1.72 \times 10^4$ Pa, is well within the elastic limit for latex [9]. This low strain value should also preclude any hysteretic effects, which may be seen at these low, and lower, pressures when testing tissue.

CONCLUSIONS
We used latex as a sample material to test the performance of a new measurement system for studying the mechanical properties of normotensive and hypertensive pulmonary arteries from rats. The test has demonstrated the high accuracy and precision that are required for testing the rat arteries. The system and the automation provided a combined standard uncertainty of $\sim 4\%$ in this measurement, and simple modifications in the future will yield even higher accuracy. The precise control and improved accuracy of the measurement should enable reliable determination of anisotropy in addition to the mechanical properties of arteries. The results of the latex test demonstrate the validity of the test system.

REFERENCES
1. Unpublished work by D. Ivy and R. Shandas, The Children’s Hospital of Denver, CO.