INTRODUCTION

Cerebral blood vessels are critical in maintaining the health and function of the brain, but their function can be disrupted by traumatic brain injury (TBI), which commonly includes damage to these vessels [1]. However, even in cases where there is not apparent mechanical damage to the cerebral vasculature, TBI can induce physiological disruptions that can lead to breakdown of the blood brain barrier or loss of cerebral autoregulation.

Definition of the properties of cerebral vessels is important to a better understanding of vascular injury thresholds. Previous investigations have shown that human cerebral arteries are stiffer than the surrounding brain tissue as well as being stiffer axially than they are circumferentially [2,3]. Longitudinal failure values have also been defined [4]. These data enable the study of vascular injury mechanisms in computer models. Further study, however, is needed to better characterize physiological response of vessels to traumatic loading.

Due to the difficulty in obtaining human vessel samples for such testing, it is desirable to have an animal model from which to gather these data. As rats are also commonly used in TBI research, this study aimed to determine the biaxial mechanical and failure properties of rat middle cerebral arteries (MCAs). Prior investigations of rat MCA have largely focused on circumferential behavior within the typical physiological range of loading [e.g. 5,6,7].

METHODS

Sample Preparation

The MCA was dissected out of 7 male Sprague Dawley rats (370 ± 40 grams). Rats were euthanized via CO₂ asphyxiation and then perfused with Hank’s Buffered Saline Solution (HBSS), followed by a 1% nigrosin dye HBSS solution. The dye allowed for the staining of the endothelial surface of the vessels without staining of the brain tissue to enhance visibility during dissection of the MCA. MCA side branches were ligated with individual fibrils from unwound 6-0 silk suture. The MCA was then cannulated with either 35 gauge stainless steel hypodermic needles or glass tip needles of the same tip diameter and then secured with intact 6-0 silk suture and cyanoacrylate glue.

Test Set-up

The mechanical testing set-up is similar to that described previously [3]. Briefly, the needles on which the MCA were mounted, and the associated mounting fixtures, were attached to a vertical linear stage. The upper fixture was suspended from a 500 gram capacity load cell. The lower fixture was mounted to the stage via a vertical, low friction sled and supported by a voice coil actuator. Movement of this voice coil moved the lower fixture vertically along the sled track, axially stretching the MCA. Associated with the lower fixture was a narrow, clear plastic water bath, filled with HBSS, surrounding the MCA and needles. The MCA was viewed via a digital video camera with a high magnification lens in order to record the change in vessel dimensions during testing. The MCA was perfused with HBSS originating from a syringe attached to a linear actuator and passing through the lower fixture, mounted MCA, and the upper fixture. Inline pressure transducers were located both proximal and distal to the mounted MCA, equidistant from the vessel. The average between these two transducers was taken to be the pressure inside the MCA, or the luminal pressure. Data and video acquisition, as well as control of the set-up, were accomplished via a custom LabView program (National Instruments).

Test Procedure

Preconditioning Tests

Following the mounting of the MCA, it was subjected to various combinations of axial stretch and pressure for preconditioning. The vessel was first stretched to $\lambda_A = 0.9$ based on the estimated in vivo length ($\lambda_{iv}$) obtained from measurements, and the
pressure was oscillated between 6.7 and 20 kPa a minimum of 5 times. This was then repeated using \( \lambda \approx 1.0, 1.1, \) and 1.2. Specimens were maintained at 13.3 kPa and \( \lambda \approx 1.0 \) between tests. Observation of the force during these tests helped to confirm the estimated \( \lambda \), as the axial force will remain constant with oscillations of pressure when the MCA is at in vivo length [8].

**Reference Geometry** A cross section from the distal end of the MCA, removed earlier during dissection and stored in HBSS, was photographed in order to calculate the reference cross-sectional area. The reference length was determined by decreasing the axial stretch on the MCA until there was obvious buckling when luminal pressure was equilibrated to atmospheric pressure. The MCA was then stretched from this buckled length to a maximum of \( \lambda \approx 1.2 \), as during preconditioning testing. The MCA length where force first registered on the load cell was considered to be the reference length.

**Characterization Tests** In order to determine the circumferential properties, the MCA was stretched to \( \lambda \approx 1.2, 1.1, \) and 1.0 sequentially. While being held at each of these stretch levels the luminal pressure was oscillated between 6.7 and 20 kPa a minimum of 5 times. In order to determine the axial properties, the MCA was subjected to luminal pressures of 20, 13.3, and 6.7 kPa sequentially. While being held at each of these pressures, the MCA was stretched from the reference length to \( \lambda \approx 1.2 \) a minimum of 5 times. The final loading phase for each test was used for analysis.

Axial failure stretch values were determined by repeating the axial properties test at 13.3 kPa but stretching the vessel axially to failure in the final cycle.

**Data Analysis**

Material properties were calculated using the same method as was used in [3]. Assuming the vessel to be a homogenous circular cylinder, the specimen stretch was calculated as:

\[
\lambda = \frac{d_1 + d_2}{d_1 + d_2}, \quad \lambda_z = \frac{l}{L},
\]

where \( \theta \), and \( z \) indicate the circumferential and axial directions respectively, and \( d_1, d_2, \) and \( l \) indicate the inner and outer diameters, and the suture-to-suture length respectively. The corresponding capital letters indicate the same values but from the zero load configuration. The inner diameter was calculated assuming the vessel wall to be incompressible and using the photographed cross-sectional area. Using these stretch values, the Mean Cauchy stresses were calculated as:

\[
T_\theta = P_l \frac{d_1}{d_2 - d_1}, \quad T_z = \frac{\lambda_z}{\lambda} \left( F + \frac{\pi d_1^2}{4} P_l \right),
\]

where \( P_l \) is internal pressure and \( F \) is the measured axial force.

**RESULTS AND DISCUSSION**

The axial and circumferential Cauchy stress-stretch responses of a representative rat MCA are shown in Fig. 1. Rat MCA displays slightly stiffer material response in the axial direction than in the circumferential direction at sub-failure stretch levels. This is consistent with behavior observed in human cerebral vessels [3].

During failure tests, the seven MCA samples displayed an axial failure stretch level of 1.334 \( \pm 0.105 \) (mean \( \pm \) standard deviation) and an axial failure stress level of 0.485 \( \pm 0.121 \) MPa (mean \( \pm \) standard deviation). Four vessels failed at or very near the sutures, while 3 failed mid-vessel. The difference in failure stress and \( \lambda_z \) was negligible between these two groups.

The response of the rat MCAs was similar to that previously observed in human cerebral arteries. Specifically, curve shapes were qualitatively similar, particularly in the observation that the vessels have a more pronounced toe region circumferentially than axially. Furthermore, the exhibited values of both the axial and circumferential stress levels for these tests are similar to those observed in human cerebral vessels [3]. Also, the axial failure stretch levels correspond well with those observed in human cerebral vessels [3,4]. However, the stress level observed at these failure points is an order of magnitude lower than seen in human vessels [3,4]. It is not clear why this would be true.

In conclusion, rat MCA has mechanical and failure properties similar to those previously reported for human cerebral vessels. These data will be useful in further study of vascular injury mechanisms and response in both animal models and isolated vessel preparations.

![Figure 1: Stretch-Stress response curves for representative MCA sample in axia (a) and circumferential (b) directions. Label subscripts indicate internal pressure in (a) and axial stretch in (b).](image-url)

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**REFERENCES**