Linking the microstructure of human middle cerebral arteries to their mechanical behavior

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Abstract

Introduction

In silico models of the cerebrovascular tree are valuable tools for advancing treatments of diseases like stroke (1). However, their reliability depends on how accurately they replicate the mechanical behavior of cerebral blood vessels. While models such as the Gasser-Ogden-Holzapfel model effectively represent arterial mechanics, their calibration is hindered by the limited data on cerebral arteries, which differ structurally from the more extensively studied large elastic extracranial arteries (2). This study aims to fill this gap by investigating the microstructure of human middle cerebral arteries and linking it to their mechanical behavior.

Materials and methods

Human middle cerebral artery tissue was obtained from six donors within 24 hours post-mortem in collaboration with the Dublin Brain Bank (Royal College of Surgeons, Dublin, Ireland). From these arteries, 32 2 mm thick ring samples were excised, and their mechanical properties were characterized through ring-extension testing in a prior investigation. This testing resulted in stress-strain curves from which mechanical properties such as linear stiffness in the elastin- and collagen-dominant regions, failure stress, and x-intercept strain were derived.

To investigate potential microstructural links to the observed mechanical behavior, a histological analysis was conducted on 7 μm-thick cross-sections stained with Hematoxylin and Eosin (H&E), Verhoeff's elastin, and Picrosirius red. Next, imaging was performed using a Leica Aperio AT2 system for the H&E and Verhoeff's elastin-stained sections, whereas the Picrosirius red-stained sections were imaged using an Olympus BX41 microscope under brightfield and polarized light (0○ and 45○).

For each stain, qualitative and semi-quantitative analyses were performed on three sections

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per sample. Medial thickness and internal elastic lamina thickness were measured at 10 points in H&E- and Verhoeff's elastin-stained sections, respectively, and normalized to the total radial thickness of the section to account for size differences. Smooth muscle cell fraction was approximated from H&E images by dividing the hematoxylin-stained area by the total medial area, using QuPath. Collagen fraction was quantified from Picrosirius red-stained sections using a custom MATLAB script. To estimate the collagen content, polarized light images at 0∘ and 45∘ were combined, as each angle captures different fiber orientations (3). The resulting composite image was used alongside the brightfield image to delineate the media-adventitia border, enabling separate collagen fraction calculations for the media and adventitia. Finally, pixel intensities, which are proportional to collagen content (3), were normalized to the maximum observed intensity (assumed to represent 100% collagen) and averaged across the tissue segment of interest to calculate the collagen fraction. Unbalanced two-way ANOVA was used to evaluate the effects of cardiovascular disease and sample location (proximal M1 vs. distal M2 segment of the artery) on the histological features.

The relationship between histological and mechanical properties was evaluated by examining the correlation between elastin-dominant region stiffness and normalized internal elastic lamina thickness, as well as between the collagen-dominant region stiffness and collagen fraction.

Results and discussion

Histological analysis showed that middle cerebral arteries share structural similarities with other arteries, including the characteristic three-layer organization (intima, media, adventitia) and the presence of collagen, elastin, and smooth muscle cells. However, distinct differences were observed when compared to large elastic extracranial arteries, such as the aorta. Consistent with prior reports (2), the external elastic lamina was absent in cerebral artery samples, though increased elasti**n** fiber content was observed in the region where the external elastic lamina would typically be found. Additionally, although the semi-quantitative analyses are still ongoing, the qualitative analysis already revealed that the media of the cerebral arteries contains fewer elastin fibers and has a higher smooth muscle cell content than large elastic extracranial arteries. These findings align with the functional demands of cerebral arteries, which prioritize precise blood flow regulation over the elastic recoil required by large elastic arteries to buffer pulsatile flow (4). It should be noted that some extracranial arteries, such as the radial artery, share a similar role in blood flow regulation and exhibit a structure more similar to cerebral arteries. Therefore, the broad term "extracranial arteries", frequently used in the literature when comparing cerebral arteries to large elastic extracranial arteries, can be misleading. A clear distinction should be made between muscular and elastic arteries.

Although the semi-quantitative analyses are still ongoing, preliminary results, along with the qualitative analysis, suggest a relationship between the histological features and the mechanical properties. Donors with cardiovascular disease (n=3) appeared to have lower elastin content and exhibited reduced normalized internal elastic lamina thickness (median: 2.33 %, IQR: 2.21–2.61 %) compared to donors without cardiovascular disease (n=3) (median: 3.10 %, IQR: 2.34–3.42 %). These histological differences align with the earlier obtained mechanical findings, where unbalanced two-way ANOVA tests revealed significantly lower x-intercept strain ($p=4.92\times10$) and higher elastin-dominant region stiffness (p=0.0097) in arteries from donors with cardiovascular disease. Additionally, initial findings indicated a higher overall collagen fraction in M1 segment samples (n=19) compared to M2 segment samples (n=13). This corresponds with the mechanical results which showed a significantly higher collagen-dominant region stiffness (p=0.038) and failure stress (p=0.002) for M1 segment samples.

In conclusion, the microstructure of cerebral arteries seems to affect their mechanical behavior, emphasizing the need for microstructurally-informed constitutive models for in silico simulations. Although multiple models like this exist for large elastic extracranial arteries, they often overlook the complexity of smooth muscle cells as elastin and collagen are seen as the main tissue constituents. Taking into account the higher smooth muscle cell content in cerebral arteries, a model with a more accurate description of these cells might be necessary.

By developing a microstructurally-informed model for cerebral arteries, it will become possible to model the entire cerebrovascular tree with vessel-size-specific tissue compositions. It has been suggested that including this vessel-size specificity is essential to accurately capture the complex relationship between blood pressure and blood flow in the brain (4). Therefore, a microstructurally-informed model, calibrated using data from this study and future studies on smaller cerebral arteries, is expected to improve the accuracy of in silico models of the cerebrovascular tree, ultimately advancing research into diseases like stroke.

References

1. Daher et al, Microvascular research, 147:104503, 2023.

2. Shafigh et al, Biomed. Mater. & Devices, 2:1175-1185, 2024.

3. Turčanová et al, BioMed Res. Int., 2021.1: 6879765, 2021.

4. Klein et al, JAHA, 11.1, 2022.

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