Unveiling Age-Related Changes in Collagen Fibers Using Machine Learning and Multiphoton Imaging

Anastasia Gkousioudi[1], Samuel Carl Halvorsen[1], Yanhang Zhang[1,2]
[1]MSTB Lab, Department of Mechanical Engineering, Boston University, Boston, United States of America
[2]Department of Biomedical Engineering, Boston University, Boston, United States of America
Email: yanhang@bu.edu

Summary

This study examines age-related changes in arterial collagen using multiphoton microscopy and machine learning. Human anterior cerebral arteries (ACAs) from donors aged 28 to 92 were imaged, and collagen fiber parameters were extracted. Principal Component Analysis (PCA) and Gaussian Mixture Model (GMM) clustering identified four distinct structural patterns. Results showed aging is related to straighter fibers, indicating arterial stiffening. This study provides a data-driven, biologically relevant assessment of collagen remodeling. These findings highlight the impact of aging on arterial microstructure, with future work incorporating biomechanical characterization to further understand the relationship between collagen structure and arterial function.

Introduction

The microstructural organization of collagen fibers in arterial walls is crucial for maintaining vascular integrity [1]. Multiphoton microscopy enables high-resolution imaging, but traditional data analysis methods struggle to quantify microstructural changes due to the inherent biological sample variability and large data volumes. In this study, using multiphoton microscopy, we imaged human anterior cerebral arteries (ACA) from human donors and extracted quantitative collagen fiber parameters. Machine learning techniques, including PCA and GMM clustering were employed to provide a data-driven approach to identify age-associated collagen microstructure alterations.

Methods

A total of 38 human ACAs from individuals aged 28 to 92 years were imaged using an FVMPE-RS confocal microscope (Olympus Life Sciences, Inc). Second harmonic generation (SHG) signals of collagen fibers were collected over an imaging depth of 60-70 μm to visualize the adventitial layer, generating a stack of images for each sample. Fiber parameters (straightness, length, angle) were extracted using CT-FIRE, and histogram-based statistical metrics (mean, median, standard deviation, skewness, and kurtosis) were calculated for each parameter across 1750 images. PCA was conducted to reduce dimensionality, and a GMM clustering algorithm was implemented to identify distinct structural patterns. A permutation test was conducted to assess the statistical significance of the identified clusters ($p < 0.05$).

Results and Discussion

Figure 1A shows the resulting clusters where each data point represents a single image characterized by its extracted fiber parameters. The total explained variance ratio of the PCA was 66.7%. PC1 was primarily associated with fiber straightness and length, PC2 with skewness and kurtosis of both straightness and length, and PC3 was mainly influenced by fiber orientation. GMM clustering identified four distinct clusters, with Cluster 1 (CL1) exhibiting the longest fiber length, while Cluster 3 (CL3) having the lowest mean age (< 57 years old; Figure 1B) and the lowest straightness.

Our findings provide strong evidence that aging is associated with straighter fibers, reinforcing the relationship between microstructural remodeling and arterial stiffening with age [2]. Unlike prior studies that primarily relied on image-based intensity metrics [3], this study employs an in-depth, data-driven clustering based on fiber-specific parameters, offering a biologically meaningful assessment of structural changes with PC1 and PC2 primarily capturing the microstructural parameters related to collagen organization.

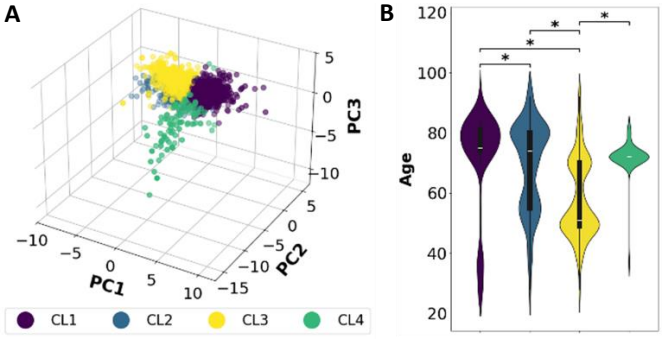

Figure 1: A) GMM clustering of arterial samples, and B) violin plot of the mean age values in each cluster (*$p < 0.05$).

Conclusions

This study introduces a novel unsupervised learning approach that provides valuable insights into the impact of aging on arterial microstructure. The machine learning-based clustering method offers a quantitative way to assess microstructural changes. Future studies will incorporate biomechanical characterization to enhance our understanding of collagen remodeling in vascular health.

Acknowledgments

The authors would like to acknowledge funding support from National Institute of Health (1R01AG075876, 2R01HL098028), and Dr. Seok-Hyun Yun for access to the confocal microscope.

References

[1] Wagenseil JE and Mecham RP (2009). *Physiol. Rev.*, **89**: 957-989.
[2] Bloksgaard M et al. (2017). *AJPHeart*, **313**: 164-178.
[3] Kistenev YV at al. (2019). *Biochem (Moscow)*, **84**: 108-123.