Journal of Biomechanics 47 (2014) 935–943

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

A custom image-based analysis tool for quantifying elastin and collagen micro-architecture in the wall of the human aorta from multi-photon microscopy

Ryan G. Koch [a,1], Alkiviadis Tsamis [a,d,f,1], Antonio D'Amore [a,c,f,g,h], William R. Wagner [a,c,d,f], Simon C. Watkins [e], Thomas G. Gleason [a,b,d,f], David A. Vorp [a,b,c,d,f,*]

[a] Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA, United States
[b] Department of Cardiothoracic Surgery, University of Pittsburgh, Pittsburgh, PA, United States
[c] Department of Surgery, University of Pittsburgh, Pittsburgh, PA, United States
[d] Center for Vascular Remodeling and Regeneration, University of Pittsburgh, Pittsburgh, PA, United States
[e] Center for Biologic Imaging, University of Pittsburgh, Pittsburgh, PA, United States
[f] McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA, United States
[g] Fondazione Ri.MED, University of Palermo, Palermo, Italy
[h] DICGM University of Palermo, Palermo, Italy

ARTICLE INFO

Article history:
Accepted 14 January 2014

Keywords:
Multi-photon microscopy
Collagen
Elastin
Micro-architecture
Quantification
Fiber orientation

ABSTRACT

The aorta possesses a micro-architecture that imparts and supports a high degree of compliance and mechanical strength. Alteration of the quantity and/or arrangement of the main load-bearing components of this micro-architecture – the elastin and collagen fibers – leads to mechanical, and hence functional, changes associated with aortic disease and aging. Therefore, in the future, the ability to rigorously characterize the wall fiber micro-architecture could provide insight into the complicated mechanisms of aortic wall remodeling in aging and disease. Elastin and collagen fibers can be observed using state-of-the-art multi-photon microscopy. Image-analysis algorithms have been effective at characterizing fibrous constructs using various microscopy modalities. The objective of this study was to develop a custom MATLAB-language automated image-based analysis tool to describe multiple parameters of elastin and collagen micro-architecture in human soft fibrous tissue samples using multi-photon microscopy images. Human aortic tissue samples were used to develop the code. The tool smooths, cleans and equalizes fiber intensities in the image before segmenting the fibers into a binary image. The binary image is cleaned and thinned to a fiber skeleton representation of the image. The developed software analyzes the fiber skeleton to obtain intersections, fiber orientation, concentration, porosity, diameter distribution, segment length and tortuosity. In the future, the developed custom image-based analysis tool can be used to describe the micro-architecture of aortic wall samples in a variety of conditions. While this work targeted the aorta, the software has the potential to describe the architecture of other fibrous materials, tube-like networks and connective tissues.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Soft fibrous tissues – e.g., ligaments, the heart, blood vessels, gastrointestinal tract and the urethra – perform functions which rely on a high degree of elasticity or compliance (Lentle et al., 2013; Mijailovich et al., 2007; Westerhof et al., 2005). The elastic properties of these tissues are supported by a complex underlying fiber micro-architecture (Halloran et al., 1995; Sokolis and Sassani, 2013; Wu et al., 2011). The two main load-bearing constituents, elastin and collagen, impart the elasticity and strength of soft fibrous tissues. Alteration of the content and/or arrangement of these fibers leads to the mechanical, and hence functional, changes associated with diseases or disorders of these tissues. Therefore it is important to extract detailed microstructural information from soft fibrous tissue to reveal possible mechanisms of diseases or disorders; i.e., relating changes in micro-architecture to tissue function and dysfunction.

Elastin and collagen are easily visualized based on their intrinsic fluorescence and second-harmonic generation, respectively (Cahalan et al., 2002; Jiang et al., 2011; Konig et al., 2005; Strupler et al., 2007).

* Corresponding author at: Swanson School of Engineering, University of Pittsburgh, 123 Benedum Hall, 3700 O'Hara Street, Pittsburgh, PA 15261, United States. Tel.: +1 412 624 8503; fax: +1 412 624 0412.
E-mail address: vorp@pitt.edu (D.A. Vorp).

[1] Co-first authors contributed equally to work.

0021-9290/$ - see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jbiomech.2014.01.027

Multi-photon micrographs of these extracellular matrix components could be processed using an appropriate image-based analysis tool to obtain micro-architectural characteristics. Prior image-analysis paradigms such as Hough transforms (Chaudhuri et al., 1993), intensity gradient-based texture analysis algorithms (Chaudhuri et al., 1993; Courtney et al., 2006; Karlon et al., 1998), direct-tracking methods (Pourdeyhimi et al., 1999; Pourdeyhimi et al., 1996a; Pourdeyhimi et al., 1996b), and fast Fourier transform (FFT)-based image-analysis (Ayres et al., 2006; Ayres et al., 2007; Ayres et al., 2008) have been successfully implemented to quantify fiber architecture of native and engineered tissues. However, these image-analysis methodologies do not offer a complete description of the fiber-network topology as they focused primarily on orientation-based information. D′Amore et al. (2010) successfully developed an image-based analysis tool to effectively characterize the architecture of engineered-tissue fiber-networks viewed with standard scanning-electron microscopy (SEM) based on a Delaunay-network skeleton and image-gradient information. The detected and quantified micro-architectural features included the fiber angle distribution, node connectivity, spatial-intersection density, and fiber diameter.

Multi-photon images of elastin and collagen, containing different levels of noise and fluorescence when compared with images from different imaging modalities such as SEM, require an optimized image-analysis tool to provide a rigorous description of micro-architectural characteristics. Characterization of fiber orientation in soft fibrous tissues would be possible using FFT, direct-tracking or texture-based methods (D′Amore et al., 2010). However, these methods would need to be modified to be compatible with the image quality from multi-photon microscopy to sufficiently describe the complicated micro-architecture of soft fibrous tissues.

The goal of this study was to develop a custom, image-based analysis tool to describe multiple parameters of elastin and collagen micro-architecture in human soft fibrous tissue samples using multi-photon images. This goal was achieved using a MATLAB language-based code that utilizes a novel fiber skeletonization approach optimized for multi-photon images of collagen and elastin. Human aorta samples were used to develop the code.

2. Methods

2.1. Human aorta specimens

To illustrate the utility of our tool on soft fibrous tissues, we used human ascending thoracic aorta (ATA) tissue specimens as a test bed. ATA specimens were obtained after informed consent according to the guidelines of our Institutional Review Board and the Center for Organ Recovery and Education (Pittsburgh, Pennsylvania). In brief, segments of ATA were either collected from organ donor/recipient subjects or obtained from patients undergoing elective ATA repair at the University of Pittsburgh Medical Center. Tissue specimens from patients aged 39–81 and aortic diameter 46–68 mm (8 males/10 females) were first perfusion-fixed in 4% paraformaldehyde. After 1.5 h, the solution was replaced with PBS and stored at 4 °C.

2.2. Multi-photon microscopy

An Olympus multi-photon microscope (Model FV10, ASW software) was used to observe elastin and collagen fibers in the ATA (Cahalan et al., 2002; Jiang et al., 2011; Konig et al., 2005). Elastin (green) and collagen (red) were automatically detected and visualized based on intrinsic fluorescence (channel RXD1, wavelength 525 ± 25 nm) and second-harmonic generation (channel RXD2, wavelength 400 ± 50 nm), respectively. Fig. 1 shows a representative multi-photon image (500×500 μm^2) from ATA.

2.3. Image-processing

To analyze the multi-photon images of ATA, a custom MATLAB image-analysis tool was developed utilizing the Image-Processing Toolbox. The input image (Fig. 2A) was selected through a user-interface dialog box. Information for a given fiber type was saved in one channel (red, blue or green). Accessing that channel provided the information to start processing (Fig. 2B).

2.3.1. Intensity-equalization and fiber-extraction

The image was smoothed and fiber edges preserved using the MATLAB function (MF) for median filtering (3×3 window, MF *medfilt2*), then contrast-enhanced using contrast-limited adaptive histogram equalization (3×3 window, MF *adapthisteq*) and a histogram adjustment (MF *imadjust*), where 1% of the image was saturated at the extremes (Fig. 2C). The image was adjusted to equalize the noise and intensity levels between inputs and to ensure the maximum available dynamic range was utilized. Noise and background information was suppressed and fibrous information enhanced using multi-scale vessel-enhancement filtering (Frangi et al., 1998). The Frangi algorithm analyzed intensity gradients, looking for tube-like vessels. The filter emphasized these components while suppressing non-tube-like information. Because of the similarity of fibers to vessels, the technique isolated the fiber content from the background material. Threshold-filtering was then performed on the image using a value of 0.4 as the cutoff-intensity by creating a binary, black and white image, and segmenting the fiber information from the background (Fig. 2D). The value 0.4, fixed for all images, was qualitatively determined to minimize fiber diameter deviations from the grayscale to binary image. A static value was able to be used because of the intensity equalization steps preceding this threshold. A Euclidean-distance transform (MF *bwdist*) was performed on the complement of the binary image to find the distance from every pixel within the fibers to the nearest edge of the fiber. A watershed-transform (MF *watershed*) was then performed to identify ridges in the distance-transformed image marking an approximation of the center of the most well-defined fibers. This image was then used as a mask on the distance-transformed image to aggregate initial fiber diameter values. The median of the fiber diameter values was chosen as the representative fiber diameter (FD). Due to artifacts at the edges of the image from the filtering techniques, FD-pixels were cropped from the four image edges. The result was a binary, black and white (BW) image.

2.3.2. Fiber-skeletonization and locations of intersections

The BW image was subjected to a series of morphological operations. These operations further smoothed the edges of the fibers and removed small holes and fiber fragments that might have been background or incomplete information. First the BW image was eroded (structuring element (SE) disk radius=FD/8, MF

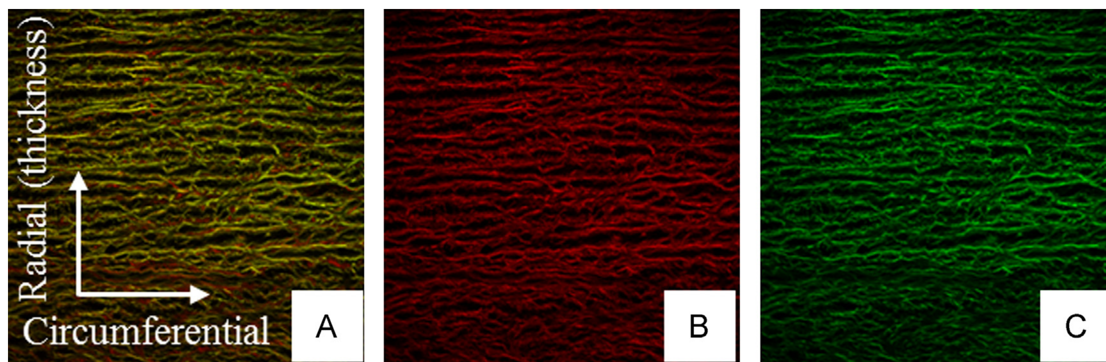

Fig. 1. Example multi-photon microscopy image (500×500 μm^2) of collagen (red) and elastin (green) fibers in the medial part of the wall of ATA. (A) Collagen and elastin fibers together. (B) Collagen fibers. (C) Elastin fibers. Pixel dwell time: 2 μs/pixel; number of pixels in the image: 1024×1024 pixels^2; laser wavelength: 830 nm; excitation power at the sample: 6%; depth from surface: 20 μm; PMT voltage: 539 V for elastin and 443 V for collagen; detector gain: 1; offset (correction for background): 9%. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Download English Version:

https://daneshyari.com/en/article/10432400

Download Persian Version:

https://daneshyari.com/article/10432400

Daneshyari.com