Multimodal Imaging of Collagen Fibril Ultrastructure

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Collagen type 1 is the most abundant extracellular matrix protein in the human body. Collagen exerts its role as a tensile material and as a substrate for cells primarily after is has assembled into fibrils and fibers. Collagen fibrils have a characteristic morphology dependent on tissue type. However, in certain diseases a dysregulation in the biochemical or biomechanical environment can lead to changes in the collagen structure. Not much is understood about the fibril structure especially in diseases which are not genetic in nature i.e. do not have an underlying mutation in the collagen or related genes. For instance, vascular diseases like abdominal aortic aneurysms (AAA) are characterized by a drastic remodeling of the vessel wall, accompanied with changes in the elastin and collagen content. At the macromolecular level, the elastin fibers in AAA have been reported to undergo significant structural alterations. While the undulations (waviness) of the collagen fibers is also reduced in AAA, very little is understood about changes in the collagen fibril at the sub-fiber level.

In this study we investigated structural changes in collagen fibrils in human AAA tissue extracted at the time of vascular surgery and in aorta extracted from angiotensin II (AngII) infused ApoE−/− mouse model of AAA. Collagen fibril structure was examined using transmission electron microscopy and atomic force microscopy (AFM). Images were analyzed to characterize the length and depth of D-periodicity, fibril diameter and fibril curvature. Tissues were also stained using a novel reagent, collagen hybridizing peptide (CHP) (which stains degraded collagen) and analyzed using a combination of AFM and fluorescent microscopy. Our results elucidate how abnormal collagen fibrils with compromised D-periodic banding were observed in the excised human tissue and in remodeled regions of AAA in AngII infused mice. These abnormal fibrils were characterized by statistically significant reduction in depths of D-periods and an increased curvature of collagen fibrils. These features were more pronounced in human AAA as compared to murine samples. Additionally, regions of abnormal collagen were located within the remodeled areas of AAA tissue and were distinct from healthy collagen regions as ascertained using CHP staining. The structural alterations in abnormal collagen fibrils appear similar to those reported for collagen fibrils subjected to mechanical overload or chronic inflammation in other tissues. Detection of abnormal collagen could be utilized to better understand the functional properties of the underlying extracellular matrix in vascular as well as other pathologies. AFM can serve as a valuable tool to not only map the structural changes but also the mechanical properties of single collagen fibrils in-situ in tissue sections.

References: Jones B, Tonniges JR, Debski A, Albert B, Yeung DA, Gadde N, Mahajan A, Sharma N, Calomeni EP, Go MR, Hans CP and Agarwal G. (2020). Collagen fibril abnormalities in human and mice abdominal aortic aneurysm. Acta Biomater. 110: 129-140

Dr. Gunjan Agarwal received her PhD in Biophysics from the Tata Institute of Fundamental Research in Mumbai, India and came to the US for her post-doctoral training at the Albert Einstein College of Medicine (Bronx, NY) followed by a year at Procter and Gamble Pharmaceuticals (Cincinnati OH). After a brief period as a research scientist at the Air Force Research Lab (WPAFB, OH), she joined the Ohio State University (Columbus, OH) as an Assistant Professor in 2003. Dr. Agarwal is currently a Professor of Mechanical and Aerospace Engineering at OSU with research focus in the domains of bioengineering and nanotechnology. She is an expert in the utilization of light, electron and atomic force microscopy (AFM) for biomedical research and directs a multi-user Bio-AFM core facility at OSU. Her primary research focus is to study extracellular matrix remodeling with a particular emphasis of the collagen fibril structure and function. Another major initiative of Dr. Agarwal's research program is to develop novel biomedical applications of the AFM. Her laboratory has applied many aspects of magnetic force microscopy (MFM) to study nanoparticle behavior and ferritin(iron) deposits in tissue sections in health and disease. She has co-authored over 50 journal articles and contributed four invited book chapters. Her research has been continuously funded by the National Institutes of Health, the National Science Foundation and the American Heart Association. She has mentored 10 PhD and 5 MS students and teaches courses in Extracellular matrix, Medical Imaging and Microscopy.