Correlative workflow for murine pulmonary valve extracellular matrix imaging

Liu, Y.[1], Lee, Y.[2], Deng, B.[1], Breuer, C.K.[3] and McComb, D.[1]

[1] Center for Electron Microscopy and Analysis, The Ohio State University, United States, [2] Nationwide Children's Hospital, Department of Tissue Engineering, United States, [3] Nationwide Children's Hospital, Department of Tissue Engineering, United States

The heart valve is a pure biomechanical tissue that responds to cardiac pressures generated by ventricular contraction. However, the reorganization of the extracellular matrix elements to accommodate repetitive strain is widely unknown in a mouse model. Collagen I is a fibrillar extracellular matrix protein that is responsible for the mechanical integrity of the pulmonary valve. It is well known the collagen matrix undergoes significant reorganization during a cardiac cycle. This spatial and temporal heterogeneity of heart valve makes it difficult to study. Two-dimensional classical optical and scanning electron microscopy images do not adequately show the spatial conformation of the pulmonary valve. Additionally, when imaging at higher resolutions, it is difficult to assess where the origin of the sample. To comprehensively study the reorganization of the extracellular matrix during the cardiac cycle we developed a correlative workflow the order to bridge the gap in length scales and link structure to physiology.

Murine pulmonary valves were fixed at various transvalvular pressures via perfusion with a solution of glutaraldehyde and paraformaldehyde in cacodylate buffer. Valves were then stained with a combination of potassium ferrocyanide, osmium tetroxide, lead aspartate uranyl acetate as a general staining protocol. Valves were then dehydrated in ethanol and embedded in Durcupan ACM epoxy resin. Micro-computed tomography (uCT) was used to generate a three-dimensional reconstruction of the heart valve, and served as a guide for identifying regions of interest and further sample processing. Once a region of identified, a microtome was used to mechanically remove excess material. From here, the specimen block was used for serial block face scanning electron microscopy (SBF-SEM) or transmission electron microscopy (TEM). SBF-SEM was used to determine organization of collagen on a local level and TEM for imaging the banding structure. Regardless of the final imaging modality, the origin of the specimen is known, and collagen orientation can be discerned with respect to a physiological axis and loading direction.