The Intrinsic Durability of the Aortic Valve Extracellular Matrix

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Statement of Purpose: The aortic valve (AV) extracellular matrix (ECM) is a critical component in understanding the native AV function, and to guide the development of novel AV replacements. For example, decellularized AV tissues are of interest since the major immunogenic cellular components are eliminated and have, initially, the necessary functional design. However, it is not known what the intrinsic durability of the AV ECM is and how this relates to decellularized AV functional limits when tissue remodeling does not take place. In the current study, we investigated the mechanical and structural properties of AV leaflets decellularized with an anionic detergent, sodium dodecyl sulfate (SDS), in order to establish a baseline. A novel bioreactor was utilized to subject the AV to cardiac exercising and allow assessment of AV ECM durability in the absence of cellular maintenance.

Methods: A flow loop bioreactor was built to subject the decellularized valve conduits to ~3.5 million cardiac cycles under sterile conditions [1]. A visually clear, cylindrical flow loop bioreactor was designed to allow for graduated left side heart pulsatile flow conditions while maintaining sterility. An AV, decellularized with SDS [2], was sutured into a silicone root with sinus design, which was then mounted into the bioreactor. Placing the AV into a silicone root allowed us to easily mount the decellularized valve in the bioreactor and was necessary in order to subject the decellularized valve to proper flow conditions. The bioreactor was then attached to the flow loop forming a closed fluid circuit. Graduated pulsatile flow mimicking left heart pressure was commenced with a bellows metering pump with an adjustable stroke volume. Phosphate buffered solution was used, with 1% antibiotic–antimycotic (Invitrogen) for sterilization and was changed every three days to maintain sterilization. The acellular AV was placed into the flow loop bioreactor and subjected to cardiac cycling for 21 days, after which the valve was inspected for deterioration due to bacteria. For mechanical evaluation we utilized planar biaxial mechanical testing to simulate physiologic loading. We used small angle light scattering (SALS) to nondestructively quantify collagen fiber architecture of both the intact and separated FM layers.

Results / Discussion: In general, decellularization with SDS preserved the mechanical properties and the overall microstructure well, with only slight disruptions of the ECM network. Repeating the above mechanical and structural assessments after 21 day time period of flow loop exercising demonstrated initial ECM durability information. The valve conduit preserved the overall morphology and valve leaflets were still able to coapt and support loads. However, areal strain and the maximum stretch ratios in the circumferential and radial directions decreased (Figs. 1a, b). These results may be due to disruption of collagen network and loss of GAGs. The collagen architecture was not affected by the cardiac cycling (Fig. 3). Future work will include increasing the period of time in which the valve is in the bioreactor and assessing the change in mechanical and microstructural properties for the durability evaluation.

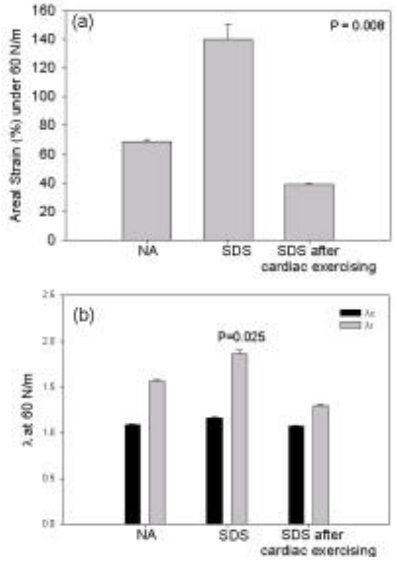

Figure 1 – (a) Net extensibility decreased after cardiac cycling. (b) The amount of stretch in both the circumferential and radial decreased.

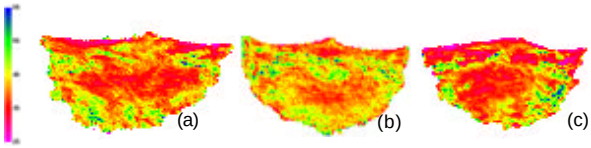

Figure 2 – (a) Collagen structure of the native AV, (b) an AV leaflet decellularized with SDS, and (c) an AV leaflet decellularized with SDS and subjected to cardiac cycling.

Conclusions: A flow loop bioreactor was developed to assess the fundamental ability of the ECM to support valve function in the absence of any cellular maintenance. We demonstrated that the flow loop bioreactor was able to mimic the heart pulsatile flow, apply cardiac cycling on the acellular AV in sterile conditions, and cardiac cycling caused changes in the mechanical and ECM network properties of the decellularized AV.

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References:

[1]Merryman et al. (2004) Advances in Tissue Engineering and Biology of Heart Valve
[2] Booth et al. (2002) J Heart Valve Dis, **11**(4): p. 457-62