

## Heart Valve Engineering: Unseeded Elastomeric Single Leaflets Retain Function and Remodel After Implant In Ovine Pulmonary Outflow Tract

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### OBJECTIVE:

Current materials for heart valve replacement and repair are limited by the inability to grow or remodel. Tissue engineered valves offer the potential to overcome these disadvantages by creating living structures, but is limited by the availability of biocompatible scaffold materials with desirable biomechanical properties. We assessed the in vivo performance of a novel scaffold poly(carbonate urethane) urea (PCUU), fabricated by electrospinning and implanted in the pulmonary outflow tract of sheep.

### METHODS:

PCUU was electrospun into elastomeric sheets of thickness ranging from 120-180  $\mu\text{m}$ . Using cardiopulmonary bypass we replaced the native anterior pulmonary leaflet with an acellular PCUU leaflet. Valve function was evaluated by epicardial echocardiography at implant and explant at weeks 1 (n=3), 3 (n=3), 6 (n=3) and 16 (n=3). Histological, immunohistochemical, molecular imaging analyses and multi-photon imaging were performed on the explanted leaflets. Cells were isolated from the explanted leaflets and characterized in-vitro.

### RESULTS:

Echocardiography demonstrated mobile functioning leaflets, with zero to mild pulmonary regurgitation. Leaflet tissue did not shrink/contract in either radial or circumferential dimensions. Histology showed persistence of scaffold material up to 16 weeks with cellular infiltration throughout the leaflet. Picrosirius red revealed mature collagen deposition along the arterial surface of the construct at 6 and 16 weeks. These findings were corroborated by multi-photon analysis showing highly aligned collagen fibers across the leaflets. Movat pentachrome showed glycosaminoglycan and elastin formation at 16 weeks. Both surfaces of the engineered leaflets were consistently covered with CD31 positive cells. The majority of cells expressed  $\alpha$ -SMA and MMP2. Sub-surface cells have shown co-localization of CD31 and  $\alpha$ -SMA. Two phenotypically and functionally distinct cell types, similar to those of native valves, were identified in-vitro.

### CONCLUSIONS:

These results suggest that: 1) PCUU can be a suitable polymer for valve bioengineering; 2) cell pre-seeding may not be required for tissue formation or remodeling for a functional engineered valve; 3) host cells seem to populate the leaflet and participate in active tissue remodeling 4) An endothelial to mesenchymal transformation process may play a