Three-Dimensional Structural Characterization of Tissue Engineered and Native Ovine Pulmonary Valves

Chad E. Eckert[1], Dane Gerneke[2], Ian LeGrice[2], Danielle Gottlieb[3], John E. Mayer[3], and Michael S. Sacks[1]
[1]University of Pittsburgh, [2]University of Auckland, [3]Children's Hospital Boston

Introduction: Tissue engineered heart valves (TEHV) have received much attention as a valve replacement therapy, offering a variety of potential long-term functional improvements over current options. Early *in vivo* and *in vitro* efforts have produced TEHV showing increasingly equivalent mechanical and structural properties compared to native valves[1]. Despite these advances, a significant gap in the literature exists regarding detailed 3D structural information of TEHV prior to implantation (*in vitro*) and after implantation (explants) as well as that of the native valve. The present work was performed to provide high resolution 3D structural data of implanted TEHV, the native pulmonary valve (PV), and pre-implant scaffold to develop an accurate understanding of developing tissue.

Methods: Three-week dynamically cultured *in vivo* samples ("pre-implant") and three month ovine pulmonary valve (PV) *in vitro* samples ("explant") were produced based on previously-described techniques[1]. Briefly, 1 mm thick 50:50 PGA:PLLA non-woven blend scaffolds were formed into 21mm diameter conduits with three internal leaflet structures and seeded with ~10^9 ovine mesenchymal stem cells. Samples were dynamically cultured for three weeks and were either stored in 10% paraformaldehyde (PF) or implanted into juvenile sheep. After three months, the implants were excised and stored in 10% PF. Native ovine PV ("native") were also excised and stored in 10% PF. Upon arrival, samples were washed in PBS, cut into 5 x 3 mm strips (from the belly region), and soaked in 0.11% picrosirius red for two days. Samples were soaked in Bouin's solution until stain no longer leeched out. A graded alcohol dehydration process was performed, followed by a graded resin pre-embedding process. Following this, samples were resin-embedded into blocks and cured for 24 hours. Blocks were mounted on aluminum plates with the tissue's full-thickness in-plane.

Using the extended volume scanning laser confocal microscope (EV-SLCM) rig at the University of Auckland[2], 1.5 x 1.5 mm full-thickness regions of samples were imaged with an in-plane resolution of 1 pixel/μm in 1 μm Z-direction steps. On average, samples were imaged through a 350-400 μm depth. With the Kr-Ar laser and a rhodamine filter set, the picrosirius red (bound to the collagen and scaffold) glowed brightly. Cell nuclei glowed due to the PF. In total, four explant tissues, two pre-implant tissues, one native tissue, and one scaffold were imaged.

With custom software, images were assembled to create full x-y plane images and stacked to form volumes. Each plane was analyzed using thresholding and gradient-based region-growing algorithms to segment collagen, void space, scaffold, and cellular material. Constituent area and volume fractions were obtained from pixel ratios. Assembled segmented volumes were visualized using Voxx (Indiana University). Custom tracking software traced individual fibers and provided fragmentation data.

Results: Segmentation yielded similar constituent area/volume fractions within each sample group; the native sample showed similar constituent distributions as in other reported techniques, though with an improved resolution. A comparison between pre-implant and explant samples showed collagen volume fraction increasing from 76.6% to 85.9%, with nuclei and scaffold volume fractions decreasing from 2.8% to 0.5% and from 5.9% to 0.8%, respectively. With the native collagen volume fraction measured at 70%, pre-implant and explant samples showed an increase. Compared to the control (unseeded scaffold), pre-implants and explants showed increased scaffold fragmentation.

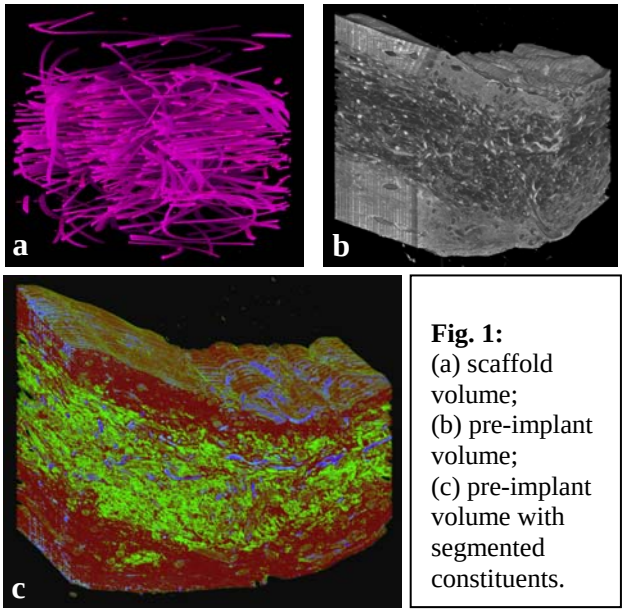

Fig. 1:
(a) scaffold volume;
(b) pre-implant volume;
(c) pre-implant volume with segmented constituents.

Conclusions: This work captured important differences between *in vivo* and *in vitro* TEHV constituents; it is the first known work to utilize EV-SLCM on TEHV. A comparison to the native valve showed structural differences that could impact long-term functionality. Importantly, this work will play a crucial role in future TEHV structural models and highlights the need for improved TEHV design.

References:

[1]Sutherland et al. *Circ*; **111**; 2783-91, 2005.
[2]Sands et al. *Microsc Res Techniq*; **65**; 227-39, 2005.

Acknowledgments:

NIH R01 HL-068816, NSF EAPSI, and AHA Great Rivers Pre-Doctoral Fellowship (C. Eckert, PI)