

TITLE: Structural characterization of fresh human, porcine, and ovine heart valve tissue for engineered valve application.

AUTHORS: P. Caruso ^{1,2}, M. Di Giuseppe¹, L. M. De Mohac¹, F. Cosentino¹, V. Balashov¹, M. Barbuto^{1,2}, M. Pilato³, G. Raffa³, B. Zuccarello², W. R. Wagner^{4,5}, A. D'Amore^{1,4,5}.

¹Fondazione Ri.MED, Via Bandiera 11, Palermo, Italy; ²Università degli Studi di Palermo, Italy; ³Department for the Treatment and Study of Cardiothoracic Diseases and Cardiothoracic Transplantation, IRCCS-ISMETT, Palermo, Italy; ⁴McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, USA; ⁵Department of Bioengineering & Surgery, University of Pittsburgh, Pittsburgh, USA.

ABSTRACT (500 WORDS, 1 figure)

- **Purpose**

The four human heart valves (HVs, Fig 1.1) exhibit substantial differences in their physiology which are reflected in their structural and mechanical properties. HV diseases remain among the most challenging clinical problems in cardiovascular medicine. Human native HVs mechanical and structural properties such as tensile and flexural stiffness, anisotropy, or even simpler factors such as leaflet thickness are largely neglected in the design of commercially available prosthetic devices and often remain poorly characterized, with data being extracted only from animal or cadaveric tissues. This gap in knowledge in valve physiology and prosthetic devices has increasingly made human cardiovascular tissues characterization a priority in biomechanics and tissue engineering.

The purpose of this study is to fully characterize human HVs structure at the organ and tissue scale and to compare these measurements with equivalent data obtained from porcine and ovine tissue. To achieve this aim, fresh human samples were obtained from heart-transplanted patients and tested within 24 hours from the tissue explant. At the organ scale, thickness topology for the aortic, mitral, pulmonary, and tricuspid (AV, MV, PV, TV) HV was measured and compared; at the microscopic scale, quantitative histology and collagen bundle diameter were quantified and compared.

- **Methodology**

Native leaflets were dissected from the valves and leaflet thickness (HVs from n=9 patients) was measured with a dial gauge (Mitutoyo) at fifteen points spanning from the free edge to the belly and commissural regions, full topology was derived using biquintic interpolation. Human HVs leaflets (n=6) were histologically evaluated using: Hematoxylin and Eosin (H&E) and Masson's trichrome staining to assess the cellularity general morphology of the leaflets' extracellular matrix (ECM) and collagen fiber architecture.

- **Results**

Results showed thickness topologies that correlate with the transvalvular pressures acting on the AV, MV, PV, and TV different anatomical locations. All four valves exhibited greater thickness in the coaptation and free edge regions (Fig. 1.2) with the MV showing a higher average thickness compared to the other valves. Collagen quantity was estimated by digital image processing. In order to measure collagen fiber bundle diameter a novel decellularization protocol, specific for HV leaflet, was developed. Next scanning electron microscopy (SEM) analysis was performed on the decellularized leaflets (Fig. 1.3), fiber angle distribution and diameter were quantified using a modified, custom-made algorithm (D'Amore et al. Biomat. 2010) and ImageJ (Fig. 1.4). Human data were finally analyzed and compared to equivalent metrics obtained from porcine and ovine HV tissue.

- **Conclusion**

The results: quantified important differences in morphology of fresh human samples that match with the different anatomical locations of the four valves; provided a comparative analysis of human vs. porcine and ovine valve tissue. The ultimate goal of this study is to establish a thorough database of human HVs structure-

function properties. This resource will contribute to advance the biomimetic-engineered tissue paradigm by providing a comprehensive description of HV structural and biomechanical parameters that can be potentially duplicated by material processing methods currently available or yet to be developed.

