Impact of Age on Bovine Pericardial Composition and Mechanics

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Statement of Purpose: Pericardium has been the versatile biomaterial of choice for cardiothoracic surgeons in the surgical suite since the 1970's (*1-2*). The success of pericardium in cardiovascular applications is attributed to its superior mechanical properties with extended durability in dynamic environments such as the aortic heart valve (*3*). Bovine pericardium is the most widely used material due to its ample availability, large size, and excellent mechanics. Although extensively utilized as a surgical patch and bioprosthetic valve leaflet material, bovine pericardial development, composition and evolution of biomechanics with age remains understudied. The intent of this investigation is to examine the impact of cattle age on macroscopic pericardial features, collagen composition and its relationship with biomechanics. Gross pericardial features such as overall size are critical from a manufacturability standpoint. Thickness has also become an increasingly important parameter for crimped transcatheter heart valves. Finally, the amount and arrangement of molecular components are the critical determinants of the mechanical properties and hence function in a medical device (*4-5*).

Methods: Pericardium was collected from cattle of various ages within 24 hours of sacrifice (15 animals per age group). Thickness was measured with a non-rotating Mitutoyo thickness gauge. Samples of fresh tissue were isolated and stored at -80°C for further analysis. Remaining tissue with fixed with glutaraldehyde by Edwards proprietary methods. To evaluate collagen content of tissues, hydroxyproline content was measured using as previously described (*6*). Briefly, samples were hydrolyzed in 6M HCl at 110° C overnight. Chloramine-T and Ehrlich's reagent were used to oxidize and label hydroxyproline. Standard immunohistochemistry was also performed to identify collagen types 1 and 3. To investigate tissue mechanics, glutaraldehyde fixed samples were rinsed in saline, cut into a standard dog bone shape and subjected to uniaxial tensile load until failure with a MTS Insight 5 in a saline bath at 37°C. Briefly, testing began with ten cycles of preconditioning to 1 MPa followed by loading to failure. Dynamic mechanical analysis (DMA) of loss and storage modulus with varying frequency was performed with a Bose ElectroForce 3200. Samples were cut into strips and subjected to frequency sweep analysis from 1 Hz (physiological) to 30 Hz (above accelerate heart valve wear test frequency).

Results: Bovine pericardial sac size and thickness (Figure 1.) increased significantly in the first six months of age (*1-2*)and then remained relatively constant. This corresponds well with the growth profile of cattle. Interestingly, at 6 months of age pericardium has reached approximate full thickness while the overall animal weight continues to increase. Collagen content of pericardium also increased most rapidly in the same time period and remained elevated with age (Table 1.) This is in agreement with previous literature that cardiovascular structures tend to become more fibrotic with age (*7*).

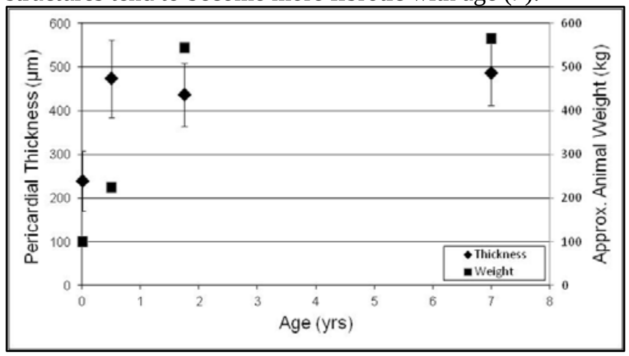

Figure 1.

Age (yrs)	Collagen Content (% dry wt)	UTS (MPa)
0.01	69 ± 14	16.08 ± 9.88
0.50	TBD	20.67 ± 7.68
1.75	94 ± 10	18.246 ± 7.31
7.00	TBD	TBD

Table 1. Collagen content and UTS (± Std Dev)

Collagen type 1 and type 3 profiles also varied with age; type 1 collagen increased while type 3 collagen correspondingly decreased. Interestingly overall static ultimate tensile strength (UTS) did not differ significantly between age groups in spite of differences in collagen content (Table 1.) Dynamic analysis revealed differences in loss modulus and storage as a function of applied frequency. At lower frequencies, loss modulus was elevated and at higher frequencies storage modulus was increased.

Conclusions: In conclusion, animal age less than six months significantly impacts the composition and mechanical properties of pericardium. Most notably, pericardium derived from younger cattle tends to be thinner, have less fibrotic collagen content and more dynamic viscoelastic character. Although differences were detected in molecular composition and DMA experiments, overall UTS was not significantly different. While this may seem inconsequential, it can have important impacts on bioprosthetic valve tissue performance and design. Even from the same species, sourcing tissue from animals of different ages for medical device utilization would require modifications of designs to accommodate different material properties.

References:

1. David TE. J Heart Valve Dis 1998;1:13-18.
2. Li X. Ann Vasc Surg 2011;25:561-568.
3. McClure RS. Ann Thorac Surg 2010;**89**:1410-1416.
4. Naimark WA. Am J Physiol1992;263:H1095-H1106.
5. Stephens EH. Tissue Eng Part A 2010;**16**:867 -878.
6. Chatterjee S. BMC Infect Dis 2005;5:45.
7. Mays PK. Mech Ageing Dev 1988;**45**:203-212.