Characterization of Host Cellular Response to Bioprostheses in Rat Subdermal Implant Calcification Model

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Introduction: Glutaraldehyde (GLUT) treated bioprosthetic heart valves (BHVs) are the only option for valve replacement with a 10-15 years implant life[2]. A major failure mode of GLUT BHVs is calcification.

The exact mechanisms of calcification are poorly understood. There are two hypotheses put forth as to how calcification progresses. Schoen et al. has previously shown that it may be a passive process of mineral aggregation over time attracted by left over cell debris, mainly phospholipids, coupled with the loss of mineralization control [1]. Zilla et al., however, has shown that calcification and structural degradation resulting in heart valve failure may be due to an active host immune response by showing infiltration of active T-cells in both subcutaneous rat implants as well as explanted failed BHVs from humans [2].

However, both hypotheses seem to have shortcomings. Levy has previously shown that GLUT treated BHVs still calcified when subcutaneously implanted in mice devoid of T-lymphocytes [3]. Alternative crosslinking chemistries such as carbodiimide prevents calcification despite the fact that cell debris is not removed [4]. Furthermore, despite most commercial BHV fabrication processes making use of delipidating and chelating agents, BHV calcification remains a predominant failure mode. This leaves much speculation to the true mechanism of calcification. The present study investigated the possible link between the host cellular response and progression of calcification in the rat subcutaneous model, a common validation test for calcification in cardiovascular biomaterials.

Methods: Freshly harvested bovine pericardium (BP) was treated with 0.6% GLUT for 24hrs and then stored in 0.2% GLUT for 6 days. GLUT BP was subcutaneously implanted into juvenile rats for 1, 3, 7, or 30 days. Samples were analyzed for mineralization via inductively coupled plasma mass spectrometry (ICP) and Alizarin red staining. Hematoxylin and Eosin staining (H&E) was utilized to evaluate fibrous capsule thickness, macrophage infiltration, and foreign body giant cell formation. Cellular phenotype infiltration was determined through immunohistochemistry (IHC) for CD80 (M1 macrophage marker) and CD163 (M2 macrophage marker).

Results: Alizarin red histology (Figure 1A) was consistent with ICP data that were indicative of calcification in BP implants. Calcification started in the medial layers of the tissue and radiated outward with evident calcification by Day 7. Host cellular migration, however, showed an initial pronounced infiltration of cells into the implant by Day 3 (Figure 1A Masson's and H&E staining). At later stages, cell infiltration seems disappeared but a moderate to high chronic inflammation response by Day 7 that persisted through Day 30 in surrounding capsule. Calcification seemed to follow once chronic inflammation set in by Day 7. IHC revealed presence of M1 macrophages dominating the inflammation response during Day 1 and 3. By Day 7, M2 macrophages dominated the response with a receded presence of M1 macrophages (Figure 1B). By Day 30, a pronounced presence of lymphocytes was observed around the fibrous capsule of the implant with little M1 and M2 macrophage presence.

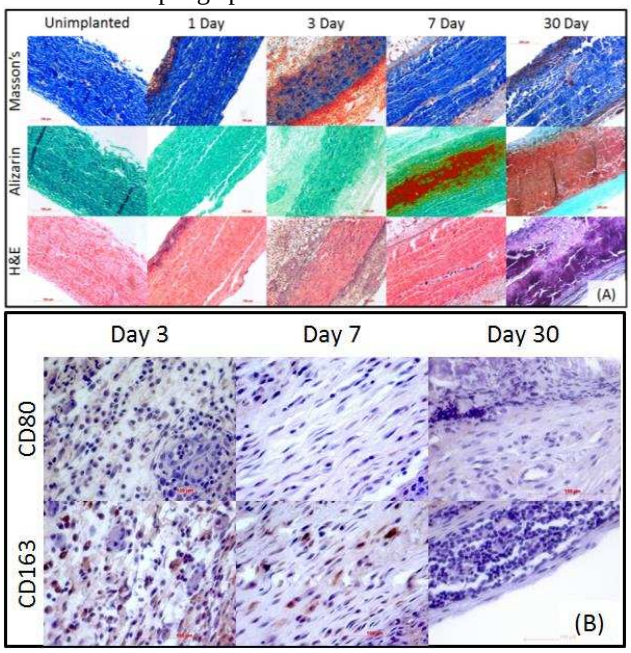

Figure 1. (A) Progression of calcification and host cellular response in GLUT BP explanted from juvenile rat subcutaneous model for 1, 3, 7, or 30 days. (B) Cellular phenotypes in fibrous capsule present at Day 3, 7, or 30. IHC stained M1 cell markers (CD80) and M2 cell markers (CD163) brown with hemotoxylin counterstain.

Conclusions: Previously undemonstrated pervasive cellular infiltration of the implant material was present during the transient inflammation response (Day 1 – 7) with the persistence of intense chronic inflammation through Day 30. Additionally, calcification was observed in the medial part of the implant and radiated outward. Ongoing work involves inhibiting cellular activity, specifically macrophages, and observing the corresponding calcification response as well as looking at the protein expression and host cellular phenotypes within the implant to discern the mechanisms of the calcification. The rat model will be compared to failed GLUT sheep valve explants as well as failed human valves to see if studies hold true across species. Understanding the mechanisms of calcification process and its underlying factors will not only lead to more focused calcification mitigation in heart valve material design but better validation testing for cardiovascular biomaterials.

References: [1] Ann Thorac Surg 2005;79:1072–80; [2] Biomaterials (2008); 29:385–406; [3] Am J Pathol 1986;122:71-82; [4] Girardot. J Heart Valve Disease, 1996. **5**(5): p. 518-25

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