CALCIFICATION OF BIOPROSTHETIC HEART VALVES AND ITS ASSESSMENT*

Jeffrey M. Gross, PhD

*Be not the first by whom the new are tried,
Nor yet the last to lay the old aside.*
—Alexander Pope, *An Essay on Criticism*

This quotation befits the surgical community as it collectively ponders how much technology to embrace with respect to current and future bioprosthetic heart valve designs. It can be argued that current regulatory guidance document governing development of these prostheses adequately safeguards against the potential for catastrophic structural failures decoupled from biologic influence, for example, stent fracture and tissue dehiscence. The current state of preclinical assessment for the “biologic degradation” of the valvular components is not as predictive given the inherent variability and complexity of the human biologic system into which these devices are implanted. Granted, preclinical evaluation in a variety of animal models is used to assess a valve design’s functionality and resistance to calcification; however, it is often difficult to extrapolate such data to the long-term human experience.

The intent of this editorial is to provide a broad overview of what is thought to be the mechanisms of calcification in bioprosthetic heart valves, list the current fixation processes in the literature, and examine how prostheses are evaluated for their antimineralization efficacy. Above all, it is my hope that this editorial will spur thought and challenge industrial, clinical, and academic scientists to seek better techniques for the evaluation of new tissue fixation processes.

Glutaraldehyde-fixed heart valves were first implanted in 1967 after it was determined that glutaraldehyde stabilized collagenous biomaterials.^{1,2} Although valves thus treated were nonthrombogenic, they failed because of calcific degeneration.^{3,4} Much has been written yet little has been proven as to the mechanisms for this calcification. It has been postulated that calcium phosphate crystals containing sodium, magnesium, and carbonate

nucleate due to devitalization of the cells and thus inactivation of the calcium pump.⁵ It has been demonstrated that calcification does initiate in the cells⁶; however, this would appear to be at odds with the fact that cusps stored in glutaraldehyde for more than 1 year no longer calcify,⁷ and valves treated with high concentrations of glutaraldehyde have diminished calcification.⁸ In both cases, cells and their remnants are present in the collagen/elastin matrix. For this reason, work has also focused on decellularization of the porcine collagen/elastin matrix.⁹ Membrane-bound phospholipids have also been associated with calcification nucleation due to alkaline phosphatase hydrolysis.¹⁰ Ethanol has been used to remove phospholipids and mitigate calcification, yet phospholipids have also been removed with chloroform-methanol yielding less than optimum antimineralization efficacy on the basis of rat subcutaneous experiments.¹¹ This would indicate that phospholipid removal alone is not sufficient to mitigate calcification. Lipid extraction can also be performed through tissue processing with detergent compounds such as sodium dodecyl sulfate. Subcutaneous rat studies indicate this approach to be efficacious, although circulatory models have demonstrated mixed results.¹² The primary concern with lipid extraction methods is the potential for collagen matrix degradation if the processes are too aggressive.¹⁰ Free aldehyde within the tissue matrix has been thought to be an initiator for calcification as well. This is supported by studies that demonstrate that aldehyde-binding agents such as alpha-amino oleic acid (AOA; Biomedical Design, Marietta, Ga), L-glutamic acid, and aminodiphosphonate prevent cusp calcification.¹³⁻¹⁶ Yet, post treatment with the amino acid lysine does not prevent cuspal calcification.¹⁷ The foregoing often conflicting information serves to underscore the complexity of the bioprosthetic valve calcification process and emphasizes the multiplicity of pathways by which calcification can initiate.

Because theories for bioprosthetic valve calcification exist without definitive proof, the approaches used by industry and researchers to seek solutions to this clinical failure modality have been based on observed phenomena. Processes have been developed and then assessed to ensure reproducibility, desired tissue biomechanics, desired surface chemistry, matrix stability, and resistance to calcification through a battery of in

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vitro and in vivo tests. A variety of treatments have been used clinically as well as experimentally. They may be broken down into two broad categories: modifications to glutaraldehyde processed tissue and non-glutaraldehyde processes. The modifications to glutaraldehyde processed tissue include but are not limited to treatments with detergents such as sodium dodecyl sulfate and Tween-80 to remove phospholipids,¹² ethanol preincubation to remove phospholipids,¹¹ covalently bound AOA, L-glutamic acid and aminodiphosphonate to bind free aldehydes,¹³⁻¹⁷ and detoxification processes using urazole¹⁸ and homocysteic acid.¹⁹ Nonglutaraldehyde processes include but are not limited to epoxy compounds,²⁰ dye-mediated photo-oxidative reactions including PhotoFix²¹ (Sulzer Carbo-medics, Austin, Tex) and carbodiimide compounds including Ultifix (Biomedical Design).²²

Some of these processes have clinical experience and some do not. Clinical data are difficult to access relative to bioprosthetic valvular calcification, because valves are not usually explanted unless demonstrable valvular performance issues are present that cause stenosis and/or incompetence. This is further confounded by the fact that one must also layer into the analysis the host of patient factors that can bias interpretations. The resulting analysis in general yields a statement as to whether a valve was calcified or not. Although this is valuable, it does not provide insight as to the mechanism for the presence of or lack of calcification. This may change, however. A recent publication by Melina and associates²³ demonstrated a real time in vivo technique using electron beam computed tomography to assess calcification in both aortic leaflets and aortic wall with stentless xenografts and homografts. This represents a first hope at getting the mechanistic question answered noninvasively in human beings.

The root cause for our inability to determine the mechanisms for bioprosthetic heart valve calcification lies in the limitations of the models we use in the pursuit of this knowledge. Current preclinical in vivo techniques for the assessment of bioprosthetic heart valve calcification revolve around analysis of explants from two animal models—the rat subcutaneous model and the juvenile sheep. Values for calcification from the rat subcutaneous model favorably trend with those from the juvenile sheep.^{10,11} Thus, despite being static and lacking blood contact, the rat subcutaneous model can be used to screen processes before committing to the expense of large animal studies. In addition to the standard 8-week implant duration reported by most investigators using the subcutaneous rat model, spatial studies should be undertaken with freshly processed and aged

shelf life valves to ensure any effect noted is in fact present as opposed to simply offset in time.²⁴ The sheep model uses an orthotopic implant. As such, it is blood contacting and integrates the unknown role of cyclic mechanical stress into the determination of calcification resistance. The downfall is that, because of the time and expense associated with conducting such studies, the use of statistically valid sample sizes is prohibitive, rendering results difficult to interpret should biologic variation occur. In general, the predictive capacity of both models suffers from differences between their lipoprotein profile and accelerated calcium metabolism as compared with human beings. Neither incorporates the cardiovascular disease effects and neither accurately reflects the coagulation pathways of human beings. The latter is important, as at present there is no validated thromboembolism model for the assessment of modified and/or new tissue fixation/stabilization technology incorporated into heart valve designs.

Given the limitations of preclinical in vivo bioprosthetic valvular calcification assessment techniques, researchers have long sought to develop in vitro methods. Calcium-rich solutions have been applied to bioprosthetic valves placed into dynamic operation in vitro at a variety of frequencies.²⁵⁻²⁷ The limitation of this technique revolves not around the ability to produce calcification but to correlate the histologic and ultrastructural manifestation of this calcification to that seen in the animal models and in human beings. In this issue of the *Journal*, Pettenazzo and associates describe their in vitro test system and results. They were able to produce extrinsic nodular and laminar calcific deposits. The intrinsic calcification noted on the basis of transmission electron microscopic analysis was associated at the microstructural level with collagen and elastin fibrils. Most notably, however, they saw minimal calcification associated with xenograft cells and cellular debris. This is at odds with evidence reported from biologic models and human explants.^{5,6} This is not to say that such modeling is not valuable. Rather, it underscores the need for additional validations with this technology. Greater sample size is necessary to ensure the model's sensitivity. Spatial studies must be completed to determine apatite initiation sites and their growth characteristics, and processes with data from animal models should be assessed in the present in vitro model to determine whether a correlation exists.

The ultimate answer is that no single model or analysis will contain the necessary predictive power to determine a bioprosthetic heart valve's potential to calcify. The key to the assessment of bioprosthetic valvular cal-

cification lies in the usage of multiple models and the understanding of their limitations. As we collectively move the state of the art forward for the assessment of calcification efficacy with bioprostheses, the patients receiving these devices will ultimately benefit because new technology will have lower risk with greater hope of demonstrating clinical efficacy. Once achieved and borne out by the scrutiny of science, we can then say, on this one issue, that Alexander Pope's first comment should not apply.

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Address for reprints: Jeffrey M. Gross, PhD, Medtronic Heart Valves, 1851 East Deere Ave, Santa Ana, CA 92705 (E-mail: grossj1@medtronic.com).

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