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Mineralization of Short Term Pericardial Cardiac Patch Grafts

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Abstract
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A layer of porous material (pseudoneointima, PNI), consisting mostly of a dense mesh of fibers interspersed with blood cells, was noted to form on the blood contacting surface of the graft. Four distinct sets of mineralization were noted in the retrieved grafts: (1) at the blood contacting surface of the PNI; (2) within the PNI at the junction between layers of PNI with differing densities; (#) near the junction of PNI and pericardium (but in the PNI); and (4) within the pericardium.

In both the PNI and pericardium the mineral was shown by EDX analysis to contain both calcium and phosphorous indicating the mineral to be a calcium phosphate. Mineralization in the PNI differed from that in the pericardium; in the PNI it was deposited in discrete regions and apparently in association with thrombi while in the pericardium it was distributed diffusely within the collagen matrix, which may influence its formation.

Disciplines
Hematology | Medical Cell Biology | Medicine and Health Sciences | Veterinary Medicine | Veterinary Microbiology and Immunobiology

Author(s)
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What relation did that crust have to the silica stones? The answer to this question may give a clue as where the stones come from.
Authors: The encrustation was crystalline and closely applied to the stone.
K.M. Kim: A number of large series of x-ray diffraction of urinary stones did not show such a relatively high incidence of silica calculi in man. Can your findings be attributed to an improved technique or to an actual increase in the incidence of silica calculi?
Authors: It is possible that our findings reflect something about the patient population from which the stones come.
L. Cifuentes-Delatte questions the possibility of simulation of silicon dioxide stones in the absence of other common components such as oxalate, struvite or Ca phosphate and feels that only silicon containing stones which were removed by surgery or the ones which also have other common urinary calculi components can be admitted in the paper.
Authors: We admit to the possibility of simulations of stones in patients. We, however, believe that except for the single case cited, the stones were bona fide urinary calculi predominantly composed of silicon dioxide. We would emphasize one of the purposes of the paper is to state our belief that silicon dioxide stones do exist in the absence of magnesium trisilicate ingestion. We feel that the inclusion of the cases discussed in the paper is valid.
K.M. Kim: Have there been studies on the kinetics of silicon dioxide crystal nucleation and growth in aqueous solutions?
Authors: Studies of this nature may be found in The Chemistry of Silica by Ralph K. Iler, John Wiley and Sons, Inc., 605 Third Avenue, New York City 10158.
K.M. Kim: Is there any known correlation between silica calculi and pneumoconiosis?
Authors: We know of no known correlation.

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MINERALIZATION OF SHORT TERM PERICARDIAL CARDIAC PATCH GRAFTS

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Abstract

Glutaraldehyde fixed patch grafts of bovine pericardium were implanted in myocardial windows in young (3-4 months old) sheep. The samples were retrieved after one to three weeks for study with scanning electron microscopy (SEM) and energy dispersive x-ray microanalysis (EDX).

A layer of porous material (pseudoneointima, PNI), consisting mostly of a dense mesh of fibers interspersed with blood cells, was noted to form on the blood contacting surface of the graft. Four distinct sets of mineralization were noted in the retrieved grafts: (1) at the blood contacting surface of the PNI; (2) within the PNI at the junction between layers of PNI with differing densities; (3) near the junction of PNI and pericardium (but in the PNI); and (4) within the pericardium.

In both the PNI and pericardium the mineral was shown by EDX analysis to contain both calcium and phosphorous indicating the mineral to be a calcium phosphate. Mineralization in the PNI differed from that in the pericardium; in the PNI it was deposited in discrete regions and apparently in association with thrombi while in the pericardium it was distributed diffusely within the collagen matrix, which may influence its formation.

KEY WORDS: Pericardium, scanning electron microscopy, x-ray microanalysis, mineralization, pseudoneointima

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Introduction

Bioprosthetic valves and vascular conduits are used extensively to correct both congenital and acquired circulatory defects. Glutaraldehyde fixed porcine valves and valves constructed of bovine pericardium are commonly utilized; these yield good long term results in adults. In children, however, significant failure rates have been reported to be primarily due to calcification of the prosthesis.[1-4,7,8] Calcification results in altered mechanical properties and functional impairment that often requires replacement of the device.

We report, herein, preliminary results of a study designed to investigate mechanisms underlying mineralization of bioprosthetic materials. Specifically, we examine the formation of mineral in bovine pericardial patch grafts implanted in young sheep. The morphology of the graft and of the mineral deposits are described.

Materials and Methods

Glutaraldehyde fixed patch grafts of bovine pericardium were implanted in myocardial windows in young (3-4 months old) sheep (Fig. 1). Clinical quality processed pericardium was supplied by Shiley Laboratories of Irvine, California. The grafts consisted of pericardium mounted on flanged epoxy rings (Fig. 2) that were surgically implanted in both atrial and ventricular walls using inflow venous occlusion. Post implantation graft retrieval periods ranged between seven days to 21 days.

Patch grafts were removed from the sheep after induction of anesthesia and anticoagulation with heparin. The graft was surgically exposed, the sheep were exsanguinated and the graft with surrounding myocardium was excised. Explants were rinsed in ice cold physiological saline, photographed, fixed in formalin-glutaraldehyde solution[6] and radiographed to locate grossly visible areas of mineralization. Triangular sections containing radiodense material were removed. The sections were rinsed briefly in distilled water and then freeze-dried. Specimens were then mounted on SEM stubs with silver paint and sputter-coated with either silver or gold. A JEOL 35C SEM and a Kevex 7000 energy dispersive x-ray microanalyzer (EDX) system were used for the analyses.

Results

The ultrastructure of bovine pericardium is well documented.[5] It consists of three layers: serosa, fibrosa and epipericardial layers. The thickest of these layers, the fibrosa, is composed of coarse, wavy fibrous connective tissue. No detectable inorganic phosphate or calcium is found in fourteen unimplanted samples of pericardium analyzed by SEM/EDX and chemical techniques.

Samples explanted between one to three weeks (Fig. 3) consist typically of a pseudoneointima (PNI), in the order of one half to two millimeters thick, covering the pericardium (PC). Microscopy reveals that the PNI is composed of a porous fibrous matrix that contains a number of different cell types. Numerous erythrocytes and some leukocytes are seen together with platelets. Backscattered electron imaging (BEI) is used to locate mineral in the explant. Typically, the PNI is seen to contain mineral (arrows); mineral is noted infrequently and in lesser quantities in the pericardium.

Figure 4 is a high power view of mineral deposits in the pericardium. Clumps of micron size particles can be seen in interfibrous spaces. EDX analysis of mineral in the pericardium indicates the presence of both calcium and phosphorous (Fig. 5). Particles in the PNI have a similar elemental composition (not shown).

Fresh thrombus formation is seen on the surface of the spongy PNI. Thrombi are most numerous on the blood contacting surface, but remnants of thrombi are also seen in the interior of the PNI. Figure 6 is a cross section of a PNI sample which has become detached from the pericardium. The arrows point to mineral deposits at the junction of higher and lower density layers of the PNI. The upper surface of the PNI is in contact with blood. Figure 7 is an EDX calcium line scan superimposed on a higher power image of the junctional region. The location of the line scan peak corresponds to the location of the mineral. It should be noted that the base line defines the scanning tract. The preferential accumulation of mineral at the junction of layers of different densities was also noted in one sample at the interface between PNI and pericardium. Figure 7 also indicates that the mineral is deposited in loculi of about 50-200μm diameter. Figure 8 is a magnified image of the edge of the loculus indicated by the arrow in Figure 7. The figure shows mineral (M) within the loculus; the mineral exists as micron-sized particles. Outside of the loculus, the fibrous matrix of the PNI can be seen. Erythrocytes are in close proximity to the mineral and associated with fibrin and platelets. The platelets and red blood cells (indicated by the arrow) appear to be calcified.

Figure 9 is a high power view of the mineral found in the junctional region of the PNI (Fig. 6, arrow). This figure also shows calcified loculi which resemble those shown in Figure 7. Figure 10 is an EDX calcium map of the identical field of view shown in Figure 9. The calcium map correlates spatially with the mineral observed by BEI (Fig. 9). A similar map was obtained for phosphorous (not shown), and it is concluded that the mineral is a calcium phosphate.

Another site of mineralization was sometimes noted on the blood contacting surface of the PNI. These mineral particle aggregates (~20 μm diameter) are shown in Figure 11. EDX analysis of the particles revealed both calcium and phosphorus.

Finally, mineralization was also observed in the pericardium. These deposits could be distinguished from mineral in the PNI by their diffuse nature and lower level of organization. It is possible that this mineral is associated with collagen fibers and its formation is controlled by the local concentration of calcium and phosphate ions. The relationship of pericardial mineral deposition to mineralization of the PNI is unknown. Experiments in progress are aimed at ascertaining whether pericardial and PNI mineralization are temporally related.

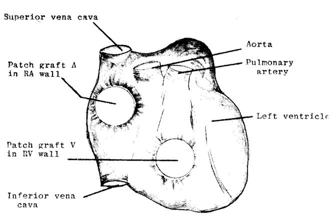

Figure 1. Schematic illustrating placement of patch grafts. Right atrium (A), Right ventricle (V).

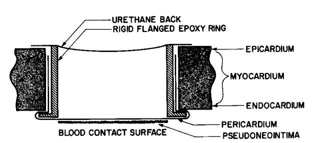

Figure 2. Schematic of saggital section of a patch graft illustrating the flanged epoxy ring backed with a urethane layer and covered with bovine pericardium facing the blood. A PNI is shown attached to the pericardium.

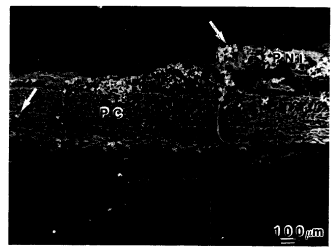

Figure 3. Low power backscattered electron image (BEI) of cross section of a pericardium patch graft after eight days implantation. A PNI is attached to the pericardium. Mineral particles (e.g., indicated by arrows) are more numerous in the PNI than in the pericardium.

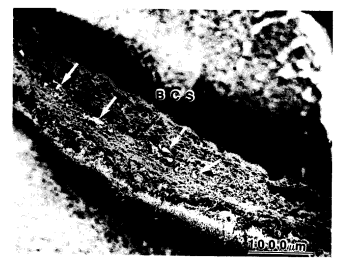

Figure 6. Low power image showing mineral particles confined to the junction between the higher and lower density regions of the PNI. BCS indicated the blood contacting surface. The brightness of the diffuse band at the lower (pericardial) surface is not due to mineral deposition.

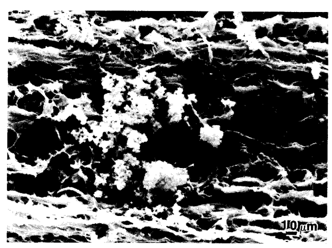

Figure 4. High power BEI view of section of pericardium showing mineral particles (seen in low power in Figure 3).

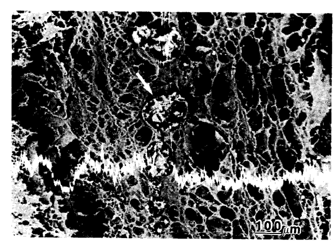

Figure 7. EDX calcium line scan superimposed on a higher power image of the junction. Note that the peak corresponds to the location of mineral deposits and the base line defines the scan line. Note also the loculated organization of the mineral (arrow).

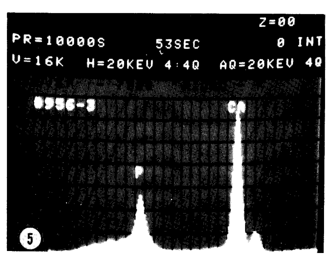

Figure 5. Typical EDX spectrum of a mineral particle (see Fig. 6). The spectrum is typical of particles found both in the pericardium and PNI.

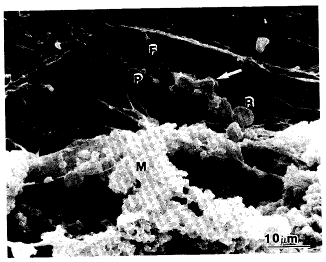

Figure 8. Magnified image of edge of loculus located by arrow in Figure 7. Note mineral (M), erythrocytes (R), fibrin (F), and platelets (P). The arrow points to a microthrombus that indicated calcification by EDX.

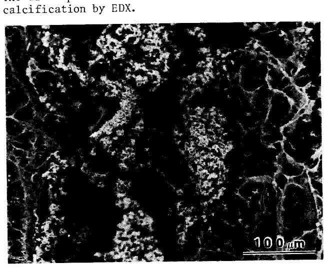

Figure 9. BEI of mineral particles.

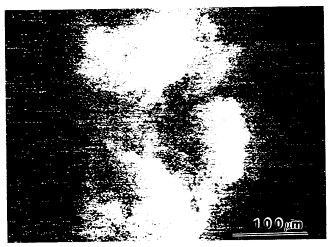

Figure 10. Calcium map of mineral seen in Figure 9.

Figure 11. BEI of the blood contacting surface of the PNI (eleven days). Clumps of mineral can be seen occupying raised loculi.

Discussion and Conclusions

This study clearly shows that soon after implantation of pericardial patch grafts a PNI forms on the blood contacting surface. The PNI is composed of a dense mesh of fibers interspersed with blood cells. At this stage of PNI development, no ingrowth of cells from the surrounding tissue was noted. In some specimens, the PNI was seen to consist of layers of differing density. The most dense zone was adjacent to the pericardium. Fresh thrombi were seen on the blood contacting surface of the PNI; remnants of thrombi could be observed throughout the interior.

A major finding of this study was that mineralization occurred within three weeks of implantation. Mineralization was seen at four distinct sites: (1) at the blood contacting surface of the PNI; (2) at the junction of PNI layers of differing density; (3) near the interface of the pericardium with the PNI (but in the PNI); and (4) within the pericardium. All three sites of PNI mineralization were similar in that the mineral was deposited in association with thrombi. It is likely that the mineralized loculi that are characteristically seen with the PNI are remnants of thrombi. Thus, as the PNI thickens and matures, these thrombi become trapped within the fibrous matrix. From the study of the surface of the PNI, it is clear that thrombi can become mineralized. With maturation of the PNI, additional mineral is accumulated and sequestered within the loculi. Another factor that may influence the development of mineral deposition may be the stratification of the PNI. Thus, diffusion barriers may exist which facilitate accumulation of calcium and phosphate ions at interfacial zones. Moreover, specific blood-borne factors may be preferentially taken up at sites of mineralization. Together, thrombus formation, ion diffusion and macromolecular adsorption may control mineralization in discrete sites in the PNI.

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Discussion with Reviewers

Reviewer I: The authors have found calcific deposits in thrombi on the glutaraldehyde-fixed patch grafts. I would like to ask if they have been able on the basis of transmission electron microscopic observations to identify the components which are associated with the calcific deposits on the thrombi.
Authors: We have not yet performed transmission electron microscopy on our specimens, but we plan to shortly.

Reviewer II: How did you identify thrombus, and what is the difference between the thrombus and the pseudoneointima?
Authors: The thrombus was identified by the presence of erythrocytes and platelets in a fibrous mesh network. The pseudoneointima instead appeared as a spongy, rather organized, dense fibrous structure interspersed with free erythrocytes, leukocytes, macrophages, fibroblast cells and occasional multinucleated giant cells.

Reviewer III: Can you demonstrate a selective accumulation of Ca and P ions in any areas immediately adjacent to a diffusion barrier before mineral deposits are present?
Authors: No. Adjacent to diffusion barriers, both ions were found together in the form of mineral.

Reviewer III: How do you explain what appears to be preferential mineralization of thrombi?
Authors: We have no explanation for this as yet.

Reviewer III: Does the same type of mineralization also occur in older animals, if the time of implantation of the bioprosthetic graft is increased?
Authors: We have not worked with older animals.

Reviewer IV: The authors state that their post-implantation graft retrievals ranged between 7 and 21 days which would suggest that the temporal nature of calcification in bioprosthetic grafts could be described. Yet, the authors do not develop this point; and further, there is no indication as to the time period associated with any of the micrographs. Did the authors gain any insight into the temporal process of calcification through their experiments?
Authors: The degree of mineralization found in the specimens retrieved after 7 to 21 days implantation showed much scatter and no sense of the temporal process of calcification was obtained. Within this short time period no temporal sequence could be ascertained. Longer time periods are presently under study to answer this question.

Reviewer IV: The calcification of pseudoneointima (PNI) has been documented in research on left ventricular assist devices where the PNI forms entirely on an artificial polymer surface. Would the authors compare their results for PNI formation on a bioprosthetic graft surface with those for PNI formation on an artificial polymer surface?
Authors: In addition to pericardial grafts, we did implant polyurethane grafts and found no morphological difference in the pseudoneointima forming in both types of grafts.

Reviewer IV: Would the authors postulate on the initiation events in the calcification process and relate their findings to graft failure in humans?
Authors: It is much too early to do this.

Reviewer I:	V.J. Ferrans
Reviewer II:	K.M. Kim
Reviewer III:	J.M. Riddle
Reviewer IV:	A.C. Nelson