Review

Cardiovascular implant calcification: a survey and update*

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Calcification of cardiovascular prosthetic implants is a common and important problem. This review provides an update based upon the Conference on Cardiovascular Implant Calcification held as part of the 13th World Congress of the International Society for Heart Research, 1989. A variety of cardiovascular prostheses are affected clinically by calcification, including bioprosthetic heart valves, aortic homografts and trileaflet polymeric valve prostheses. In addition, experimental studies have demonstrated calcification of artificial heart devices in ventricular assist systems in long-term calf studies. The pathophysiology of this disease process is incompletely understood. A common element between the various types of cardiovascular implant calcification is the localization of calcific deposits to devitalized cells and membranous debris. Prevention of cardiovascular implant calcification by either biomaterial modifications or regional drug therapy (controlled release) is being investigated.

Keywords: Prostheses, calcification, polymers

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Calcification of cardiovascular implants and diseased cardiovascular tissues is common[1]. By definition, pathological calcification refers to the deposition of calcium phosphates or other calcific salts at sites which would not normally have become mineralized. Calcification of cardiovascular implants often results in clinical device failure due to mechanical dysfunction, vascular obstruction, or embolization of calcific deposits. At present, the pathogenesis of this disease process is incompletely understood and there are no satisfactory preventive measures or therapies to reverse cardiovascular implant calcification. This article, reviews and updates our growing understanding of cardiovascular implant calcification; it was stimulated by presentations and discussions in conjunction with the Conference on Cardiovascular Implant Calcification held as part of the 13th World Congress at the International Society for Heart Research at the University of Michigan, Ann Arbor, in May, 1989.

CLINICALLY IMPORTANT CARDIOVASCULAR CALCIFICATION

Calcific degeneration impairs the usefulness of a variety of clinical and experimental cardiovascular implants. Calcific degeneration of porcine bioprosthetic heart valves is their principal mode of failure, and calcification also impairs the durability of bovine pericardial bioprostheses[1-4]. Valved aortic homografts frequently develop calcification of both the aortic media and to a lesser extent the aortic valve leaflets; these calcific deposits may lead to functional compromise[5,6]. Calcification is also important in causing functional compromise of other types of biological valve replacements, such as human dura mater or fascia lata valves[1-3]. Similarly, the use of trileaflet polymeric valves composed of various synthetic elastomers such as polytetrafluorethylene (Teflon) or polyurethane has been frustrated by calcification[6-9]. Calcification of synthetic vascular grafts occurs occasionally, but is rarely of clinical significance. Calcification of the flexing polymeric bladders of experimental ventricular assist devices and total artificial hearts is frequent and can cause failure of these devices[10-12]; however, mineralization of blood pumps in humans has not yet been reported, despite increasing implant durations.

Dysfunction due to calcific degeneration of bioprosthetic heart valves has been the subject of clinicopathological studies, which in turn have stimulated experimental investigations (see later). Although heart valve bioprostheses fabricated from porcine aortic valve or bovine pericardium frequently fail as a result of calcific degeneration, bioprosthetic heart valve calcification occurs more rapidly in children and young adults than in the elderly[1-4]. As many as 50% of porcine aortic valve bioprostheses can be expected to fail by 10–15 yr post-operatively in adults, but after only 3 yr in children[1-4]. The reasons for age-dependent calcification are uncertain. Furthermore, patients with disordered calcium metabolism, as in renal disease, are also at an increased risk for bioprosthetic valve calcification. Pathological analyses of failed valves reveal dystrophic calcific deposits within the cusps which involve both cellular remnants and collagen fibrils (intrinsic mineralization)[1-4]. Calcific deposits are most extensive at regions of greatest haemodynamic stress, such as the commissures and annular attachments. Although bioprosthetic valve calcification causes either stenosis or regurgitation or both, regurgitation due to tearing at calcific deposits is most frequent, and calcific emboli can also occur. There is no satisfactory means short of re-operation for either treating this disorder once it occurs or preventing it. Valvuloplasty of native heart valves, particularly mitral valves, has met with some success[13-15]. However, balloon dilatation of calcified bioprosthetic valves has also resulted in significant, although incomplete, relief of calcific obstruction in some cases[16-20], but with significant risks of tearing and calcific emboli[18-20]. These complications limit the safe use of this technique primarily to valves on the right side of the heart[18,20]

EXPERIMENTAL MODELS

Calcification of implanted cardiovascular biomaterials has been studied through a variety of animal models (*Table 1*), which are reviewed below. In general, animal models of cardiovascular implant calcification simulate human disease states in both morphological characteristics and pathophysiology. Several unique model systems have become available; in particular, the pathology of mineralization of bioprosthetic heart valve tissue implanted subdermally in rats is comparable pathologically to that of human circulatory retrievals, despite having a different (non-circulatory) implant environment.

Bioprosthetic tissue

Bioprosthetic heart valve calcification has been reproduced experimentally with subdermal implants in rats[21,22], rabbits[23], or mice[24], and orthotopic or conduit-mounted valve replacements in large animals such as sheep[25] or calves[26]. Both circulatory and non-circulatory bioprosthetic implants yield mineralization with morphological features comparable to those of clinical specimens. Investigations of bioprosthetic valve calcification using these animal models demonstrate that this disease process occurs as an interaction of host, implant and mechanical factors. This review emphasizes considerations of host and implant factors. The possible importance of mechanical factors in the mechanism of bioprosthetic calcification is less well understood, and the reader is referred to the limited basic research on this subject by others[27-29].

Younger animals calcify bioprosthetic valve implants more rapidly than do older subjects, thereby mimicking the clinical age dependence[30]. An increased serum phosphate level has been noted in younger animals and may, in part, explain this age effect[30]. The most important implant factor identified is glutaraldehyde fixation[30,31]; porcine aortic valves not pretreated with glutaraldehyde remain uncalcified in the rat subdermal model. Although glutaraldehyde probably serves to stabilize calcifiable structural components,

Table 1 Experimental models of cardiovascular calcification

Type	Implant	Species	Duration	Ref.
Bioprosthetic heart valves	Subdermal	Rat	3 wk	21, 22
	Orthotopic valve	Calf or sheep	3–5 months	25, 26
Polyurethane	Ventricular assist/ artificial heart	Calf	3–5 months	35
	Trileaflet orthotopic valve	Calf or sheep	3–5 months	9, 35
Aortic homografts	Subdermal	Rat	3 wk	40
	Vascular graft	Rat	3 wk	38
	Vascular graft	Dog	5 months	42
	Vascular graft	Sheep	5 months	41

other mechanisms of glutaraldehyde-potentiated mineralization may also exist.

The earliest experimental calcific deposits in rat subdermal implants of porcine bioprosthetic heart valve tissue have been noted in the cells devitalized by glutaraldehyde pretreatment within the cusps with later involvement of collagen fibrils (i.e. intrinsic mineralization)[21, 30]. In pericardial tissue, deposition of calcium phosphates also initially involves cell remnants followed by collagen[21]. The kinetics of mineralization of these materials are similar. Studies using a new high-resolution, high-sensitivity, transmission electron microscopy technique (electron energy loss spectroscopy (EELS)) confirm that the devitalized cells of bioprosthetic heart valve tissue are rich in phosphorous and that during the initial 48 h of a rat subdermal implant, influx of calcium leads to the formation of calcium phosphates at these sites[32]. Late involvement of collagen could be due to either extension of calcific deposits from the initial cell-oriented mineral nuclei, or to an independent extracellular matrix mineralization mechanism. As calcification proceeds, initial calcium phosphate deposits grow and the number of nuclei increases. Eventually, macroscopic calcific deposits coalesce to form nodules, which dissect between tissue planes, often ulcerating and grossly deforming the valve structure. This morphology is similar to that of clinically expanded, failed valves.

Morphological examination of circulatory explants of bioprosthetic heart valves from sheep[25] and calves[26] reveals morphology comparable to that of both clinical explants and material derived from the subdermal model. However, it has not been possible as yet to study the early time-course of bioprosthetic heart valve calcification in a circulatory model. These circulatory studies are expensive, thereby restricting the number of specimens available, and the specimens are difficult to retrieve, limiting the use of sophisticated morphological techniques. Other pathological observations noted in clinical and experimental circulatory explants are not necessarily of relevance to the pathogenesis of clinical bioprosthetic heart valve calcification. For example, extrinsic (surface oriented) calcific deposits occasionally occur on the surface of bioprosthetic heart valve leaflets in sheep explants. These extrinsic deposits are found to occur in either mural thrombotic deposits or the vegetations of infective endocarditis, or fibrous tissue overgrowth, as occasionally noted clinically. Furthermore, insudation of blood into the cusps (cuspal haematoma) has also been noted in both experimental and clinical circulatory explants, although not in direct association with calcification[33]. Cartilage formation within valve leaflets has also been rarely reported in bioprosthetic heart valves explanted from sheep mitral valve replacements; however, this has not been described in either subdermal explants or clinical valves[34].

Polymer calcification: ventricular assist devices (or artificial hearts) and trileaflet valves

Polymer (i.e. polyurethane) calcification in blood pumps and trileaflet valves occurs with a common pathophysiology in both types of implants[7-12, 35-37]. Calcification typically occurs initially in the flexing portion of the blood pumps in experimental ventricular assist or total heart systems, particularly along the lines of highest flexion stress, but regardless of the surface material used[10-12]. Scanning electron microscopy demonstrates that early calcifying lesions of the polyurethane blood-contacting surface are composed of irregular and slightly elevated plaque-like areas[8, 10, 12, 37]. Two hypotheses are proposed for the mechanism of polyurethane calcification: (1) direct adsorption of calcium and phosphate by the polymer surface with subsequent subsurface crystallization[10], and (2) initiation of calcification in adherent degraded cellular components (e.g. erythrocytes, inflammatory cells, platelets) on the surface[12]. Animal model studies of this type of cardiovascular calcification, usually involving calves or sheep, yield considerable variability in calcification within and between studies[9-12, 35-37].

In experimental ventricular assist devices implanted in calves, mineral deposition occurs both on the blood-contacting surface and within polyurethane bladder conduit walls[10-12, 35]. Devitalized cells and related debris have been demonstrated in association with extrinsic calcific lesions. Localization of left ventricular assist and total heart device calcification to areas of highest stress may be caused by locally intense haemodynamic forces leading to cell trauma, with further blood cells and platelet deposition[12, 37]. Calcification may also be associated with thrombus deposited on roughened Dacron–polyurethane composite blood-contacting surfaces, designed to promote formation of a uniform adherent thrombus (pseudointima)[10]. Calcification affecting irregular polyurethane blood-contacting surfaces may be primarily due to entrapped cellular elements within fibrin thrombi. Textured polyurethane blood-contacting surfaces in cardiac assist devices seeded with fetal calf fibroblasts before device implantation, have reduced thrombus-associated calcification[35]. Cell seeding may achieve this effect by fostering a uniform fibrotic surface which for unknown reasons, is associated with minimal calcific deposits.

Calcifications of polymeric trileaflet valves and blood pumps share many features. Calcification of trileaflet polymer valves in sheep and calf studies is typically observed at locations associated with more mechanical activity along valve leaflet commissures and on leaflet surfaces along flexure lines[8, 36]. Furthermore, the calcific deposits are discernible not only on the polyurethane leaflet surface, but are also associated with deposits of subsurface origin[37].

Aortic homografts

Both rat subdermal and circulatory (rat, sheep and dog) models of aortic homograft calcification have been investigated. Limited pathological studies in these investigations demonstrate prominent calcification of the aortic wall and, to a lesser extent the valve leaflets[38-42], comparable to clinical specimens[5, 6, 43, 44]. The observed aortic wall mineralization involves calcification of elastin as a prominent feature. A recent series of rat aortic homograft implant studies have further demonstrated intrinsic calcification of the aortic homograft valve leaflets in rat circulatory studies[38]. This investigation also suggested an immunological basis for the calcification, demonstrating significantly more severe valvar calcification in homografts to transgeneic rats compared with syngeneic rats[38].

MECHANISMS OF CARDIOVASCULAR CALCIFICATION

The pathophysiology of cardiovascular implant calcification is complex and incompletely understood. However, some mechanistic factors (*Table 2*) are shared by the various types of cardiovascular implant calcification.

Table 2 Mechanistic factors in cardiovascular implant calcification

Factor	Ref.
Membrane and cell fragments	45, 46
Cell death or dysfunction	47
Alkaline phosphatase	48
Acidic phospholipids	50, 51
Structural proteins: collagen and elastin	52, 53
Mineral phase transitions	54, 55
Calcium-binding proteins	56-61
Extracellular matrix	45
Endogenous inhibitors	72

Role of cells and membranes

Chief among the common elements in the various types of cardiovascular implant calcification, is the presence within the calcifiable substrate and early lesions of devitalized cells, cellular debris, and subcellular vesicle-like organelles[45]. It has been hypothesized that these various cell-derived components initiate calcification in direct analogy to the matrix vesicles of endochondral skeletal mineralization[46]. Matrix vesicles, which are membranous structures derived from the surfaces of hypertrophic chondrocytes, are thought to initiate calcification at the mineralization front by focally concentrating calcium within their already phosphate-rich structures[46, 47]. These vesicles have high levels of alkaline-phosphatase[45-47] and have the ability to concentrate calcium and increase phosphorus during mineralization. Matrix vesicle-like structures have been shown to occur in human calcific aortic stenosis[45], atherosclerotic plaque[45], and may be at least in part comparable to the early cell-oriented calcifications noted in human[47] and experimental bioprosthetic heart valves[21, 22, 30].

Calcification of cells and cell fragments is due to a distortion of normal physiology. Normally, living mammalian cells maintain an intracellular calcium concentration which is approximately one ten-thousandth that of extracellular levels[1-3]. In physiological states, despite passive entry of calcium into cells through several types of calcium channels, this steep gradient is maintained by the outward pumping activity of several ATP-dependent calcium-ion exchange systems and intracellular calcium buffering mechanisms[1-3]. Cell death (necrosis) or dysfunction of diverse causes impairs the normal ability to expel intracellular calcium, while passive influx could not only continue to take place, but also would be accelerated due to membrane injury. Thus, cell necrosis or damage (including damage due to fixation with glutaraldehyde) causes both increased calcium influx (by not restricting calcium flow to specific channels) and decreased efflux (by impairing calcium exclusion mechanisms). The membranes of the cell serve as sites, rich in phosphorus, capable of reacting with exogenous calcium, in analogy to matrix vesicles. Thus, a net calcium influx, perhaps coupled with the activity of alkaline phosphatase, or other intracellular phosphatases leading to the hydrolysis of phosphoesters, is very likely the initiating mechanism of calcium phosphate crystallization in cardiovascular implants.

Recently, alkaline phosphatase has been demonstrated histochemically within and on the surface membranes of devitalized cells of bovine pericardium both before and after glutaraldehyde fixation[48]. Thus, alkaline phosphatase is not only intrinsic to this type of cardiovascular implant material but also is located at the sites where initial calcification occurs. Alkaline phosphatase has been hypothesized to facilitate the initiation of bone mineralization both by the hydrolysis of phosphoesters, thereby raising regional phosphate concentrations, and by the hydrolysis of inhibitors of calcification, such as pyrophosphate. Recent investigations of the disease hypophosphatasia highlight the importance of alkaline phosphatase in skeletal mineralization[49]. The lethal infantile form of hypophosphatasia is characterized by skeletal hypomineralization and calcium imbalance, with absence of circulating bone-type alkaline phosphatase activity, is due to a single base substitution in the genome for alkaline phosphatase[49]. This missense mutation results in the synthesis of a dysfunctional enzyme molecule, thereby leading to the full-blown clinical syndrome.

Role of phospholipids

Acidic phospholipids such as phosphatidyl inositol and phosphatidyl serine are present at high levels in cells at the mineralizing front in bone and in pathological calcifications[50, 51]. However, phospholipids and phosphoproteins form a nucleation complex with calcium and phosphate, which may initiate bone mineralization; such calcium–phospholipid–phosphate complexes have been demonstrated in pathological calcification[50, 51]. Detergent pretreatment of bioprosthetic heart valves, shown to inhibit mineralization (see later), likely acts by the detergent-mediated extraction of phospholipids and other proteolipids, which takes place under these conditions.

Role of collagen and elastin

Structural protein mineralization occurs in both bioprosthetic heart valve and aortic homograft calcification. In animal model studies[21, 22, 30] as well as clinical retrievals of bioprosthetic heart valves[1-4], collagen-oriented calcification is usually noted to occur significantly later and perhaps secondary to cell-oriented calcific deposits. Calcification of collagen is prominent following long-term implantation. However, subdermal implants of Type I collagen sponges in rats also calcify[52] in the absence of cells devitalized by glutaraldehyde and this observation suggests that collagen calcification may occur independently of cell-oriented mineralization.

Elastin (in addition to cell- and collagen-associated) calcification is characteristically observed in the media of aortic homografts in rat subdermal explants, dog right ventricle to pulmonary artery grafts, and human clinical specimens[38-44]. Although limited pathological studies of aortic homograft calcification have been carried out, it is apparent that both cell-oriented and elastin calcification occur and that these phenomena may be mechanistically related[40, 53].

The calcium phosphate mineral phase

Poorly crystalline apatitic mineral resembling hydroxyapatite, the predominant mineral phase of bone, is present in a number of different cardiovascular calcifications[31, 54, 55]. However, other investigations have indicated less mature mineral phases, and thus suggest an orderly sequence leading to hydroxyapatite formation. In particular, octacalcium phosphate has been hypothesized to be a possible precursor to more mature mineral phases in a variety of clinical cardiovascular calcific deposits, including atherosclerotic plaque bioprosthetic valve calcification. Thus, calcium phosphate deposition proceeds from the formation of amorphous calcium phosphate, perhaps initiated by calcium phospholipid phosphate complexation (see earlier), to the

formation of more complex phases, including either brushite or octacalcium phosphate or both, finally resulting in mature hydroxyapatite deposition.

Calcium-binding proteins containing either aminomalonic acid or γ-carboxyglutamic acid

Calcium-binding proteins containing γ-carboxyglutamic acid (Gla) result from post-translational carboxylation of specific glutamic acid residues by several protein-specific vitamin K-dependent carboxylases[56-58]. This class of calcium-binding proteins includes the vitamin K-dependent coagulation factors and an as yet emerging class of Gla-containing bone proteins. The physiological functions of the Gla-containing bone proteins are not known. Gla proteins are present in virtually all types of cardiovascular calcification studied thus far, but are not detectable in non-mineralized vascular tissues. Indeed osteocalcin, the most abundant bone Gla protein, is present in calcific atherosclerotic plaque, native calcific valvular aortic stenosis and calcified bioprosthetic heart valves[59]. Although the role in cardiovascular calcification, if any, of the Gla-containing proteins is uncertain, it has been shown that inhibition of Gla-containing protein synthesis with vitamin K antagonism results in a significant reduction in the Gla protein levels in cardiovascular tissue, but does not deter pathological calcification[30].

The past decade has also yielded a growing interest in the possibility that other calcium-binding proteins are important in the formation of cardiovascular calcification. Calcium-binding proteins containing aminomalonic acid have been shown to be present in calcified cardiovascular tissue, but are not present in non-mineralized samples[60,61]. Aminomalonic acid is thought to be biosynthesized as a result of either an as yet unknown carboxylase or through the mediation of oxygen free radical modifications of glycine or alanine residues. The pathophysiological role of proteins containing aminomalonic acid is as yet unknown.

PREVENTION OF CARDIOVASCULAR CALCIFICATION

No effective therapy presently exists for removal of established cardiovascular calcific deposits and no clinically useful preventive measures are available. However, several preventive strategies (*Table 3*) are effective experimentally (see below) for inhibiting bioprosthetic valve tissue calcification; these approaches may ultimately be of clinical importance. Nevertheless, criteria need to be considered to evaluate both efficacy and safety for each of the approaches to be considered (*Table 4*).

Table 3 Prevention of cardiovascular implant calcification

Agent	Application	Mechanism	Ref.
Ethanehydroxy-diphosphonate (controlled release)	Bioprostheses	Restrict mineral phase	65, 66
Phosphocitrate	Bioprostheses	Restore endogenous inhibitor	72
Aminodiphosphonate pretreatment	Aortic homografts Bioprostheses	Restrict mineral phase	67, 68
Detergent pretreatment	Bioprostheses heart valves	Phospholipid extraction	62, 63
Protamine pretreatment	Bioprostheses heart valves	Charge modification	73
Al^{3+} or Fe^{3+} pretreatment	Bioprostheses heart valves	Restrict mineral phase	32, 69

Table 4 Bioprosthetic heart valve antimineralization treatments: criteria for efficacy and safety

Efficacy
Determination of specific mechanisms of action
Effectively inhibits calcification
Dose-response relationship established
Effect not lost or inactivated during function
Neutralized component not reaccumulated
Does not merely delay onset of mineralization
Valve has adequate performance (i.e. unimpaired durability and hydrodynamics)

Safety: Does not
Cause adverse blood-surface interactions
- haemolysis
- platelet adhesion
- complement activation
- inflammatory cell activation
- binding of vital serum factors

Enhance local or systemic inflammation
- foreign body reaction
- immunological reactivity
- hypersensitivity

Cause local or systemic toxicity

Detergents

Detergent pretreatment of bioprosthetic heart valve tissue inhibits subdermal bioprosthetic leaflet calcification and delays the onset of circulatory deposits in some but not all studies[62,63]. The mechanism of action of detergent mitigation of calcification is unknown, but it may be due to either the extraction of membrane lipids, net surface charge modification, or removal of endogenous alkaline phosphatase. Detergents have been widely used to extract and purify alkaline phosphatase from tissue and thus detergents probably remove the intrinsic enzyme from detergent-incubated bioprosthetic tissue. Absorption of acidic phospholipids or extrinsic alkaline phosphatase or both from blood during *in vivo* function of an implant could cause reversal of detergent-mediated inhibition of calcification. Detergent pretreatment has been more effective for preventing experimental calcification of porcine aortic bioprostheses than pericardial bioprostheses[63]. The reasons for this are not known; progression of experimental bioprosthetic valve calcification is virtually identical in both types of biomaterials. At present, detergent-pretreated bioprosthetic valves are in clinical trials.

Diphosphonates

Diphosphonates are synthetic analogues of pyrophosphate, and are potent calcification inhibitors[64]. Their mechanism of action is thought to be due to inhibition of hydroxyapatite crystal growth, although there is evidence that diphosphonates may also act by inhibiting alkaline phosphatase[64]. By several modes of administration, these compounds inhibit experimental bioprosthetic valve calcification. In the rat subdermal model, systemic ethanehydroxydiphosphonate (EHDP) administration, in concentrations sufficient to inhibit markedly bioprosthetic calcification, results in severe inhibition of bone growth[65]. However, adverse effects are avoided by using low-dose diphosphonate administration with polymeric drug carriers[66] localized to the valves in both the rat subdermal model and in sheep tricuspid valve replacements.

Aminopropanehydroxydiphosphonate binds covalently to residual aldehyde functional groups of glutaraldehyde-pretreated bioprosthetic tissue via an amino-aldehyde reaction, and significantly diminishes calcification in rat

subdermal implants[67,68]. However, circulatory studies of aminodiphosphonate-pretreated bioprosthetic valves have failed to show inhibition of calcification, and this result is most likely due to the instability of the glutaraldehyde binding to the bioprosthetic tissue as well as unstable aminodiphosphonate binding to the residual aldehyde functions.

Aluminium chloride and ferric chloride

Observations that patients with renal failure treated with haemodialysis develop severe osteomalacia after trace-level exposure to Al^{3+} suggested the strategy of pretreating bioprosthetic cusps with $AlCl_3$ to prevent calcification[68-70]. The mechanism of action by which Al^{3+} causes osteomalacia has been thought to result from an inhibition of hydroxyapatite crystal growth[70]. $FeCl_3$ has also been associated, although in rare instances, with osteomalacia in cases of iron overload in renal insufficiency[71]. Recent studies demonstrate that preincubation of bioprosthetic tissue in $FeCl_3$ or $AlCl_3$ prevents implant calcification without adverse effects[32]. Morphological studies of bioprosthetic tissue pretreated with aluminum chloride reveal localization of both Al^{3+} and Fe^{3+} to devitalized cells[32,59]. Thus, Al^{3+} and, perhaps Fe^{3+}, may inhibit the membrane-linked calcification events at these important sites, which are the loci of the initial calcific deposits.

Other inhibitors

Phosphocitrate, an endogenous inhibitor of calcification, has been demonstrated to inhibit bioprosthetic tissue calcification (bovine pericardial) in the rat subdermal model[72]. However, this was only possible with local controlled release administration; systemic administration was not effective. The mechanism of action of phosphocitrate is incompletely understood. It is thought to act either by inhibiting hydroxyapatite nucleation or alkaline phosphatase or both[72]. Protamine sulphate pretreatment of bioprosthetic tissue is another relatively recent and promising approach for preventing calcification as demonstrated by rat subdermal studies[73]. Covalent bonding of protamine to bioprosthetic tissue is hypothesized to inhibit implant calcification due to its imparting a net positive surface charge, thereby repelling calcium.

FUTURE DIRECTIONS

Emerging areas of research may play an important role in the strategies necessary to understand and control cardiovascular implant calcification (*Table 5*). There is a need for calcification-resistant biomaterials, both tissue-derived biomaterials as well as synthetic elastomers such as polyurethane. In addition, preventive drug therapy may focus increasingly on implantable controlled release systems and immobilized drugs, since both of these approaches offer the possibility of optimizing local drug concentrations, while eliminating the possibility of side-effects which may be associated with systemic anticalcification therapy. If these preventive strategies come to fruition, the use of a wide variety of cardiovascular implants, particularly bioprosthetic valves, could vastly increase.

Table 5 Future directions

- Calcification-resistant biomaterials
- Preventive therapy: controlled release; immobilized drugs
- Treatment of developing and established calcifications: targeted pharmacological agents

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