CHAPTER 3

Degradation of Materials in the Biological Environment
3.4 PATHOLOGICAL CALCIFICATION OF BIOMATERIALS

- Biomaterials and prosthetic devices, particularly those used in the circulatory system, but also at other sites, may be affected by the formation of nodular deposits of calcium phosphate or other calcium-containing compounds, a process known as calcification or mineralization. *(Ch.6.4: Table 1)*
- In many cases, this causes device failure. Calcification has been encountered in association with both synthetic and biologically derived biomaterials in various clinical and experimental settings, including bioprosthetic or homograft cardiac valve substitutes and vascular replacements, blood pumps used as cardiac assist devices, breast implants, intrauterine contraceptive devices, urinary prostheses, and soft contact lenses.
- Deposition of mineral salts of calcium occurs as a normal process in bones and teeth (physiologic mineralization). Moreover, it is desirable that some implant biomaterials calcify, e.g., osteoinductive materials used for orthopedic or dental applications.
- However, non-skeletal tissues and the biomaterials that comprise other medical devices are not intended to calcify (e.g., heart valves, breast implants), since mineral deposits can interfere with their function.
- Therefore, calcification of these tissues or biomaterials is abnormal or pathologic.
• The mature mineral phase of **biomaterial-related** and other forms of **pathologic calcifications** is a poorly crystalline calcium phosphate known as **apatite**.

• It closely **resembles** calcium hydroxyapatite, the mineral that provides the structural rigidity of bone and has the chemical formula **Ca_{10} (PO_{4})_{6} (HO)_{2}**.

• Indeed, we will see later that many features are shared between biomaterials calcification, other pathologic calcifications on the one hand and normal bone mineralization on the other. **Pathologic calcification** is also common in **native arteries and heart valves**, where it occurs as an important feature of the serious diseases **atherosclerosis** and **degenerative aortic stenosis**, respectively.

• Pathologic calcification is further classified as either **dystrophic** or **metastatic**, depending on its setting. **Dystrophic calcification** is the deposition of calcium salts (usually calcium phosphates) in **damaged or diseased tissues or biomaterials** in individuals with normal calcium metabolism. In contrast, **metastatic calcification** is the deposition of calcium salts in **previously normal tissues** in individuals with **deranged mineral metabolism** (for example, with elevated blood calcium levels).

• The conditions favoring dystrophic and metastatic calcification can act **synergistically**; thus, in the presence of **abnormal mineral metabolism**, calcification associated with **biomaterials or injured tissues** is enhanced.
Moreover, the ability to form bone is physiologically regulated through adjustment of enhancing and inhibiting substances, many of which circulate in the blood.

In young individuals the balance appropriately favors bone formation. However, this same chemical environment favors enhanced calcification of biomaterials in the young.

The cells and extracellular matrix of dead tissues are the principal sites of pathologic calcification.

Calcification of an implant biomaterial can occur deep within the tissue (intrinsic calcification) or at the surface, associated with attached cells and proteins (extrinsic calcification).

An important instance of extrinsic calcification is that associated with tissue heart valve infection (prosthetic valve endocarditis).
The spectrum of pathologic biomaterials and medical device calcification

Heart Valves and Vascular Replacements

• Calcific degeneration of glutaraldehyde-pretreated porcine bioprosthetic heart valves is the most clinically significant dysfunction of a medical device due to biomaterials calcification.

• The predominant pathologic process is intrinsic calcification of the valve cusps, largely initiated in the deeply seated cells and the tissue from which the valve was fabricated and often involving collagen.

• Calcification leads to failure most commonly by causing cuspal tears, less frequently by cuspal stiffening, and rarely by inducing distant emboli.

• Overall, more than half of porcine bioprostheses fail within 12-15 years.

• Calcification is more rapid and aggressive in the young; for example, the rate of failure of bioprostheses is approximately 10% in 10 years in elderly recipients, but is nearly uniform in less than 4 years in most adolescent and preadolescent children.
• Calcification has also complicated the clinical use and experimental investigation of heart valves composed of other tissues (e.g., bovine pericardium) and polymers (e.g., polyurethane).

• In some young individuals with congenital cardiac defects or acquired aortic valve disease, human allograft/homograft aortic (or pulmonary) valves surrounded by a sleeve of aorta (or pulmonary artery) are used.

• Allograft valves are valves that are removed from a person who has died and transplanted to another individual; the tissue is usually cryopreserved but not chemically cross-linked.

• Allograft vascular segments (without a valve) can be used to replace a large blood vessel.

• Whether containing an aortic valve or nonvalved, allograft vascular tissue can undergo severe calcification, particularly in the wall; calcification can lead to allograft valve dysfunction or deterioration.

• Synthetic vascular replacements composed of Dacron or expanded polytetrafluoroethylene (e-PTEE) also calcify in some patients.
Polymeric Bladders in Blood Pumps

- Deposition of calcific crystals on flexing bladder surfaces (which are usually composed of polyurethane) occurs in and may limit the functional longevity of blood pumps used as ventricular assist systems or total artificial hearts.
- Massive deposition of mineral leading to failure has been noted in experimental animals, and a lesser degree of calcification has been encountered following extended human implantation.
- Mineral deposits can result in deterioration of pump or valve performance through loss of pliability or the initiation of tears. Blood pump calcification, regardless of the type of polyurethane used, generally predominates along the flexing margins of the diaphragm, emphasizing the important potentiating role of mechanical factors in this system.
- Calcific deposits associated with blood components can occur either within the adherent layer of deposited proteins and cells (pseudointima) on the blood-contacting surface (extrinsic mineralization) or below the surface (intrinsic calcification).
- In some cases, calcific deposits are associated with microscopic surface defects, either originating during bladder fabrication or resulting from cracking during function.
Breast Implants

• Calcification of silicone gel breast implant capsules occurs as discrete calcified plaques at the interface of the inner fibrous capsule with the implant surface. Capsular calcification has also been encountered with breast implants in patients with silicone envelopes filled with saline.

• Calcification could interfere with effective tumor detection and diagnosis, which could potentially delay treatment, particularly in patients who have breast implants following reconstructive surgery for breast cancer.

• In a study of breast implants removed predominantly for capsular contraction, 16% overall demonstrated calcific deposits, including 26% of implants inserted for 12-20 years and all those ≥23 years.

• Capsular mineralization has also been associated with the Dacron patches used on silicone gel implants in the 1960s and early 1970s to anchor implants to the chest wall in an attempt to prevent implant migration and sagging. Ivalon (polyvinyl alcohol) sponge prostheses, used quite extensively during the 1950s, were also frequently associated with calcification. In Japan, where augmentation mammoplasty was frequently performed using injection of foreign material (liquid paraffin from approximately 1950 until 1964, and primarily liquid-silicone injections thereafter), the incidence of calcification has been much higher. One study showed calcification in 45% of breast augmentations which were done by injection.
Intrauterine Contraceptive Devices

- Intrauterine contraceptive devices (IUDs) are composed of plastic or metal and placed in a woman’s uterus chronically to prevent implantation of a fertilized egg.
- Device dysfunction due to calcific deposits can be manifested as contraceptive failure or device expulsion. For example, accumulation of calcific plaque could prevent the release of the active contraception preventing agent—either ionic copper from copper-containing IUDs or an active agent from hormone-releasing IUD systems.
- Studies of explanted IUDs using transmission and electron microscopy coupled with X-ray microprobe analysis have shown that surface calcium deposition is ubiquitous but variable among patients.
Urinary Prostheses

- **Mineral crusts** form on the surfaces of polymeric prostheses to alleviate urinary obstruction or incontinence. Observed in male and female urethral implants and artificial ureters, this problem can lead to obstruction and device failure.
- The mineral crust consists of either hydroxyapatite or struvite, an ammonium- and magnesium-containing phosphate mineral derived from urine. There is some evidence that encrustation may both result from and predispose to bacterial infection.

Soft Contact Lenses

- **Calcium phosphate deposits** can opacify soft contact lenses, typically composed of poly(2-hydroxyethyl methacrylate)(HEMA).
- Growing progressively larger with time, they are virtually impossible to remove without destroying the lens.
- Calcium from tear fluid is considered to be the source of the deposits found on HEMA contact lenses, and calcification may be potentiated in patients with systemic and ocular conditions associated with elevated tear calcium levels.
ASSESSMENT OF BIOMATERIALS CALCIFICATION

• Calcific deposits are investigated using morphologic and chemical techniques.
• **Morphologic techniques** facilitate detection and characterization of the microscopic and ultrastructural sites and distribution of the calcific deposits and their relationship to tissue or biomaterials structural details.
• Such analyses yield important **qualitative** (but not quantitative) **information**. In contrast, **chemical techniques**, which require destruction of the tissue specimen, permit both **identification** and **quantitation** of bulk elemental composition and determination of crystalline mineral phases.
• However, such techniques generally **cannot** relate the **location** of the mineral to the details of the underlying tissue structure.
• The most comprehensive studies characterize both morphologic and chemical aspects of calcification.
Morphologic Evaluation

- Morphologic assessment of calcification is done by means of several readily available and well-established techniques that range from macroscopic (gross) examination and radiographs (X-rays) of explanted prostheses to sophisticated electron energy loss spectroscopy.
- Each technique has advantages and limitations; several techniques are often used in combination to obtain an understanding of the structure, composition, and mechanism of each type of calcification.
- Careful visual examination of the specimen, often under a dissecting (low power) microscope, and radiography assess distribution of mineral in explanted bioprosthetic heart valves and ventricular assist systems.
- Specimen radiography typically involves placing the explanted prosthesis on an X-ray film plate and exposing to an X-ray beam in a special device used for small samples (e.g., we use the Faxitron, Hewlett-Packard, McMinnville, CA, with an energy level of 39 keV for 1 min for valves). Deposits of mineral appear as bright densities that have locally blocked the beam from exposing the film.
- Light microscopy of calcified tissues is widely used identification of mineral is facilitated through the use of either calcium or phosphorus-specific stains, such as alizarin red (which stains calcium) or von Kossa (which stains phosphates).
• These **histologic stains** are readily available, can be easily applied to tissue sections embedded in either paraffin or plastic, and are most useful for confirming and characterizing suspected calcified areas which have been noted by routine **hematoxylin and eosin staining** techniques.

• **Sectioning** of calcified tissue that has been **embedded in paraffin** often leads to considerable artifact due to fragmentation; embedding of tissue with calcific deposits in **glycolmethacrylate polymer** yields superior section quality.

• **Electron microscopic** techniques, which involve the bombardment of the specimen with a highly focused electron beam in a vacuum, have much to offer in the determination of early sites of calcific deposits.

• In transmission electron microscopy (TEM), the beam traverses an **ultra-thin section (0.05 µm)**; observation of the **ultrastructure** (submicron tissue features) of calcification by TEM facilitates the understanding of the **mechanisms** by which calcific crystals form.

• Scanning electron microscopy (SEM) images the specimen **surface**, and can be coupled with elemental localization by **energy-dispersive X-ray analysis** (EDXA), allowing a **semiquantitative** evaluation of the local progression of calcium and phosphate deposition in a site-specific manner.
• Electron energy loss spectroscopy (EELS) couples transmission electron microscopy with highly sensitive elemental analyses to provide a most powerful localization of incipient nucleation sites and early mineralization.

• In general, the more highly sensitive and sophisticated morphologic techniques require more demanding and expensive preparation of specimens to avoid unwanted artifacts.

• Forethought about and careful planning of specimen handling optimizes the yield provided by the array of available techniques, and allows multiple approaches to be used on a single specimen.
Chemical Assessment

- **Quantitation** of calcium and phosphorus in biomaterial calcifications permits characterization of the **progression of deposition**, comparison of severity of deposition among specimens and determination of the effectiveness of preventive measures.
- However, such techniques **destroy the configuration** of the specimen during preparation.
- **Calcium** has been quantitated by **atomic absorption spectroscopy** of acid hydrolyzed or ashed samples.
- Recently, highly sensitive multielement integrated coupled plasma (ICP) instrumentation has become available. This permits **high-resolution quantitation** of not only calcium, but **other relevant elements**, such as aluminum and ferric ion in the same sample.
- **Phosphorus** is usually quantitated as phosphate, using a **molybdate complexation** technique with spectrophotometric detection.
- The **crystalline form** of calcium phosphate (mineral phase) can be determined by **X-ray diffraction**. **Carbonate-containing mineral phases** may also be analyzed by **infrared spectroscopy**.
PATHOPHYSIOLOGY

General Considerations

- The **determinants of biomaterial mineralization** include factors related to (1) host metabolism, (2) implant structure and chemistry, and (3) mechanical factors.
- Natural **cofactors and inhibitors** may also play a role (see below).
- The **most important** host metabolic factor is related to **young age**, with more rapid calcification taking place in immature patients or experimental animals.
- Although the relationship is well established, the **mechanisms** accounting for this effect are **uncertain**.
- The **structural elements** of the biomaterial and their modification by processing may be important implant factors for **bioprosthetic tissue** is the pretreatment with glutaraldehyde, done to preserve the tissue. It has been hypothesized that the cross-linking agent glutaraldehyde stabilizes and perhaps modifies **phosphorus-rich calcifiable structures** in the bioprosthetic tissue.
• These sites seem to be capable of mineralization upon implantation when exposed to the comparatively high calcium levels of extracellular fluid.

• Calcification of the two principal types of biomaterials used in bioprostheses—glutaraldehyde-pretreated porcine aortic valves or glutaraldehyde-pretreated bovine pericardium—is similar in extent morphology, and mechanisms.

• In both physiologic and pathologic calcification, nucleation of apatite crystals is more difficult than subsequent growth, which occurs relatively easily since the concentrations of both calcium and phosphorus in blood and extracellular fluid are near saturation.
Regulation of Pathologic Calcification

- Calcification has typically been considered a passive, unregulated, and degenerative process.
- However, the observations of matrix vesicles, hydroxyapatite mineral, and bone-related morphogenetic and noncollagenous proteins in pathological calcifications have suggested that the mechanisms responsible for pathologic calcification may be regulated, similarly to normal mineralization of bone and other hard tissues.
- In normal blood vessels and valves, inhibitory mechanisms outweigh procalcification inductive mechanisms; in contrast, in bone and pathologic tissues, inductive mechanisms dominate.
- In the process of normal bone calcification, the growth of apatite crystals is regulated by several noncollagenous matrix proteins including (1) osteopontin, an acidic calcium-binding phosphoprotein with high affinity to hydroxyapatite that is abundant in foci of dystrophic calcification; (2) osteonectin, and (3) osteocalcin, and other γ-carboxyglutamic acid (GLA)-containing proteins, such as matrix GLA protein (MGP).
- Naturally occurring inhibitors to crystal nucleation and growth may also play a role in biomaterial and other cardiovascular calcification.
Specific inhibitors in this context include osteopontin and high-density lipoprotein (HDL, the “good” cholesterol). An active area of research is the role in pathological mineralization of naturally-occurring mineralization cofactors, such as inorganic phosphate bone morphogenetic protein and proinflammatory lipids and other substances (e.g., cytokines) as well as inhibitors.

The noncollagenous proteins osteopontin, TGF-beta 1, and tenascin-C involved in bone matrix formation and tissue remodeling have been demonstrated in clinical calcified bioprosthetic heart valves, natural valves, and atherosclerosis, suggesting that they play a regulatory role in these forms of pathologic calcification in humans.

Evidence for the active regulation of cardiovascular calcification also derives from tissue culture models of vascular cell calcification, which mimic pathologic vascular calcification in vivo, and genetic studies in mice. For example, osteopontin inhibits and proinflammatory lipids and cytokines enhance the mineralization of smooth muscle cell cultures.

In transgenic mouse models, in which the gene for the matrix GLA protein (MGP) was knocked out or the osteopontin gene was inactivated, severe calcification of blood vessels resulted. Moreover, inhibition of matrix remodeling metalloproteinases inhibits calcification of elastin implanted subcutaneously in rats.
Experimental Models for Biomaterials Calcification

- **Animal models** have been developed for the investigation of the calcification of bioprosthetic heart valves, aortic homografts, cardiac assist devices, and trileaflet polymeric valves.
- Experimental models used to investigate the pathophysiology of bioprosthetic tissue calcification and as a preclinical screen of new or modified materials and design configurations include tricuspid or mitral replacements or conduit-mounted valves in sheep or calves, and isolated tissue (i.e., not in a valve) samples implanted in and around the heart and subcutaneously in mice, rabbits, or rats.
- In both circulatory and noncirculatory models, bioprosthetic tissue calcifies progressively with a morphology similar to that observed in clinical specimens, but with markedly accelerated kinetics.
- **Static in vitro models** of biomaterials calcification have been investigated but have generally nor been useful. However, several groups have used flexing vale models for bioprosthetic and polymeric vale calcification, in which the morphology of the resulting mineralization seems more representative of pathologic calcification that occurs in vivo.
Compared with the several years normally required for calcification of clinical bioprostheses, valve replacements in sheep or calves calcify extensively in 3 to 6 months. However, expense, technical complexity, and stringent housing and management procedures pose important limitations to all the circulatory models using large animals.

In addition, implantation in the heart requires the use of complex procedures such as cardiopulmonary bypass as well as a high level of surgical expertise and postoperative care.

These limitations stimulated the development of subdermal (synonym subcutaneous—under the skin) implant models. In subdermal bioprosthetic implants in rats, rabbits, and mice,

- (1) calcification occurs at a markedly accelerated rate in a morphology comparable to that seen in circulatory explants;
- (2) the model is economical so that many specimens can be studied with a given set of experimental conditions, thereby allowing quantitative characterization and statistical comparisons; and
- (3) specimens are rapidly retrieved from the experimental animals, facilitating the careful manipulation and rapid processing required for detailed and high resolution analyses.
• The subcutaneous model is a technically convenient and economically advantageous vehicle for investigating host and implant determinants and mechanisms of mineralization, as well as for screening potential strategies for its inhibition (anticalcification).

• Promising approaches may be investigated further in a large-animal valve implant model. Large-animal implants as valve replacements are also used
  – (1) to elucidate further the processes accounting for clinical failures,
  – (2) to evaluate the performance of design and biomaterials modifications in valve development studies,
  – (3) to assess the importance of blood/surface interactions, and
  – (4) to provide data required for approval by regulatory agencies.

• Polyurethane calcification has also been studied with subdermal implants in rats. Subcutaneous implants may also be used to investigate calcification of biomaterials intended for clinical use in other anatomic sites, for example, polyhydroxyethylmethacrylate hydrogels used in soft contact lenses.
Pathophysiology of Bioprosthesis Heart Valve Calcification

- Data from valve explants from patients and subdermal and circulatory experiments in animal models using bioprosthetic heart valve tissue have elucidated the pathophysiology of this important clinical problem and enhanced our understanding of pathologic calcification in general.

- The similarities of calcification in the different experimental models and clinical bioprostheses suggest a common pathophysiology, independent of implant site.

- Calcification appears to depend on exposure of a susceptible substrate (often containing phosphorus) to extracellular fluid containing calcium; both mechanical factors and local implant-related or circulating substances may play regulatory roles.

- However, since the morphology and extent of calcification in subcutaneous implants is analogous to that observed in clinical and experimental circulatory implants, despite the lack of the dynamic mechanical activity that occurs in the circulatory environment, it is clear that dynamic stress promotes but is not prerequisite for calcification of bioprosthetic tissue. Interestingly, in the subcutaneous model, calcification is enhanced areas of tissue folds, bends, and areas of shear, suggesting that static mechanical deformation also potentiates mineralization, and unpublished results.
Although these data suggest that local tissue disruption mediates the mechanical effect, the precise mechanisms by which mechanical factors influence calcification are uncertain.

Moreover, no definite role has been demonstrated for circulating macromolecules or cells and many lines of evidence suggest that neither nonspecific inflammation nor specific immunologic responses appear in favor bioprosthetic tissue calcification.

Nevertheless, a potential role for inflammatory and immune processes has been postulated by some investigators. Proponents of an immunological mechanism for failure cite the evidence that (1) experimental animals can be sensitized to both fresh and cross-linked bioprosthetic valve tissues, (2) antibodies to valve components can be detected in some patients following valve dysfunction, and (3) failed tissue valves often have brisk mononuclear inflammation; no causal immunologic basis has been demonstrated for bioprosthetic valve calcification.

Nevertheless, in experiments in which vale cusps were enclosed in filter chambers that prevent host cell contact with tissue but allow free diffusion of extracellular fluid and implantation of valve tissue in congenitally athymic (“nude”) mice, who have essentially no T-cell function, calcification morphology and extent are unchanged.
• Clinical and experimental data detecting antibodies to valve tissue after failure probably reflect a secondary response in valve damage rather than a cause of failure.

• The initial calcification sites in bioprosthetic tissue are predominantly dead cells and cell membrane fragments.

• This occurs because the normal handling of calcium ions is disrupted in cells which have been rendered nonviable by glutaraldehyde fixation.

• Normally, plasma calcium concentration is 1 mg/ml (approximately $10^{-3}$ M) since the membranes of healthy cells pump calcium out, the concentration of calcium in the cytoplasm is 1,000-10,000 times lower (approximately $10^{-7}$ M).

• Cell membranes and intracellular organelles are high in phosphorus (as phospholipids, especially phosphatidylserine, which can bind calcium; they can serve as nucleators of calcific crystals.

• Mitochondria are also enriched in calcium. Other initiators under various circumstances include collagen and elastic fibers of the extracellular matrix, denatured proteins, phosphoproteins, fatty acids, blood platelets, and bacteria.

• We have hypothesized that cells calcify after glutaraldehyde pretreatment because this cross-linking agent stabilizes all he phosphorus stores, but the normal mechanisms for elimination of calcium from the cells are not available in glutaraldehyde-pretreated tissue.
Once initial calcification deposits forms, they can *enlarge and coalesce*, resulting in grossly mineralized *nodules* that can cause a prosthesis to malfunction.

In addition to the calcification of valve cusps, calcification of the adjacent aortic wall portion of glutaraldehyde-pretreated porcine aortic valves and valvular allografts and vascular segments is also observed *clinically and experimentally*.

Mineral deposition occurs *throughout the vascular cross section* but is accentuated in the *dense bands* at the inner and outer media, and cells and elastin (which itself not generally a prominent site of mineralization in cusps) are the major sites.

In *nonstented porcine aortic valves* that have greater portions of aortic wall exposed to blood than in the *currently used stented valves*, calcification of the aortic wall is potentially *deleterious*.

It could stiffen the foot, adhering hemodynamic efficiency, cause nodular calcific obstruction, potentiate wall rupture, of provide a nidus for emboli. Moreover, some anticalcification agents (see later) including 2-amino-oleic acid (AOA) and ethanol prevent experimental cuspal but not aortic wall calcification.
Calcification of Collagen and Elastin

- Calcification of the extracellular matrix structural proteins collagen and elastin has been observed in clinical and experimental implants of bioprosthetic and homograft valvular and vascular tissue and has been studied using a rat subdermal model.
- Collagen-containing implants are widely used in various surgical applications, such as tendon prostheses and surgical absorptive sponges, but their usefulness is compromised owing to calcium phosphate deposits and the resultant stiffening.
- Cross-linking by either glutaraldehyde or formaldehyde promotes the calcification of collagen sponge implants made of purified collagen but the extent of calcification does not correlate with the degree of cross-linking. In contrast, the calcification of elastin appears independent of pretreatment.
Three general strategies have been investigated for preventing calcification of biomaterial implants:

1. **Systemic therapy** with anticalcification agents;
2. **Local therapy** with implantable drug delivery devices; and
3. **Biomaterial modifications**, whether by removal of a calcifiable component, addition of an exogenous agent, or chemical alteration.

Investigations of an anticalcification strategy must demonstrate not only the effectiveness of the therapy but also the absence of adverse effects.

Adverse effects in this setting could include systemic or local toxicity, tendency toward thrombosis on infection, induction of immunological effects or structural degradation, with either immediate loss of mechanical properties or premature deterioration and failure.

Indeed, there are several examples whereby an antimineralization treatment contributed to unacceptable degradation of the tissue. The treatment should not impede normal valve performance, such as hemodynamics and durability.
• As summarized in more detail in Ch.6.4: Table 4, a rational approach for preventing bioprosthetic calcification must integrate safety and efficacy considerations with the scientific basis for inhibition of calcium phosphate crystal formation.

• This will of necessity involve the steps summarized in Ch.6.4: Table 5, before appropriate clinical trials can be done.

• Experimental studies using bioprosthetic tissue implanted subcutaneously in rats have clearly demonstrated that adequate doses of systemic agents used to treat clinical metabolic bone disease can prevent its calcification.

• However, because these agents may interfere with calcium metabolism or growth of calcific deposits, systemic drugs are associated with many side effects, including interruption of physiologic calcification (i.e., bone growth), and animals receiving doses sufficient to prevent bioprosthetic tissue calcification suffer growth retardation.

• Thus, the principal disadvantage of the systemic use of anticalcification agents for preventing pathologic calcification relates to side effects on bone. This difficulty can be avoided by localized drug release using coimplants of a drug delivery system adjacent to the prosthesis, in which the effective drug concentration is confined to the site at which it is needed (i.e., near the implant) and systemic side effects would be prevented.
Studies incorporating EHBP (Ethane hydroxybisphosphonate) in nondegradable polymers, such as ethylene-vinyl acetate (EVA), polydimethylsiloxane (silicone), and polyurethanes, have shown the effectiveness of this strategy in animal models.

This approach, however, has been difficult to implement in a clinically useful manner. The approach that most likely to yield an improved clinical valve involves modification of the substrate, either by removing or altering a calcifiable component or binding an inhibitor.

Forefront strategies should also consider (1) a possible synergism provided by multiple anticalcification agents and approaches used simultaneously; (2) new materials, and (3) the possibility of tissue-engineered heart valve replacements.

The agents most widely studies, for efficacy, mechanisms, lack of adverse effects, and potential clinical utility, are summarized hereafter and in Ch.6.4: Table 6.

Combination therapies using multiple agents may potentially provide synergy of beneficial effects to permit simultaneous prevention of calcification in both cusps and aortic wall, particularly beneficial in stentless aortic valves.
Inhibitors of Hydroxyapatite Formation

**Biophosphonates**

- Ethane hydroxybisphosphonate (EHBP) has been approved by the FDA for human use to inhibit pathologic calcification and to treat hypercalcemia of malignancy.
- Compounds of this type probably inhibit calcification by poisoning the growth of calcific crystals.
- Either cuspal pretreatment or systemic or local therapy of the host with diphosphonate compounds inhibits experimental bioprosthetic valve calcification.
- **Controlled clinical trials** have orally administered bisphosphonates have demonstrated the ability to stabilize osteoporosis.
- These agents, such as Alendronate, are hypothesized to act by stabilizing bone mineral. However, the effects of such agents on bioprosthetic valve or other pathologic biomaterial calcification are not yet known.
Trivalent Metal Ions

- Pretreatment of bioprosthetic tissue with iron and aluminum, inhibits calcification of subdermal implants with glutaraldehyde-pretreated porcine cusps or pericardium.
- Such compounds are hypothesized to act through complexation of the cation with phosphate, thereby preventing calcium phosphate crystal formation and growth.
- Both ferric ion and the trivalent aluminum ion inhibit alkaline phosphatase, an important enzyme used in bone formation, and this may also be a component of the mechanisms by which they prevent initiation of calcification.
- Furthermore, recent research from our laboratories has demonstrated that aluminum chloride prevents elastin calcification through a permanent structural alteration of the elastin molecule. Iron and aluminum may also active when released from polymeric controlled-release implants.
Calcium Diffusion Inhibitor

*Amino-oleic Acid*

- 2-α-Amino-oleid acid *bonds covalently* to bioprosthetic tissue through an *amino linkage to residual aldehyde functions* and *inhibits calcium flux* through bioprosthetic cusps.
- AOA is effective in mitigating cusp but *not aortic wall calcification* in rat subdermal and cardiovascular implants. This compound is used in an FDA-approved porcine aortic valve.

Removal/Modification of Calcifiable Material

*Surfactants*

- Incubation of bioprosthetic tissue with *sodium dodecyl sulfate* (SDS) and other detergents extracts the *majority of acidic phospholipids*; this is associated with reduced mineralization, probably resulting from *suppression of the initial cell-membrane oriented calcification*.
- This compound is used in FDA-approved porcine valve.
**Ethanol**

- Ethanol preincubation of glutaraldehyde-cross-linked porcine aortic valve bioprostheses prevents calcification of the valve cusps in both rat subdermal implants and sheep mitral valve replacements.
- Pretreatment with 80% ethanol (1) extracts almost all phospholipids and cholesterol from glutaraldehyde-cross-linked cusps, (2) causes a permanent alteration in collagen conformation as assessed by attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR), (3) affects cuspal interactions with water and lipids, and (4) enhances cuspal resistance to collagenase.
- Ethanol is in clinical use as a porcine valve cuspal pretreatment in Europe, and its use in combination with aluminum treatment of the aortic wall of a stentless valve is under consideration.

**Decellularization**

- Since the initial mineralization sites are devitalized connective cells of bioprosthetic tissue, some investigators have removed these cells from the tissue, with the intent of making the bioprosthetic matrix less prone to calcification.
Use of **Tissue Fixatives** Other Than Glutaraldehyde and Modification of Glutaraldehyde Fixation

- Since previous studies have demonstrated that conventional glutaraldehyde fixation is conducive to calcification of bioprosthetic tissue, several studies have investigated modifications of and alternatives to conventional glutaraldehyde pretreatment.

- Paradoxically, fixation of bioprosthetic tissue by extraordinarily high concentrations of glutaraldehyde (5-10 x those normally used) appear to inhibit calcification.

- Residual glutaraldehyde residues in bioprosthetic tissue can be neutralized (detoxified) by treatment with lysine or diamine; this inhibits calcification of subdermal implants.

- Non-glutaraldehyde cross-linking of bioprosthetic tissue with epoxides, carbodiimides, acylazides, and other compounds reduces their calcification in rat subdermal implant studies.

- Photooxidative preservation inhibits experimental calcification, possibly owing to the formation of unique calcification-resistant cross-links.
Alternative Materials

- Polyurethane trileaflet valves have been fabricated and investigated as a possible alternative to bioprostheses or mechanical valve prostheses.
- Despite versatile properties, such as superior abrasion resistance, hydrolytic stability, high flexural endurance, excellent physical strength, and acceptable blood compatibility, the use of polyurethane has been hampered by calcification, thrombosis, tearing, and biodegradation.
- Although the exact mechanism of polyurethane calcification is as yet unclear, it is believed that several physical, chemical, and biologic factors (directly or indirectly) play an important role in initiating this pathologic disease process.

Tissue Engineered Heart Valve Replacements

- In the approach called tissue engineering, an anatomically appropriate construct containing cells seeded on a resorbable scaffold is fabricated in vitro in a bioreactor, then implanted.
- Progressive tissue remodeling in vivo is intended to ultimately recapitulate normal functional architecture.
• Autologous tissue-engineered valve cusps have been implanted in the pulmonary valve position in lambs, demonstrating the initial feasibility of the concept of a tissue-engineered heart valve leaflet.

• With this concept, the cells comprising the prosthesis are intended to be viable and capable of renewal, thus theoretically inhibiting-calcification.

• Heart valves utilizing this strategy have been implanted in growing sheep for extended periods (to 20 weeks) without calcification.

CONCLUSIONS

• Calcification of biomaterial implants is an important pathologic process affecting a variety of tissue-derived biomaterials as well as synthetic polymers in various functional configurations.

• The pathophysiology has been partially characterized with a number of useful animal models; a key common feature is the involvement of devitalized cells and cellular debris.

• Although no clinically useful preventive approach has been proven to be safe and effective, several strategies based on either modifying biomaterials or local drug administration appear to be promising in some contexts.