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Modern view on calcification of xenogenic bioprosthetic heart valves and their anti-calcification treatment strategies

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Abstract

Aim. *The aim of this review was to analyze publications describing studies focusing on the pathophysiological mechanisms of calcification of bioprosthetic heart valves, and to substantiate new and promising methods of calcification prevention for the implantable medical devices.*

Material and methods. *Databases and electronic libraries such as PubMed, Google Scholar and eLibrary were used for searching relevant articles. Search queries included the following word combinations: "bioprosthetic heart valves", "structural valve degeneration", "calcification", "cyclic loading", "inflammation", "proteolysis", "proteolytic enzymes", "decellularization", "anticalcification treatment". The references in relevant articles were used for the search as well. Preference was given to works published from January 2013 to January 2023.*

Results. *We have considered the key aspects of bioprosthetic heart valves calcification and the main strategies of calcification prevention. Calcification of bioprosthetic heart valves incorporates a complex set of mechanisms that includes, but is not limited to: 1) binding of calcium in*

chemically stabilized biomaterial by free groups of the preservative; 2) precipitation of calcium on residual donor cells and cell debris; 3) pro-calcifying changes in biological material due to proteolysis, mechanical and oxidative stress; 4) cell-mediated biomineralization. Despite modern advances in biopreservation, such as treatment with chemical agents that prevent the deposition of calcium, the problem of bioprosthetic heart valves calcification still prevails. The cause of it lies in the heterogeneity of the pathophysiological mechanisms behind the mineralization of biomaterial: currently developed methods of calcification prevention cannot block all ways of bioprosthetic heart valves calcification.

Conclusion. *Calcification of bioprosthetic heart valve leaflets is a complex process that underlies the main cause of dysfunction of the medical devices. Supposedly, a new innovative approach that involves polymer hydrogel filler in biomaterials can completely prevent its calcification.*

Keywords: bioprosthetic heart valves, structural valve degeneration, calcification, hydroxyapatite, calcium-binding proteins, cyclic loading, immune rejection

Conflicts of Interest. The authors declare no conflict of interest.

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BHVs, bioprosthetic heart valves
SVD, structural valve degeneration

Introduction

The gold standard for the treatment of severe forms of acquired heart defects is the replacement of the affected valves [1]. As a rule, xenogeneic bioprosthetic heart valves (BHVs) made of chemically stabilized tissues of animal origin (porcine aortic valve, porcine or bovine pericardium), as well as mechanical substitutes produced of man-made materials (metal alloys and pyrolytic carbon) are used for this purpose [2]. Currently, most surgeons and patients prefer xenogeneic BHVs, since they compare favorably with their mechanical counterparts in their low thrombogenicity, avoiding the risks of lifelong anticoagulant therapy [2]. However, BHVs have short lifespan as they are prone to structural valve degeneration (SVD) [3]. In more than half of the cases, it is manifested by valve calcification, which leads to a limited mobility of leaflets, a decrease in the effective opening area, and valve obstruction [3]. According to statistics, from 20% to 50% of BHVs require replacement due to the development of SVD after 15 years of functioning [2]. Due to the significant risks associated with valve replacement and failure, the durability of the latter considerably limits the feasibility of bioprosthetics in people under 65 years of age, whose life expectancy exceeds the average life span of implants [1].

Since calcification is the main culprit in BHVs dysfunction, the main efforts of their manufacturers and researchers are aimed at developing methods to reduce the rate of calcification of biomaterials. Thus, the approaches introduced into the modern manufacturing of

modern BHVs include: **1)** anti-calcification treatment of biomaterial with chemical agents that mask the negatively charged free groups of preservative agents and thus prevent calcium precipitation (see below); **2)** elimination of primary nuclei of hydroxyapatite crystal nucleation (residual tissue lipids, dead donor cells and cellular debris) through tissue delipidation and decellularization. However, despite certain advances in this area, the problem of BHVs calcification is still far from solved. Apparently, the cause of this is that the above methods, while allowing the exclusion of biomaterial factors from the mineralization process, do not eliminate the impact of pro-calcifying factors in the recipient's blood on the implants. Consequently, failures in the development of resistant to calcification BHVs can be naturally explained: the methods used today only partially suppress the mechanisms responsible for the development of calcification.

We should note that over the past 20 years, views on the mechanisms of BHVs calcification have been seriously revised. In particular, it was possible to identify the involvement of circulating recipient factors in this process, including a number of osteogenic calcium-binding proteins and matrix-degrading enzymes, as well as cells and molecular components of the immune system. And if earlier BHVs calcification was presented to researchers as a passive degenerative-dystrophic process caused by suboptimal chemical stabilization of biological tissue and mechanical stress, today it is considered as a phenomenon behind which lies a complex of synergistically acting heterogeneous mechanisms [4]. Understanding this concept among researchers and manufacturers of BHVs is obviously a necessary condition for the development of new effective methods for suppressing valvular calcification.

This review is focused on the analysis of current information on the

triggers and pathophysiological mechanisms of BHVs calcification. We also reviewed the latest achievements in the development of anti-calcification protection of biomaterials, highlighting the most promising trends in the search for a final solution to the problem of bioprosthetic valve calcification.

Literature search strategy

This review has analyzed relevant articles that describe the pathophysiological mechanisms of calcification of BHVs stabilized with glutaraldehyde or ethylene glycol diglycidyl ether. The reviewed studies are presented in the databases and electronic libraries PubMed, Google Scholar, and eLibrary. Search queries are based on combinations of the words "bioprosthetic heart valves", "structural valve degeneration", "calcification", "cyclic loading", "inflammation", "proteolysis", "proteolytic enzymes", "decellularization", "anti-calcium treatment" and their Russian-language analogues. In addition, the search for studies was carried out using the reference lists given in relevant articles. When analyzing the literature, preference was given to the articles published from January 2013 to January 2023. Earlier publication being the primary sources of the discussed concepts and containing important historical data have also been reviewed.

Biomaterial factors in calcification of bioprosthetic heart valves

The biological component of xenogeneic BHVs is represented by tissue of animal origin, which has been treated with special preservative substances [5]. Preservative reagents stabilize collagen fibers, increasing the mechanical strength of the biomaterial and its resistance to enzymatic and oxidative degradation, and also contribute to the partial destruction of or shielding from immunoreactive animal antigens. The need to use

preservative reagents is dictated by the rapid degeneration of biomaterial in the human body in the absence of its chemical fixation. In particular, the first attempts to replace heart valves with unstabilized porcine xenografts, performed by a team of surgeons led by Alain Carpentier in the mid-60s of the last century, showed extremely unsatisfactory results: 40% of the xenografts developed dysfunction within 6 months after implantation and only about 30% of these continued to function normally after 3 years [6]. Histological analysis of the excised valves demonstrated that the main cause of their degeneration was an immune reaction, accompanied by the breakdown of the collagen base of the biomaterial and rupture of the leaflets [6].

The creation of full-fledged commercial BHVs became possible thanks to the method of preserving biomaterials with glutaraldehyde proposed by A. Carpentier [7]. The use of this chemical agent to stabilize biological tissues made it possible to slow down SVD and significantly increase durability of BHVs [7]. Thus, for the first bioprostheses, freedom from SVD after 5 years of functioning was equal to 77% when implanted in the mitral position and 89% when implanted in the aortic position [7]. However, over time it became clear that the treatment of BHVs with glutaraldehyde promotes the development of calcification of the leaflets [4]. This problem persists when stabilizing biological tissue with ethylene glycol diglycidyl ether, which was proposed as an alternative to glutaraldehyde in the mid-90s as a more calcification-resistant type of preservative reagent [8].

The nature of the calcium-binding activity of glutaraldehyde and ethylene glycol diglycidyl ether is based on the structure of the molecules of these substances and the leading mechanism of their interaction with collagen fibers. The principle of biological tissue stabilization with the preservative reagents under consideration is the formation of covalent

bonds between their molecules and the functional groups of amino acids that make up collagen [5]. The molecules of glutaraldehyde and ethylene glycol diglycidyl ether are bifunctional (that is, they have two identical binding sites at opposite ends of the carbon chain), due to which they can form chemical bridges between the polypeptide chains of collagen, ensuring their chemical cross-linking [5]. In this case, some preservative reagent molecules interact with collagen through only one binding site, as a result of which free aldehyde or epoxy groups are present in the chemically stabilized biomaterial. The latter carry a negative charge and trap calcium cations from the environment, promoting dystrophic calcification. In particular, a direct relationship between the number of free aldehyde groups and the degree of calcification of glutaraldehyde-treated bovine pericardium was demonstrated in an experiment *in vivo* using a rabbit model of intramuscular implantation [9].

It should be noted that in addition to the formation of free preservative reagent groups, the chemical fixation of the biomaterial provokes procalcifying changes in its structure. In particular, the treatment causes the biological tissue to lose collagen-associated mucopolysaccharides, which are associated with collagen fibers through so-called "hole zones". It is well known that these areas are the main centers of nucleation and growth of hydroxyapatite crystals along collagen fibers during physiological mineralization of bone tissue [10]. It is logical to assume that the loss of mucopolysaccharides and the exposure of areas prone to calcification may be an important factor in the formation of calcium deposits in BHVs. We should add that glutaraldehyde and ethylene glycol diglycidyl ether do not stabilize elastic fibers, and therefore they remain vulnerable to the destructive effects of cyclic loads, proteolytic and oxidative enzymes. This precedes and predetermines the elastin-oriented calcification of the biomaterial [11].

Another factor that plays a role in the process of chemically stabilized biological tissue calcification is the presence of residual donor cells and cellular debris in it. The results of *in vitro* studies have shown that when a culture of porcine aortic valve interstitial cells is fixed with glutaraldehyde, their gradual mineralization occurs, accompanied by a depletion of calcium ions in the culture medium [12]. Electron microscopy has been used to visualize deposits of hydroxyapatite crystals on the inner surface of plasma membranes of dead cells and apoptotic bodies [12]. Data obtained in a subcutaneous implantation rat models confirmed that donor cells act as the main nuclei of hydroxyapatite nucleation, while the stabilized matrix serves as a substrate for further growth of calcifications [13].

The mechanism of calcification of fixed cells appears to be based on the influx of calcium ions from the environment into phosphate-rich cytosol after their death. Living cells maintain lower calcium concentrations in the cytosol compared to the extracellular fluid. This ionic asymmetry is achieved thanks to the active transport system, represented by a calcium-dependent ATPase and a sodium-calcium ion exchanger, which transport calcium ions through the plasmalemma from the cell to the environment. Chemical treatment leads to cell death and cessation of ion pumps activity, resulting in a flow of calcium ions directed into the cell along a concentration gradient. In addition, calcium is released from its intracellular stores.

It has been shown that in glutaraldehyde-treated porcine aortic valve fibroblasts, intracellular calcium concentrations can exceed those in living cells by approximately a million times [12]. Since cell membranes and other intracellular structures are rich in phosphates, calcium ions accumulating in the cell begin to concentrate on their surface, forming calcium-phosphate complexes. Thus, the influx of calcium into the

phosphate-rich cytosol creates a special localized microenvironment with high concentrations of calcium-phosphate products sufficient to initiate precipitation of hydroxyapatite.

Despite the existence of the methods for additional processing of biological tissue, which are used in the production of modern BHVs and are designed to neutralize the participation of biomaterial factors in calcification, the latter remains one of the main causes of prosthetic valve dysfunctions [14]. Also noteworthy is the relationship between the rate of BHVs calcification and the metabolic or immune status of recipients. In particular, taking calcium-containing drugs or the presence of diseases (for example, hyperparathyroidism and renal failure), accompanied by increased levels of circulating calcium-phosphate complexes in the blood, are associated with a more rapid progression of SVD [4]. A tendency towards accelerated calcification of BHVs is characteristic of young recipients compared to older ones, which can be explained by the greater reactivity of their immune system and higher intensity of calcium metabolism [4]. Based on the above, we should conclude that, in addition to biomaterial factors, recipient factors make a significant impact on calcification of BHVs.

Recipient factors and their contribution to calcification of bioprosthetic heart valves

The impact of recipient factors on the mineralization of BHVs has been studied rather superficially, and many conclusions are based on indirect evidence. Nevertheless, based on the available information, it would be right to assert that some plasma proteins and cellular elements of blood are directly involved in calcification of bioprostheses.

Back in the 80–90s of the last century, it was established that BHVs explanted due to calcification contain bone matrix proteins, such

as osteopontin and osteocalcin, which are not seen in non-calcified valves [15–17]. Recent immunohistochemical studies have confirmed the earlier obtained results; besides, some other regulators of bone metabolism, including bone sialoprotein and alkaline phosphatase, have been identified in BHVs leaflets [18]. The sources of proteins turned out to be populations of osteoblast-like cells, although the diffuse pattern of sample staining with antibodies against osteopontin, osteocalcin, and bone sialoprotein demonstrated by the authors indicates the imbibition of these substances into the valve tissue from the blood plasma of recipients [18].

Bone matrix proteins have a high calcium-binding activity [19]. Being immobilized on the surface of collagen fibers, they can initiate the nucleation of hydroxyapatite crystals in BHVs leaflets, similar to what happens during the formation of bone tissue [19]. Moreover, these proteins act as signaling molecules that activate pro-osteogenic changes in smooth muscle cells and fibroblasts [20]. No studies *in viv*o have been performed so far aimed at testing the hypothesis about the participation of bone matrix proteins in the mineralization of prosthetic valves. Nevertheless, the coincidence of the expression patterns of such proteins in explanted BHVs with calcification of biological tissues, as well as the identification of a direct correlation between their concentration levels in the tissue and the extent of their calcification, suggests the stated hypothesis to be true [15–18].

In addition to bone matrix proteins, various proteases may be involved in the mineralization processes of BHVs. Numerous studies demonstrate that the leaflets of excised valves contain dense cellular infiltrates consisting of macrophages, giant multinucleated cells, neutrophils, T and B lymphocytes [21–24]. Immune cells are the source of a wide range of matrix-degrading enzymes, including aggressive collagenases and elastases from the group of cysteine cathepsins and

matrix metalloproteinases [21]. Moreover, some proteolytic enzymes (for example, gelatinase B) abundantly enter the prosthetic biomaterial from the blood perfusing it [21]. Although the mechanism of enzyme interactions with biological tissue does not imply per se the formation of calcium deposits, the proteolytic cleavage of extracellular matrix fibers is responsible for procalcifying changes in its structure.

Proteolytic degradation of collagen and elastin fibers helps exposing the areas vulnerable to calcification and accelerates mineralization, as demonstrated by studies conducted *in vitro* and *in vivo* [25, 26]. Apparently, this process can be intensified in the environment rich in oxidative enzymes and reactive oxygen species and also created by immune cells infiltrating the implants.

It is worth noting that in addition to the production of osteogenic calcium-binding proteins, matrix-degrading enzymes and oxygen radicals, the recipient cells take part in forming the calcium deposits, undergoing mineralization after apoptosis [23]. This is confirmed by the results of electron microscopic studies of BHVs, which demonstrate the presence of crystalline structures on the surface membranes of apoptotic macrophages, which suggests their involvement in the processes of nucleation and growth of hydroxyapatite crystals [27].

Finally, blood lipid deposits accumulated in BHVs leaflets during their functioning can act as hydroxyapatite nucleation nuclei [28].

Mechanical stress as an intensifier of calcification of bioprosthetic heart valves

Native heart valves and their substitutes function in an extremely aggressive mechanical environment. Each valve opens and closes about 40 million times throughout the year, while valves go through 3 billion cycles over their lifetime [29]. During the cardiac cycle, the heart valve

leaflets are subjected to variable mechanical loads caused by wall shear stress when the valve is open, bending deformations of the tissue that occur during its opening and closing, as well as the tension on the leaflets under the action of blood back pressure when they are closed [30].

It is generally accepted that the mechanical stress experienced by BHVs makes a significant impact on the intensification of their calcification process. *In vivo,* calcium deposits in BHVs tissues are formed predominantly in the areas that are subject to the greatest mechanical loads [31, 32]. Results of *in vitro* tests show that upon incubation in a calcinating solution and exposure to cyclic loads, the mineralization of the bovine pericardial plates begins at the bending sites [33]. Clinical observations also indicate a close relationship between mechanical stress and calcification. In particular, faster rates of calcification are observed in patients with hypertension and those receiving small-diameter BHVs whose effective opening area is not large enough relative to the body surface area (the so-called patient-prosthetic mismatch) [34]. Also, the significance of mechanical stress in the mineralization of prosthetic valves was demonstrated by the data obtained from studying the pairs of identical xenoaortic BHVs, which were included in the design of the Heart Mate XVE device (Thoratec Corporation, USA) used to assist the left ventricle heart functioning [35]. It was shown that inflow BHVs experiencing greater closure pressure compared to the outflow ones were calcified more often and had a larger extent of calcification [35].

The mechanism of intensification of hydroxyapatite deposition in BHVs under the impact of mechanical stress is based on the accumulation of fatigue damage in the structure of the biomaterial fibrous matrix. Molecular damage to collagen fibers in glutaraldehyde-treated bovine pericardial samples has been shown to occur after 20 million cycles,

which is equivalent to only 6 months of valve operation *in vivo* [36]. Since the biological tissue of BHVs is not capable of regeneration, degenerative changes in the collagen network that occur due to cyclic loads are irreversible. In this case, a break of the material structural integrity facilitates the diffusion of calcium ions through voids and microcracks in its thickness, and also promotes the formation of hydroxyapatite crystals on damaged areas of collagen and elastic fibers [4]. Likewise, fatigue damage and delamination of the fibrous matrix of the valves facilitates the penetration of monocytes and other blood cellular elements into them. Noteworthy is that, according to microscopic studies, cellular infiltrates are located predominantly in the areas with a disorganized and fragmented matrix [21–24]. It should also be added that cyclic loads provoke the loss of chemical cross-links in stabilized biological tissue, gradually reducing its resistance to proteolytic degradation [37].

It is important to note that calcification of the leaflets and associated valve stenosis lead to increased mechanical stress. Initially, BHVs provides a physiological blood flow, but as calcification develops, the transprosthetic gradient increases and the flow velocity increases many times over. The high linear velocity of blood flow causes an excessive increase in the shear stress acting on the valve leaflets. Thus, a degeneration closed cycle is formed: an increase in the rigidity and thickness of the valves due to progressive calcification leads to a gradual decrease in their mobility and the formation of valve stenosis, causing an increasing deviation of the hydrodynamic transprosthetic flow parameters from normal values; this in turn contributes to additional damage to the biomaterial and accelerates its calcification.

Preventing the calcification of xenogeneic bioprosthetic heart valves calcification: in search of new solutions

In their search for solving the problem of calcification of biological tissues in BHVs, investigators work in several directions in accordance with theoretical ideas about the causes and mechanisms behind this phenomenon. In general, five main strategies can be distinguished: **1**) the search for new calcification-resistant reagents; **2**) the neutralization of free aldehyde and epoxy groups to reduce the calcium-binding potential of biological material fixed with standard preservation agents; **3**) the removal of primary hydroxyapatite nucleation centers from the tissue structure; **4**) fabricating the BHVs using low-immunogenic tissues of genetically modified animals; **5**) creation of composites by combining biological tissue with polymer hydrogels.

Back in 1987, a group of researchers led by Gershon Golomb studied the effect of glutaraldehyde treatment on the process of calcification of biological tissues and concluded that it would be possible to completely suppress the mineralization of BHVs only by substituting for another reagent [38]. Since that time, a number of alternative stabilizers have been identified that are less prone to binding calcium ions compared to glutaraldehyde. These include substances of plant origin (genipin and tannic acid) and bacterial origin (reuterin), methacrylic anhydride and carbodiimides [39–43]. However, despite promising results of preclinical trials based on the use of these compounds, methods for preserving biomaterials have not reached the stage of clinical trials. Probably, the low interest in further testing of such preservative reagents can be explained by the doubts from manufacturers of BHVs about their reliability due to poor knowledge in both fundamental and applied aspects. In addition, the stabilization of biomaterial using these reagents is difficult for implementation in mass production and is extremely

expensive, which raises questions about its economic feasibility. Among alternative approaches to glutaraldehyde stabilization, only the epoxy preservation method based on the use of ethylene glycol diglycidyl ether has been introduced into the production of BHVs. Epoxy-treated biomaterial is considered more resistant to calcification; and bioprostheses fabricated of it show satisfactory durability even in young patients [44–46]. However, ethylene glycol diglycidyl ether-fixed valves have not been widely used and are currently commercially available only in the Russian Federation [8].

The advantages of glutaraldehyde and ethylene glycol diglycidyl ether (high rate of chemical interaction, reliability of cross-linking, solubility in water, and low cost) over other preservative reagents have prompted researchers and manufacturers of BHVs to search for a compromise solution. However, most of research works were devoted to methods for deactivating aldehyde and epoxy groups, which are largely responsible for dystrophic calcification. Thus, to date, several methods have been developed for additional treatment of stabilized biological tissue with various amino derivatives, such as aminodiphosphonates and 2-amino oleic acid, which are used in manufacturing of the commercial BHVs [47]. Use of these approaches made it possible to shift the timing of the calcification development in modern BHVs by 5–7 years compared to earlier models, although they did not completely solve the problem of calcification.

In addition to chemical deactivation of free preservative reagent groups to reduce the rate of calcification of BHVs, decellularization has been actively investigated in recent years. The principle of this method is based on the destruction and subsequent washing away of residual donor cells through physical and chemical action [48]. Decellularization makes it possible to eliminate part of the hydroxyapatite nuclei contained in the

tissue (dead donor cells and their fragments), as well as reduce its immunogenicity due to the removal of xenoglycans associated with cell membranes. *In vivo* studies using a model of orthotopic implantation of BHVs in the mitral and pulmonary positions in sheep demonstrated a lower tendency of decellularized valves to calcification as compared to non-decellularized valves [49, 50], but this approach has received limited distribution in commercial production. Apparently, the reason for this is the lack of optimal protocols that make it possible both to remove donor cells from biological tissue, and also to preserve its original structure without loss of strength properties.

Due to the growing popularity of the hypothesis on the participation of immune system cellular and molecular agents in the calcification of BHVs, researchers have begun to develop immunologically inert implant. It is known that the biological tissue stabilization with glutaraldehyde or ethylene glycol diglycidyl ether, and the decellularization are not enough to completely eliminate the immunogenicity [51]. Animal antigens associated with donor cells and the extracellular matrix, such as galactose-alpha-1,3-galactose and N-glycolylneuraminic acid, remain in the structure of the chemically altered tissue, promoting the development of a humoral and cellular immune response to implants [51]. One solution to the described problem is the breeding of genetically modified pigs and bulls that do not express the most immunoreactive xenogeneic antigens and can act as donors of low-immunogenic biomaterial [52].

Based on modern ideas about SVD, it can be assumed that the use of genetically modified animal tissues in the production of BHVs will help increase their durability due to a decreased effect of antibody binding and a decreased cellular infiltration. Although this hypothesis remains clinically untested, its correctness is indirectly confirmed by data

from a number of *in vitro* and *in vivo* studies. In particular, the valves and pericardium of galactose-alpha-1,3-galactose and N-glycolylneuraminic acid knockout pigs do not bind human serum antibodies, unlike tissues from wild-type pigs [53, 54]. Baboons implanted with BHVs fabricated of the valves from genetically modified pigs did not develop a specific humoral response, unlike monkeys in the comparison group that received standard bioprostheses [55]. Finally, enzymatic removal of galactose-alpha-1,3-galactose from the structure of bovine pericardium contributed to a twofold decrease in the level of calcification compared to unchanged pericardium when implanted subcutaneously into mice deficient in this saccharide [56]. It is important to note that the wear-resistance property of BHVs fabricated by using tissue from genetically modified pigs is comparable to that of modern commercially available models, as indicated by *in vitro* tests for 200 million cycles and the results of implantation in the mitral position in sheep for 90 days [57, 58].

It should be noted that the strategies described above, although they can reduce the passive deposition of hydroxyapatite in biological material and the inflammatory cellular response, do not prevent the imbibition of various procalcifying agents from the recipient’s blood into the biological tissue. Potentially, this problem can be solved by a recently proposed innovative approach based on the formation of composite materials by combining biological tissue with polymer hydrogels [59, 60]. The essence of this method is to fill the collagen matrix of the tissue with a biostable and biocompatible polymer hydrogel (for example, based on polyethylene glycol diacrylate or polyvinyl alcohol). The latter creates a physical barrier for cellular blood elements and substances circulating in the plasma, including ionized calcium, penetrating into the thickness of the biomaterial. Studying *in vivo* a modified with polyethylene glycol diacrylate bovine pericardium in a subcutaneous implantation model in

rats demonstrated a three-fold decrease in calcium content in the tested samples compared to controls [59]. Similarly, fragments of epoxy-treated xenogeneic pericardium, additionally modified with cryostructured polyvinyl alcohol, compared to samples in the control group, contained 5 and 3 times less calcium after incubation in a calcium-saturated solution for 3 and 6 weeks, respectively [60].

Conclusion

This review provides evidence in favor of the statement that calcification of BHVs is a complex multifactorial process realized through several heterogeneous synergistically acting mechanisms, both passive degenerative-dystrophic, and active immunologically mediated. Currently noted success associated with a significant increase in the service life of modern BHVs compared to earlier models has been achieved by the improved valve design and approaches to anti-calcification treatment of the biological component. The latter are aimed primarily at deactivating free chemical groups of preservative agents, as well as eliminating the primary nuclei of hydroxyapatite crystals, but these approaches do not affect the immunological pathways of calcification development. Many researchers express the opinion that the possibilities of these approaches for solving the problem of calcification are practically exhausted.

Among the recently proposed strategies in the development of anti-calcification protection of BHVs, the modification of biological tissue with polymer hydrogels deserves a close attention. Potentially, this approach can eliminate the impact of all the recipient pro-calcifying factors (ionized calcium, osteogenic proteins dissolved in plasma, proteases and other substances, as well as cellular elements of the blood) on the biological tissue, preventing calcification. However, there is

currently no data on the cycle resistance of such composite materials and therefore it is not yet known whether hydrogels retain their insulating properties when subjected to cyclic loading.

Based on the above, we can conclude that the method of filling the internal structure of a biomaterial with polymer gels is the most promising trend for future research, since, unlike other approaches, it has the potential to eliminate the effect of all mechanisms of calcification development.

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