Early Degeneration of Aortic Bioprosthetic Valves

Chemical interactions, prevention and treatment

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Bioprosthetic heart valves ensure a better quality of life compared to mechanical valves but are prone to degeneration with leaflet calcification and tearing. Valvular leaflets mineralization process is initiated within interstitial cells devitalized by glutaraldehyde with formation of calcium phosphate hydroxyapatite crystals. The aim of the current paper is to analyse early bioprosthetic valve degeneration occurring in three patients that needed bioprosthetic valve explantation and reimplantation of a mechanical valve. The three patients, aged 29, 64 and 78 years presented early degeneration of the implanted valve at 30, 36 and 52 months after the intervention in the context of unavoidable risk factors like young age, chronic kidney disease and small stented valve. Computed tomography examination demonstrated calcifications of the bioprosthetic valve and histological examination confirmed the findings by identifying tissue degeneration with calcification in all cases. Early bioprosthetic valve degeneration is a serious issue in cardiovascular surgery and a major cause of valve failure requiring reintervention. Several methods could be used to delay degeneration like systemic therapy with general anti-calcification agents, the use of locally delivered drugs or tissue pretreatment with special substances (ethanol, nonionic detergents, tridecyl alcohol ethoxylate). No optimal decellularization method for all types of tissues has been yet discovered. In conclusion, early bioprosthetic valve degeneration is a reality that should be taken into account in high risk patients. Current tissue pretreatment with glutaraldehyde is not perfect as it accelerates degeneration with formation of hydroxyapatite calcifications. New methods are needed to achieve efficient decellularization while preserving the functional characteristics of the transplanted tissue.
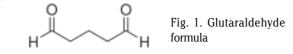
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The hemodynamic performance of valvular substitutes (mechanical prostheses and bioprostheses) for the treatment of acquired valve diseases is well established. Mechanical prostheses have been in use since 1960 and have seen several improvements in their profile. All these models have a major common disadvantage: that of being thrombogenic, and therefore of requiring lifetime anticoagulant treatment to allow the proper functioning of the prosthesis and avoids thromboembolic events [1, 2].

Bioprostheses were introduced in the 1970s and do not share this disadvantage and therefore do not require anticoagulant treatment and ensure a better quality of life for the patient. However, tissue deterioration of the bioprostheses is inexorable with the years, with appearance of calcifications or tearing, responsible for stenoses and/or valvular leaks requiring a reintervention. Ten years after surgery, 70 to 80% of bioprostheses remain functional, compared to only 40% after 15 years. The degeneration is accelerated in younger patients. In addition to young age, pregnancy and renal failure also accelerate the degeneration of bioprostheses [3, 4].

Degenerative lesions, leaflet calcification more or less associated with tearing secondary to collagen matrix disruption first occur at commissural junction zones predisposed to high pressures. The mechanism of this calcification thus appears mixed: chemical and mechanical due to stress lesions [5, 6].

Valvular leaflets mineralization process is initiated within interstitial cells devitalized by glutaraldehyde (GA) - $C_5H_8O_2$, (fig. 1).

Fig. 1. Glutaraldehyde formula

Glutaraldehyde causes a disappearance of the mesothelium and an alteration of the interstitial cells.

Devitalized cells become unable to maintain low intracellular calcium levels in the presence of high extracellular free calcium concentrations. Thus, the formation of calcium phosphate hydroxyapatite crystals $(Ca_{10}(PO_4)_6(OH)_2)$ begins at the level of the devitalized support cells. The phosphorus ions necessary for the formation of the first mineralized calcium phosphate nuclei come from the organic phosphorus present in the cell membranes due to the action of alkaline phosphatases [7].

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Experimental part
Materials and methods

The authors analysed three patients aged 29, 78 and 64 years respectively who benefited of aortic valve replacement with a bioprosthetic valve in 2007, 2017 and 2018. The study was conducted in accordance to the Helsinki Declaration and to some published models [8-10]. Patient details are presented in table 1.

All three patients registered prosthesis degeneration with stenosis within 30 to 52 months after implantation and needed redo surgery with bioprosthesis valve explantation and reimplantation of a mechanical valve. All surgical interventions were performed at the Cardiovascular Institute from Iasi, Romania and explanted valves were analysed by a histopathologist.

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Patient	Age at implantation	Diagnosis	Degeneration delay	Bioprosthesis type	Bioprosthesis size	Others
P.E.	64	SAS	52 months	Hancock II	21	HVC
G.I.	78	SAS	36 months	Hancock II	23	CKD stage III
M.M.	29	SAI	30 months	Edwards Carpentier	23	-

SAS – severe aortic stenosis, SAI – severe aortic insufficiency, CKD – chronic kidney disease, HVC - Hepatitis C virus

Results and discussions

The earliest degeneration occurred at 30 months after implantation in a young patient (29 years) with no significant comorbidities who required implantation of a bioprosthetic valve for severe aortic insufficiency (class III NYHA heart failure) despite guideline indications pleading for a mechanical aortic valve.

The next patient presenting with early degeneration was 78 years old at the time of surgery. He presented with severe, degenerative, aortic stenosis and several comorbidities such as multivascular disease (atherosclerosis), permanent atrial fibrillation, grade III arterial hypertension, moderate mitral regurgitation, stage III chronic kidney disease. The patient was frail with limited mobility and given the unavailability of transcatheter aortic valves (TAVI) at the time of surgery (2009) the main surgeon decided to implant surgically a bioprosthetic valve. The postoperative evolution was marked by complications such as prolonged inotropic support and severe azotate retention requiring dialysis. Because of slow recovery in the setting of his prior frailty, the patient was submitted to a recovery clinic when discharged.

The last patient was 64 years at the time of surgery and presented several comorbidities such as viral hepatitis (virus C), mitral stenosis with left atrial dilatation, atrial fibrillation and right ventricular dysfunction, stable angina pectoris.

Before explantation of the degenerated bioprosthetic valve, all three patients benefited from a computed tomography examination with calcium scoring calculation (Aortic Valve Calcium Scoring) for the bioprosthetic valve on non-contrast images (Siemens Definition Flash 2×128 Stellar detectors). All patients registered very high values, 963 for P.E., 1012 for G.I. and 987 for M.M.

Explanted valves were analysed both macroscopically and microscopically after being fixed in formalin, decalcified and embedded in paraffin. All specimens were stained with hematoxylin-eosin (HE). The main cause of valve degeneration was tissue degeneration with calcification in all cases.

Early bioprosthetic valve degeneration is a serious issue in cardiovascular surgery and a major cause of valve failure requiring reintervention. According to a recent up-to-date published in 2017, several predictors of bioprosthethic valve degeneration have been identified, both patient-related (younger age, higher body mass index, smoking, diabetes, dyslipidemia, renal insufficiency), and valve-related factors (persistent left ventricular hypertrophy, smaller prosthesis size, prosthesis-patient mismatch) [11]. The three patients from our study presented such risk factors: M.M. was very young when first operated, G.I. presented stage III chronic kidney disease and P.E. benefited from the smallest stented valve (no prosthesis-patient mismatch). Sometimes, such situations can be managed by implanting a mechanical valve but in other cases, like in our patients, implantation of a bioprosthetic valve cannot be avoided. G.I. was old at the time of surgery, frail and with several comorbidities increasing the risk of anticoagulation related complications. P.E. had a small native aortic valve associated to a reduced body mass index and surface area requiring implantation of a small valve. In the same time, given his liver disease, lifetime anticoagulation had to be avoided so a bioprosthetic valve was the only choice. M.M. on the other hand, insisted for a bioprosthetic valve despite surgeon's arguments for a mechanical valve. In such cases when patient-related factors cannot be managed and implantation of a bioprosthetic valve is mandatory the only solution remains to improve bioprosthetic valve manufacture process.

Homologous or heterologous tissue biomaterials require pretreatment that attenuates their antigenicity, sterilizes them, and protects them from enzymatic degradation. GA is the most used bioprosthetic tissue treatment medium. It is an aliphatic dialdehyde capable of binding to the free amino groups of the tissue components, especially collagen. The formation of covalent bonds between the collagen fibers makes it possible not only to stabilize the extracellular matrix and to preserve it from possible enzymatic degradation but also to improve its mechanical capabilities [12].

The mechanism of dystrophic calcification involves a reaction between extracellular calcium and phosphorus ions of cell membranes. Normally the plasma extracellular calcium concentration is 1mg/mL and when the cell is healthy with a good Ca^{2+} pump function, the cytoplasmic calcium concentration is 1000 to 10000 times lower. These physiological mechanisms that maintain low intracellular calcium content in the presence of high free calcium concentrations are altered by the cellular devitalization following GA treatment. The phosphorus ions necessary for the formation of the first mineralized nuclei come from the organic phosphorus of cell membranes and other intercellular structures thanks to the enzymatic action of alkaline phosphatases. Thus, the first crystals of calcium phosphate hydroxyapatite grow coalescing to become real nodules that eventually thicken and stiffen the valvular leaflets [13, 14].

Collagen and elastin can serve as sites for the formation of calcium phosphate mineralized nuclei independently of cellular structures. Tissue pretreatment with glutaraldehyde or formaldehyde are factors that promote the calcification of the extracellular matrix because they have a certain toxicity and do not allow to fix uniformly all the constituent elements of this matrix [6, 13-16]. Similar effects of glutaraldehyde and its derivatives have been described for medical devices also in other fields of interventional procedures [17, 18]

Various studies have hypothesized that the inflammatory and the immunological responses [19-21] could also play a role in the mineralization process of bioprosthetic tissues. Antibodies against donor valvular tissue components have been found in patients with structural deterioration of the bioprosthesis. Histological examination of these explanted valves often reveals the presence of inflammatory

infiltrates with mononuclear cells. The accelerated calcification of bioprostheses is partly linked to these immunoallergic mechanisms. Clinical observations have shown that prolonged corticosteroid therapy would slow the calcification of bioprostheses in young patients, thus supporting the hypothesis of involvement of immunological and inflammatory mechanisms in the degeneration of valvular tissue implants [22].

Recent studies have shown that hypercholesterolemia promotes calcification of cardiac bioprostheses. Increasing serum cholesterol level would be an activating factor in the calcification process of valvular bioprosthetic tissues [23].

Many strategies have been developed to improve the durability of bioprostheses based on the pathogenic mechanisms that determine their degeneration. The means for treating bioprosthetic tissues to optimize their durability and mechanical properties include extracellular matrix fixation techniques, and different methods for preventing mineralization of valvular tissue [17]:

-systemic therapy with the use of general anti-calcification agents;

- the use of locally delivered drugs;

-tissue pretreatment methods that act on tissue components that may be involved in the mineralization process.

The various drugs generally used to treat bone metabolism disorders such as calcium chelators (diphosphonates, 1-biphosphonates) are also effective in preventing the calcification of bioprosthetic tissues in rats. These drugs are involved in phosphocalcic metabolism and the physiological mechanisms of bone formation. Therefore, their general use also leads to inhibition of bone production and growth retardation [24]. To overcome this inconvenient, the local use of these drugs was advocated through an implant system that allows delivery of the anti-mineralization substance on demand. These methods are used successfully in animal models by associating 1-bisphosphonates with non-degradable polymers such as polydimethylsiloxane (silicone). Other inhibitors of hydroxyapatite formation are trivalent metals like iron (Fe) and aluminum (Al) [25] that can bind to phosphates and inhibit the formation of calcium-phosphate complexes, and calcium diffusion inhibitors (ethylenediaminetetraacetic acid).

Tissue pretreatment can also be improved. The use of alcoholic solutions including ethanol at different concentrations has shown its effectiveness in the prevention of calcification of valve tissue. Ethanol is used to extract cholesterol and phospholipids from cell membranes that after enzymatic degradation by alkaline phosphatases provide the phosphorus ions involved in the formation of mineralized crystals [26]. Other methods target decellularization process. Courtman et al. have proposed the use of a nonionic detergent associated with an enzymatic extraction process. After this treatment, the acellular matrix is composed of collagen, elastin and glycosaminoglycans [27]. *Mendoza-Novelo et al.* [28] compared three decellularization processes: tridecyl alcohol ethoxylate, reversible alkaline swelling and the nonionic detergent. The histological results showed a significant reduction of cellular antigens for the three decellularization processes attesting to their effectiveness. Unfortunately, the content of the extracellular matrix in glycosaminoglycans also decreases significantly. The use of a hypotonic buffer such as sodium dodecyl sulfate (SDS), protease and nuclease inhibitors help protecting the integrity of glycosaminoglycans and matrix proteins such as collagen.

Depending on the procedure used and its objectives, the effects of decellularization are more or less deleterious to tissue architecture and integrity. The processes of decellularization are essentially differentiated by the alterations of the extra-cellular matrix that they can cause [29]. This is why the choice of an efficient decellularization method should depend on the type of target tissue and the aims of the treatment.

At present, there is no optimal decellularization method for all types of tissues. However, depending on the target tissue and the site of implantation of the biomaterial, it is possible to use a suitable method for achieving efficient decellularization while preserving the functional characteristics of the tissues.

Additional treatment is required after decellularization to stabilize the mechanical and biological properties of the extracellular matrix of tissues that undergoes significant changes with a significant risk of rapid enzymatic resorption after implantation.

Thanks to a better knowledge of the pathogenic mechanisms of the structural degeneration of the valvular tissues and in particular of the role of treatment methods like GA in their determinism, research has been directed towards the development of new non-aldehyde alternative methods (Genipin, epoxy compounds, carbodiimides, glycerol, acyl azide, cyanimide and photooxidation) [30, 31]. However, these different methods were evaluated in vitro and compared to GA. A last possibility is to cover the decellularized fabric with a polymer film. Subsequent studies should compare these different methods taking into account the conservation of tissue properties, cytotoxicity and other deleterious effects of these treatments.

Conclusions

In conclusion, early bioprosthetic valve degeneration is a reality that should be taken into account in high risk patients. Current tissue pretreatment with glutaraldehyde is not perfect as it accelerates degeneration with formation of hydroxyapatite calcifications. New methods are needed to achieve efficient decellularization while preserving the functional characteristics of the transplanted tissue.

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