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In-vitro calcification study of polyurethane heart valves

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ABSTRACT

Tri-leaflet polyurethane heart valves have been considered as a potential candidate in heart valve replacement surgeries. In this study, polyurethane (Angioflex®) heart valve prostheses were fabricated using a solvent-casting method to evaluate their calcification resistance. These valves were subjected to accelerated life testing (continuous opening and closing of the leaflets) in a synthetic calcification solution. Results showed that Angioflex® could be considered as a potential material for fabricating prosthetic heart valves with possibly a higher calcification resistance compared to tissue valves. In addition, calcification resistance of bisphosphonate-modified Angioflex® valves was also evaluated. Bisphosphonates are considered to enhance the calcification resistance of polymers once covalently bonded to the bulk of the material. However, our *in-vitro* results showed that bisphosphonate-modified Angioflex® valves did not improve the calcification resistance of Angioflex® compared to its untreated counterparts. The results also showed that cyclic loading of the valves' leaflets resulted in formation of numerous cracks on the calcified surface, which were not present when calcification study did not involve mechanical loading. Further study of these cracks did not result in enough evidence to conclude whether these cracks have penetrated to the polymeric surface.

1. Introduction

One of the major causes of mortality worldwide is related to heart valve disease and the associated complications with its treatment. Heart valve replacement procedures have been performed successfully since 1960, even though 10-year survival rates still range from 37 to 58% [1]. In 2004 only, approximately 90,000 valve replacement operations were performed in USA on patients with valvular heart disease [2]. Currently, defective heart valves are replaced either by mechanical or bioprosthetic valves [3]. Metal or carbon alloys are the primary constituents of mechanical valves and, based on their structure, are classified as caged-ball, single-tilting-disk, or bileaflet-tilting-disk valves [4]. The Starr–Edwards [5,6] caged-ball can be considered the first generation mechanical valve while the Medtronic Hall [7] and St. Jude Medical [8] with centrally located occluders or leaflets can be considered as the second-generation valves. Finally, the tri-leaflet polyurethane valves can be considered as the third generation of the mechanical valves, which have peripherally located leaflets that eliminate many of the flow disturbances associated with the other mechanical valve designs [9]. The two most popular bioprostheses are the porcine xenograft [10], and the bovine pericardial valve [11].

Heart valve replacement surgery, using either of the two types of clinically approved valves, is known to be the best method for treating patients suffering from valvular disease. However, there are serious drawbacks with their usage. Mechanical valves require daily anticoagulation therapy to reduce thromboembolic complications [12,13], while biological valves are less thrombogenic than mechanical valves, they undergo tissue degradation due to mechanically induced fatigue damage, tear mostly because of the stress concentration due to its fixation and changes in the mechanical properties of the valve tissue, and leaflet calcification, leading to valve failure over time [12,14–16]. As a result of tissue degradation, durability of bioprosthetic valves range from 5 to 20 years, which leads to reoperation for the recipient [17]. To address these issues, several studies have considered new design and new materials for heart valves which require lower levels of anticoagulation compared to some of the existing mechanical valves and have a longer life-span than tissue valves [18–20]. In recent years, tri-leaflet polymeric heart valves have received a significant attention because of their synthetically enhanced mechanical properties and their improved fluid flow. Among different types of polymers, polyurethane is the most popular choice for biomedical applications, given its biocompatibility and reasonable tensile strength [21]. In addition to these properties, application of this material in the design of heart valves should consider its biostability, haemocompatibility, antithrombogenecity and resistance to degradation and calcification [18]. Furthermore, a heart valve material should be able to withstand many cycles of stress and deformation before failure [22]. It has been

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demonstrated that polyurethanes have the desired characteristics suitable for design of many implantable devices [23] and may also be a proper choice to be considered for heart valve fabrication. In addition, polyurethane heart valves may be less susceptible to calcification compared to bioprosthetic heart valves, which are pretreated with glutaraldehyde before implantation. This makes them immunologically inert and improves the tissue durability. However, it is believed that glutaraldehyde treated tissues are susceptible to calcification, which is a major contributor in bioprosthetic heart valve failure. Glutaraldehyde treatment devitalizes the cells and these residual cells become the primary sites for calcification. Calcium phosphate is the calcification mineral which forms through the reaction of the calcium ion in extracellular fluid with the membrane-associated phosphorus ion [24].

The mechanism for polyurethane calcification is not yet completely understood. There are different hypotheses related to polyurethane calcification. It has been hypothesized that polyurethane heart valve calcification is due to deposition and attachment of cellular debris and thrombi on the surface of the material [25,26]. However, it has been shown that there is no need for thrombus formations in order for a polyurethane surface to get calcified. This has been verified in rat subdermal implants [27,28]. Imachi et al. [29] hypothesized that when polyurethane is subjected to repeated stretching, micro-gaps form on the polyurethane surface. Therefore, blood proteins and/or phospholipids are trapped in these micro-gaps providing a source for calcium ion interaction, leading to formation of calcium phosphate ($Ca_3(PO_4)_2$). However, in our previously published results, we have shown that polyurethane surfaces can become calcified even in the absence of mechanical loading [30].

Different approaches have been taken towards reducing the calcification of bioprosthetic heart valves. They include pretreatment of the valves with metallic salts, detergents, bisphosphonates or by covalent attachment of other anti-calcification agents [31–34]. Similarly, to make polymers more resistant to calcification, anti-calcification agents, such as bisphosphonates, have been considered as an additive to the polymeric material [18]. Bisphosphonates prevent nucleation and growth of calcium-phosphate, thus it is expected that the bisphosphonate modified polymers provide more resistance to calcification. Alferiev et al. [35] developed a novel method to covalently bond bisphosphonate and diethylamino groups to BioSpan® (The Polymer Technology Group Inc., Berkeley, CA), which is a proprietary high strength elastomeric biomaterial. It was shown that bisphosphonate-modified BioSpan® is less susceptible to calcification. Based on this result, and with the opportunity to modify Angioflex® with the bisphosphonate that was provided for us by Ivan Alferiev's group, we hypothesized that the new modified material *might* provide a similar calcification resistance as to BioSpan®. To test this hypothesis, heart valves made from treated (modified) and untreated materials were cyclically loaded on a Valve Accelerated Life Tester and their calcification process were investigated.

2. Experimental procedures

2.1. Materials

Angioflex® (a proprietary polyether-based polyurethane material that has been developed by ABIOMED Inc., and successfully used in the design of their implantable replacement heart) and bisphosphonate/diethylamino modified Angioflex® (BP-Angioflex®) were used in this study to understand the effects of material modifications on the calcification process. It has been postulated that BP-Angioflex® provides a better calcification resistance than the untreated material. The details of material processing for preparation of BP-Angioflex® can be found elsewhere. All chemicals used in this study were acquired from Sigma-Aldrich (St. Louis, MO).

2.2. Calcification metastable solution

Two different calcification solution compounds have been identified for *in-vitro* experiments in this study. Golomb and Wagner's compound [36] is the one used here, while Starcher and Urry's [37] compound was previously used by Deiwick et al. [38] for their *in-vitro* calcification studies of bioprostheses.

Golomb and Wagner's Compound: The calcification metastable solution consists of 3.87 millimole (mM) $CaCl_2$, 2.32 mM K_2HPO_4, yielding a ratio of calcium to phosphate (Ca/PO_4) = 1.67, and 0.05 M Tris Buffer (in this study $C_4H_{11}NO_3$) solved in one (1) *l* of reverse osmosis (RO) water [36].

Starcher and Urry's Compound: Solution consists of 20 mM barbital buffer, pH 7.41, containing 55 mM KCl, 1.25 mM KH_2PO_4 and 1.5 mM $CaCl_2$ (yielding a Ca/PO_4 ratio of 1.2)[37].

We have compared the Ca/PO_4 content for these two compounds in [30]. Based on that comparison, Golomb and Wagner's compound, which we have used in this study, is more physiologically representative of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) and is more aggressive than its counterpart (hydroxyapatite is the most common form of calcium minerals in the vascular calcification process). Therefore comparing the calcification resistance of a material subjected to this solution with that of exposed to Starcher and Urry's would underestimate calcification resistance [30].

2.3. Valve Accelerated Life Tester (VALT)

The Valve Accelerated Life Tester (Vivitro Labs Inc., Victoria, B.C., Canada) is designed to assess heart valve durability. It consists of 6 test chambers and a unique Scan-Valve for rapid monitoring of pressures across the valve (upstream and downstream of the valve). Valves are mounted on pistons in a chamber filled with Golomb and Wagner's Compound [36]. A shaker via a control module drives the pistons at a selectable frequency. Fig. 1 shows a VALT setup.

2.4. Methods

To setup the experiments for this study, each chamber, containing a polyurethane heart valve, was filled with Golomb and Wagner's compound, up to where its cap fitted. The VALT was turned on for one (1) min to bring all the air bubbles to the surface. Each chamber was then de-aired before the test by sucking the air out using a syringe through the allotted small opening in each chamber. To make sure that chambers were completely filled with the calcification solution, a little of the solution was added back to the chamber using the same syringe filled with fresh solution until it started to leak from the opening. At this point, the opening was closed quickly

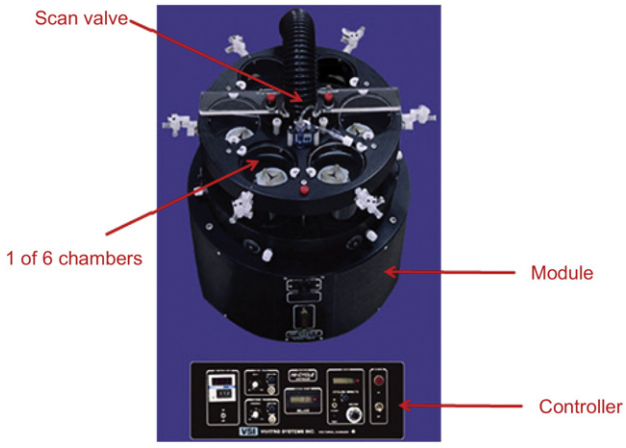

Fig. 1. Valve Accelerated Life Tester (VALT) setup.