A Comprehensive Study on the Dynamic Calcification of Bioprosthetic Heart Valves Utilizing Bovine Pericardium

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Abstract

The present study aimed to assess the dynamic calcification behavior of bioprosthetic heart valves. The researchers developed a fast, reliable, and reproducible method to evaluate the calcification potential of biomaterials. They applied biomechanical forces to influence calcification and tissue characteristics in the heart valves. It was found that primary tissue failure and valve leaflet calcification can significantly reduce the lifespan of bioprosthetic heart valves. To study heart valve replacement and calcification, the researchers utilized an advanced atomic absorption spectroscopy method (AAS). This technique allowed for accurate measurement of calcification levels. Dynamic tests were found to induce greater calcification compared to static tests. Overall, calcification testing using the developed method proved to be useful in economically screening new materials or modifications of existing materials before conducting in vivo testing. This research contributes to understanding the calcification behavior of bioprosthetic heart valves and potentially improving their longevity.

Key Words: Dynamic calcification, Bioprosthetic heart valves and Atomic absorption spectroscopy (AAS)

Introduction

Patients with heart valve illness often experience calcification, which is a major cause of failure in biological heart valve prostheses. Factors such as tissue devitalization, mechanical stress, age, renal function, pregnancy, and other unknown variables can influence the extent and progression of calcification. There are two types of calcification: intrinsic and extrinsic. Intrinsic calcification involves the precipitation of calcium and phosphate ions within the tissue, while extrinsic calcification occurs within the thrombus, including platelets in the blood.

Various methods are employed to prepare tissues suitable for heart valve fabrication, including tissue fixation, de-cellularization, and anti-calcification processes. The anti-calcification and tissue dehydration treatment aims to reduce calcification by reducing residual glutaraldehyde, extracting phospholipids, and altering collagen conformation. This approach increases tissue resistance to collagenase enzymes and eliminates the need for storage in liquid sterilants. To evaluate the functional dynamics of a bioprosthetic heart valve and its propensity for calcification, it is tested in an accelerated pulsatile valve tester using a calcifying fluid. The goal is to develop a heart valve with reduced calcification and improved longevity for patients with heart valve diseases. The accelerated pulsatile valve tester operates at a frequency of 300 cycles per minute (5 Hz) and a temperature of 37°C. The synthetic calcifying fluid in the tester is

changed weekly to prevent contamination or clouding.

At 30-day intervals, the samples were visually inspected using an optical microscope. This study aims to create a heart valve that minimizes calcification and provides a better quality of life for patients. By understanding the mechanisms of calcification and optimizing tissue preparation and testing methods, researchers and medical professionals strive to improve the success rate and longevity of biological heart valve prostheses.

Material and Methods

The animal tissue from a bovine pericardium was used to prepare the heart valve. An investigation was conducted using bioprosthetic heart valves, calcifying fluid, forceps, glass beakers, volumetric flasks, pH meters, and weighing scales to assess the quantity of dynamic calcification. Tri acid, tri base, dipotassium phosphate (K2HPO4), calcium chloride (CaCl2), and filtered water were the chemicals utilized to make the synthetic calcifying fluid. With the appropriate concentration of chemicals (mixture of 7.88 gm tri-acid, 6.06 gm K_2HPO_4 and 4.29 gm of $CaCl_2$ in 1000 mL of purified water) synthetic calcifying fluid was prepared.

Test Samples

Sr No.	Sample Name	Size of Heart Valve (mm)
01	Regular heart valve	23
02	Improved anti-calcification treated heart valve	
03	Anti-calcification treated heart valve stored in 0.625% glutaraldehyde for 6 months	
04	Dry tissue heart valve	

Novel accelerated pulsative valve tester as shown in the fig.01 was used to perform calcification study of bioprosthetic heart valves. Total six bioprosthetic heart valves were arranged in the chamber of pulsative valve tester containing synthetic calcifying fluid. Out of these 06 heart valves, two valves were fabricated using fixed bovine pericardium tissue. Another two valves were fabricated from tissue which was fixed and further treated with series of improved anti-calcification processes such as heat treatment, blocking and reduction, etc. Some anti-calcification treated heart valve samples stored in 0.625% glutaraldehyde for 6 months were also taken in consideration for analyzing calcification. Remaining two valves were treated with additional treatment i.e. dehydration process which aid in storage of valve. The dehydrated tissue valves not required liquid medium for the storage and thus prevents calcification occurs due to glutaraldehyde. In dry tissue valve, the tissue was initially undergone fixation process followed by anti-calcification process and subsequently dehydration process. A synthetic calcifying fluid was prepared which resembles body's calcification mechanism. Before placing the bioprosthetic heart valves inside the testing chambers, all the chambers were sterilized. Approximately 2.5 L of synthetic calcifying fluid was filled in each chamber. At weekly intervals, the synthetic calcifying fluid was changed to prevent contamination from any microbial agents. The machine has a facility to accommodate two valves in one chamber containing the synthetic calcifying fluid. The fluid is kept in circulation and further the chamber kept pulsating which provides valves functional parameters such as valve opening and closing. The pulsating mechanism provides differential pressure required for valve same as body function.

Fig.01 Accelerated pulsative valve tester

After placing the bioprosthetic heart valves in an *in vitro* accelerated pulsatile valve tester, testing parameters were set as mentioned in table-1. Accelerated pulsatile valve tester runs at frequency of 300 cycles/minutes (5 Hz) at 37ºC temperature with synthetic calcifying fluid. The valves are subsequently examined for evaluating tissue characteristics and the degree of calcification on the bioprosthetic heart valve after the completion of the corresponding accelerated cycles (200 million) in accordance with the set interval.

Sr No.	Test	Parameters
1	Fluid	Synthetic calcifying fluid pH - 7.4
2	Pressure difference	▲ p = 90 mmHg + 20/-0 mmHg (aortic valves) ▲ p = 120 mmHg + 20/-0 mmHg (Mitral valves)
3	Rate/Frequency	70 cycles per minute (Real Time) 300 cycles per minute (Accelerated Time)
4	Temperature	37 °C
5	Period	Up to 1.5 years

Table.1 Test parameters

Visual inspection and Atomic absorption spectroscopy were the two methods used to examine the calcification of bioprosthetic heart valves.

1. Visual Inspection

After the completion of corresponding accelerated cycles (200 million) according to the predetermined time interval, visual inspection of the bioprosthetic heart valve was done to check potential changes in tissue properties.

2. Atomic Absorption Spectroscopy (AAS)

AAS is an analytical technique that measures the concentration of elements. For the assessment of calcium content, samples of bioprosthetic heart valves were dried to a constant weight in a desiccators oven and incubated with HNO_3 (0.75 mol/L) at 68 ^oC for 15 hours. After centrifugation at 2500 rpm, the centrifuged fluid was diluted, and the calcium content was determined by means of atomic absorption spectroscopy. Elemental concentrations were expressed throughout as mg/g of dry tissue weight. This technique can also measure down to parts per billion of a gram in a sample.

Results and Discussion

Growth of calcification formation can disrupt a human body's natural biological processes. Calcification is the most important causes of structural deterioration of glutaraldehyde fixed bioprosthetic valves. In this research, regular and anti calcification heart valve were stored in fluid (e.g glutaraldehyde) and dry tissue heart valve was stored without fluid. The detected elements such as calcium and phosphorous from the heart valve were observed through atomic absorption spectroscopy (AAS) in ppm which is illustrated in table.2. Anti-calcification treated valve also showed good results but the problem was with storage condition i.e. in liquid medium which can cause calcification in long term.

Sr. No	Sample Name	Calcium Content (ppm)	Phosphorus Content (ppm)
01	Regular heart valve	15	9.80
02	Anti-calcification treated heart valve	3.38	2.06
03	Anti-calcification treated heart valve stored in 0.625% glutaraldehyde for 6 months	3.90	2.40
04	Dry tissue heart valve	3	1.70

Table-2 Calcium and Phosphorous content

Hence, we obtained more amount of calcification in regular heart valve, very less amount of calcification in improved anti-calcification treated heart valve and anti-calcification treated heart valve stored in 0.625% Glutaraldehyde for 6 months and very negligible amount of calcification in dry tissue heart valve. It is summarized in the table-3.

Sr. No.	Sample	Visual Inspection
01	Regular heart valve	More amount of calcification was observed
02	Improved anti-calcification treated heart valve	Very less amount of calcification was observed
03	Anti-calcification treated heart valve stored in 0.625% glutaraldehyde for 6 months	Very less amount of calcification was observed

04	Dry tissue heart valve	Very negligible amount of calcification was observed

Table-3 Summary of calcification detection

The calcification on heart valve is depicted in fig.01, 02, 03, and 04 respectively, based on their level of detection.

1. Regular heart valve

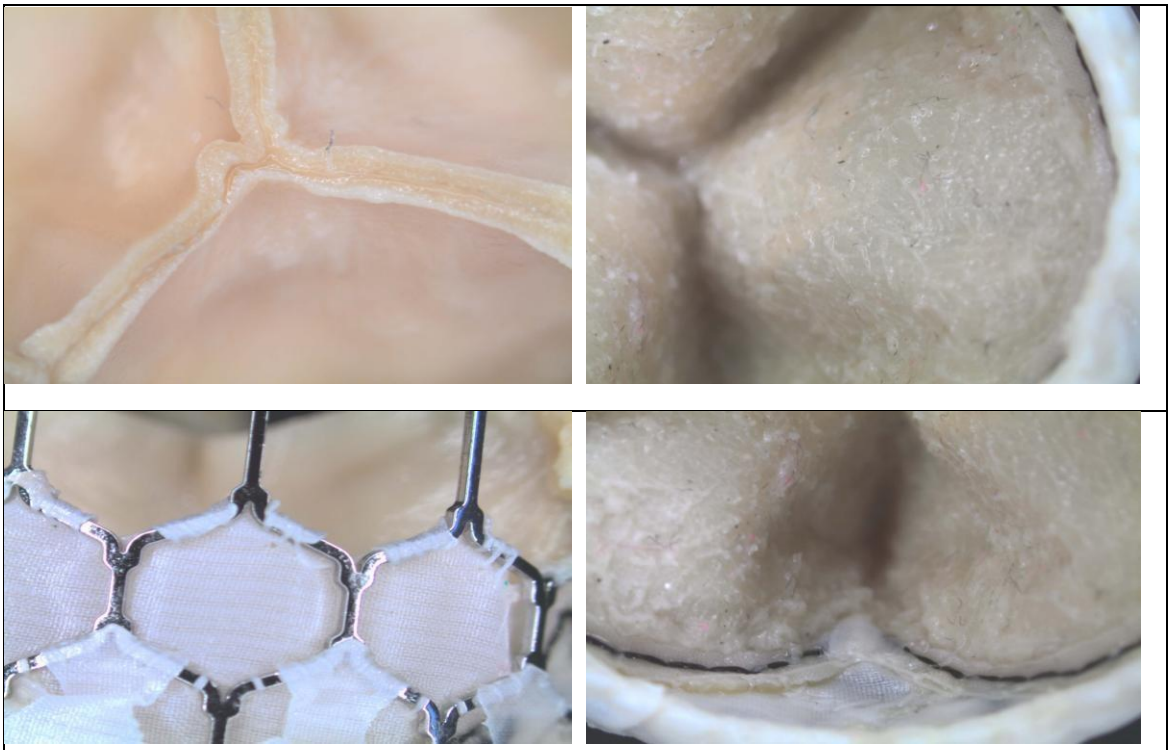

Fig.01 – More amount of calcification was observed

2. Improved anti-calcification treated heart valve

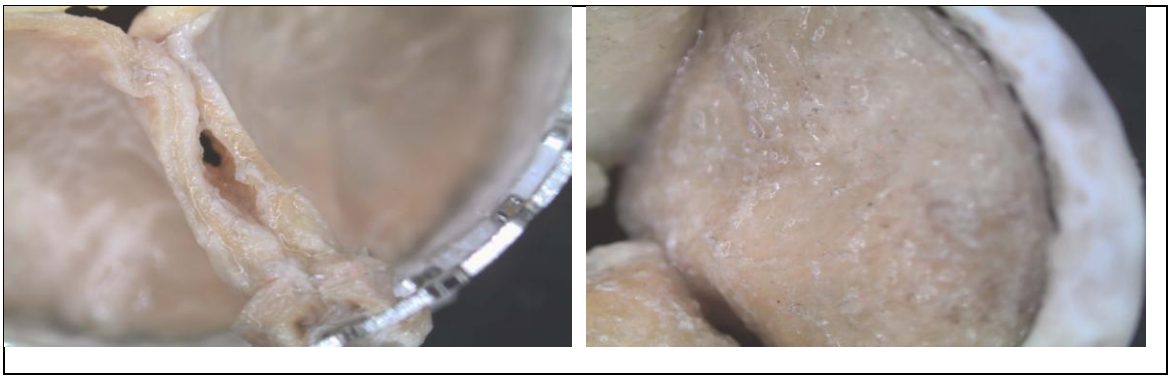

Fig.02-Very less amount of calcification was observed

3. Anti-calcification treated heart valve stored in 0.625% glutaraldehyde for 6 months

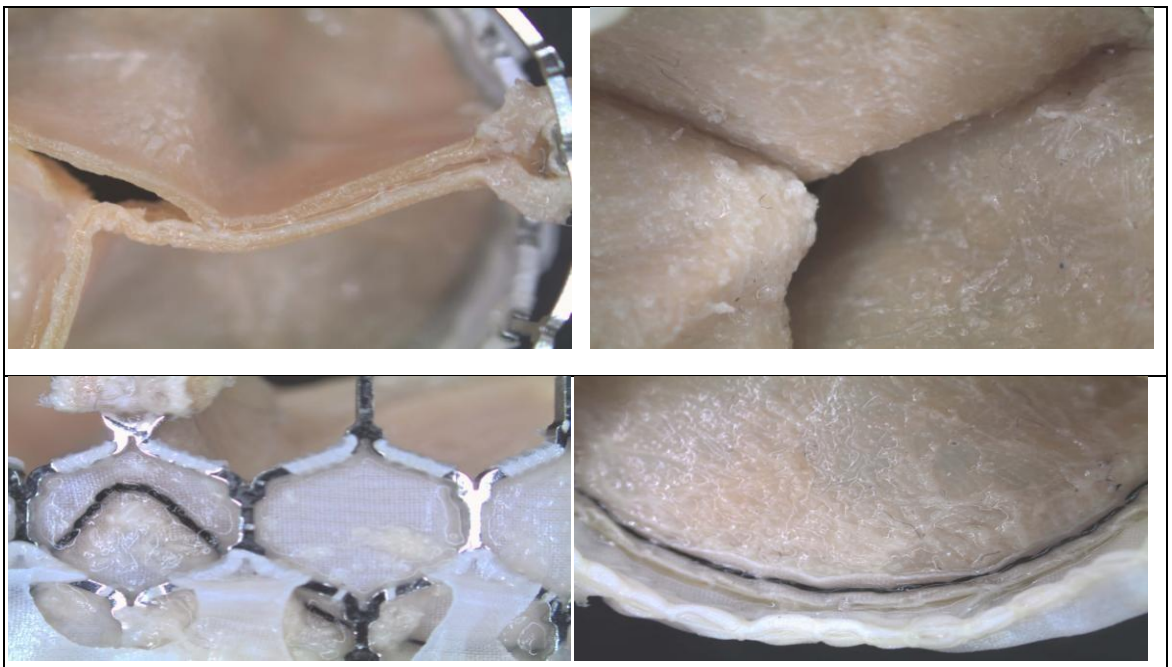

Fig.03 Very Less amount of calcification was observed

4. Dry tissue heart valve

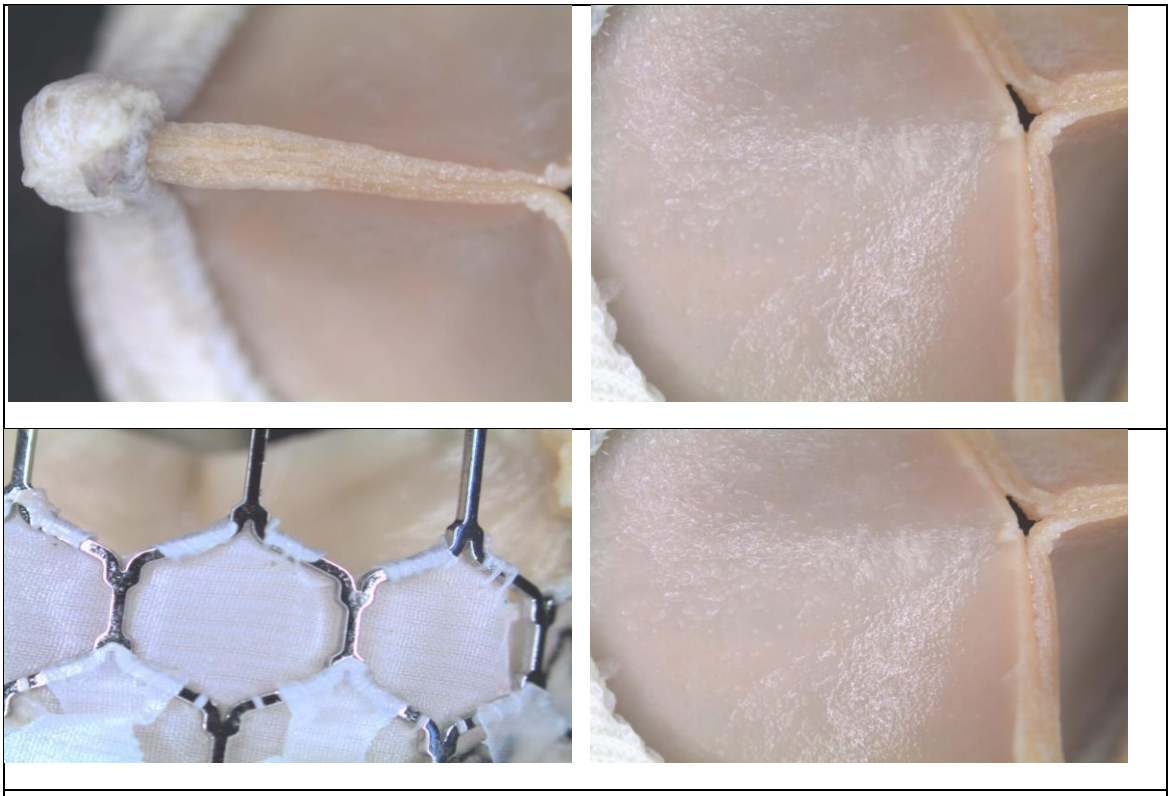

Fig.04 - Very negligible amount of calcification was observed

Conclusion

In conclusion, based on the extensive analysis conducted in the comprehensive study on the "Dynamic Calcification of Bioprosthetic Heart Valves" utilizing bovine pericardium, it has been determined that, the dry tissue heart valves exhibit very negligible calcification formation. This characteristic contributes to their exceptional valve performance, durability, and overall safety for patients in comparison to regular and anti-calcification treated heart valves. Furthermore, the study did not identify any valve-related complications. Consequently, the utilization of dry tissue heart valves holds significant potential for the treatment of heart valve diseases. Nonetheless, further investigation through longer-term clinical follow-up is necessary to validate and solidify these promising findings.

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