

A Study On In Vitro Calcification of Polyurethane

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ABSTRACT

To Determine the major factor which causes the accelerated calcification of the severe flexing area of the artificial heart sac, comparative study under well defined in vitro situation were carried out. The results show that the effect of static mechanical stress is not so important. According to the data, change of surface area caused by the applied mechanical stress is one of the important factors of the heavy calcification of the severe flexing area of the artificial heart sac.

INTRODUCTION

Calcification of the Polymeric surface is a common phenomenon which every material experiences during the time it is implanted in biological system. Calcification is sometimes beneficial if the implanted prosthesis is a hard tissue. But if the implanted artificial organ is a soft tissue like cardiovascular prosthesis, such a calcification eventually destroys the implanted cardiovascular prosthesis. Therefore, in order to develop a safe and stable cardiovascular prosthesis, it is very important to prevent the calcification of a cardiovascular prosthesis. Physical, chemical and biological factors affect the calcification process. Currently the mechanism is obscure and there are only several hypotheses.

Thoma et al. suggested that the ether oxygen of a PTMG polyol molecule interacts with the calcium ion and leads to crown ether formation.[1] Several groups postulate a close connection between mechanical stress and amount of calcium deposition.[2-3]

It is well-known that the regions of continued high mechanical flexure exhibits more heavy calcification than the other regions. The two

major factors which accelerate the calcification process in that region are already known - One is the mechanical stress and the other is the crack on the surface. However, which one is more significant in the acceleration of calcification process is uncertain.

To evaluate the role of each factor in the acceleration of calcification, a comparative study under a well-known and controllable environment is needed. Two kinds of experimental system have been made to evaluate the role of each factor and the effect of negatively charged sulfonate groups on the polymer surface in the calcification process as well. One of the systems is called the static system and the other is called the dynamic system. In the static system, polyurethane samples are calcified under static mechanical stress. On the other hand, in the dynamic system, polyurethane experiences changing mechanical stress while it is being calcified. After some time, the calcified polyurethane samples had been taken out from both systems to test.

MATERIALS AND METHODS

1. Experimental Setup

1-1. Static System

Polyurethane samples (DOW chemical, Hydroxyl end group, PELLETHANE 2363-80AE,) for the static system were made as follows.

8%(weight percent) polyurethane/DMAC (N-N dimethylacetamide) solution was used to make a polyurethane film. The solution was prepared and degassed under vacuum condition. Then, the solution was poured into the mold with great care to avoid trapping further air bubbles, and placed in a drying oven, and dried at 40° C for 7 days. The

testing specimens were cut out of the polyurethane film (thickness 0.1mm) to the proper size. After being manufactured, the specimens were stored in a vacuum desiccator with silica at room temperature.

Next, the length of these samples were extended to the extension rate of 0%, 100%, and 400% and placed in sample holders to impose different static stress to the samples. The extended samples were immersed into big glass beaker which contains a calcium solution. The system was placed on a heating magnetic stirrer as shown in Fig 1. The solution was refreshed once a week and was being stirred continuously until the end of this experiment. The temperature was maintained at 37 ± 30 C. The testing solution was metastable, containing 5.14 mmol/l $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 3.08 mmol/l KH_2PO_4 , in 0.1 mmol/l Tris buffer, buffered to pH 7.40. In this solution the calcium concentration was twice the mean total serum level, and the ration of Ca/Po₄ was 1.67, as in hydroxyapatite (HAP). All solutions were made up using deionized water.

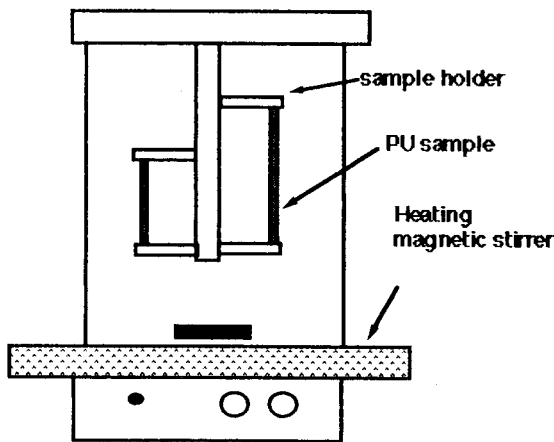


Fig 1 A schematic diagram of the static system

1-2 Dynamic System

To impose changing stress onto polyurethane samples like that of total artificial heart blood sac, a mock circulation system which imitates the pre- and afterload of the human body system have been made (Fig 2).

The polyurethane sac was made using dipcoating methods. The dipmolds consisted of wax and silicon coating. The mold was dipped into a 16% (weight percent) polyurethane/DMAC (N-N dimethylacet amide) solution in a dried clean

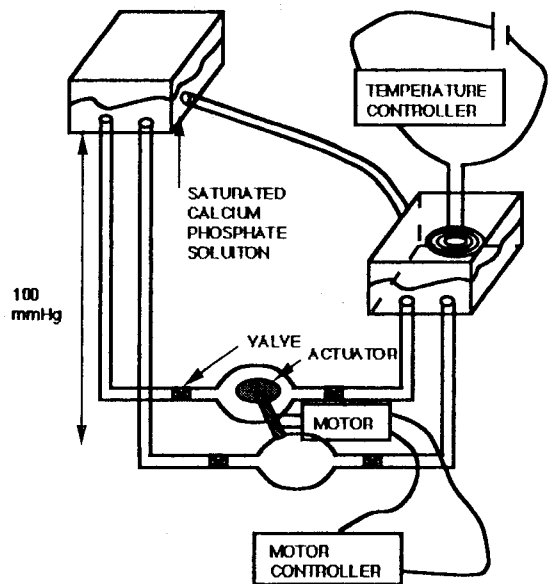


Fig 2 A schematic diagram of the dynamic system

chamber and was dried in the chamber for 24 hours until the next dipping. After 8-10 times dipping, the sac has been denuded from the mold and dried for 7 days. The thickness of the manufactured sac was about 1.8 mm. The shape of the sac is shown in Fig.3.

The polyurethane valve used in the experiment was a sinkhole valve. The thickness of the polyurethane leaflet was 0.2 mm. The leaflet was cut out of a membrane which was made by the same methods as we used when making polyurethane film for the samples of the static testing.

The preparation of PU-PEO-SO₃ by bulk modification is as follows : First, the reaction of amino terminated PEO (MW-1000) with propane solution is performed at 50° C for 5 hours to get sulfonated PEO (H₂N-PEO.SO₃). Consecutively, H₂N-PEO.SO₃ is reacted with hexamethylene diisocyanate (HMDI) at 50° C for 3 hours to yield isocyanated PEO.SO₃ (OCN.PEO-SO₃). Finally, OCN.PEO-SO₃ is grafted to PU dissolved in dymethylacetamide (DMAC) for 3 days at 50° C to produce PU-PEO-SO₃.

The obtained PU-PEO-SO₃ was dissolved in DMAC (2.5 %, w/v) and coated on Sinkhole bileaflet polymer valve sac

The testing solution contained 2.57 mmol/l

$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1.54 mmol/l KH_2PO_4 , in 0.05 mmol/l Tris buffer, buffered to pH 7.40. In this solution the calcium concentration was similar to the mean total serum level, and the ration of Ca/Po_4 was 1.67, as in hydroxyapatite (HAP). All solutions were made using deionized water.

manufactured, all components were assembled and 20l of a saturated calcium phosphate solution was used as the testing fluid. The temperature of the testing solution was maintained at $37 \pm 1^\circ \text{C}$. During the testing period, systolic volume was maintained at 100ml, heart rate was 80 beat per min, mean aortic pressure was 100 mm Hg, and mean atrium pressure was 10 mm Hg. The pumping had been done for one month.

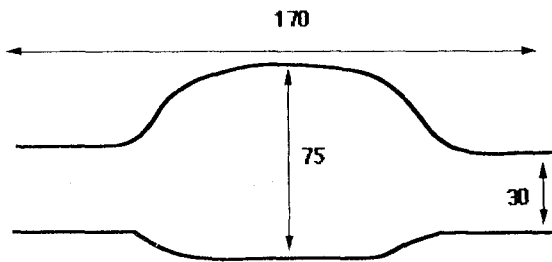


Fig 3 Diagram of the shape of the sac

2. Characterization

The surface morphology and the amount of calcium and phosphorus atoms on the samples after calcification were determined using a scanning electron microscope (SEM, Hitachi 2500C) and Induction Coupled Plasma (ICP). In the SEM observation, samples were mounted and sputter-coated with carbon using an ion coater and observed at an accelerating voltage of 20 kV.

The deposited calcium and phosphorus had been dissolved in a 0.6 N HCl solution for 7 days at 60°C . The amount of dissolved calcium and phosphorus was measured using an ICP.

RESULTS AND DISCUSSION

The morphology of the polyurethane surface after calcification testing is shown in Fig 4. As shown in the figure, polyurethane surface is covered with calcium and phosphorus and the morphology of the polyurethane surface was changed to a more rough one. There are many cracks on the surface,

but it is uncertain whether the cracks are restricted to the calcium layer only or extends to the polyurethane layer as well.

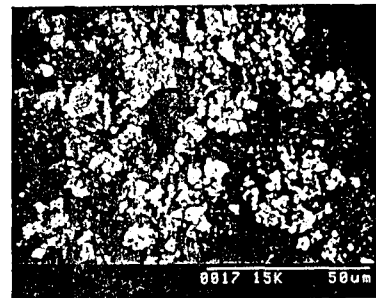
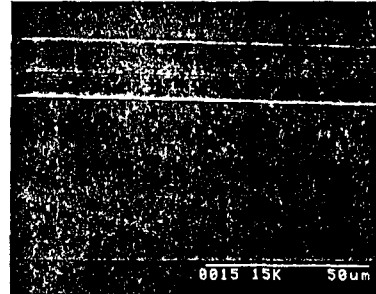


Fig 4 SEM photograph of the sample surface.

After the calcifying periods, the amount of deposited calcium was estimated according to the deformation rate. The calcified polyurethane sheet of the static system and the polyurethane sac of the dynamic system were cut into 1 cm^2 samples. For the samples of the static system, there are no difficulties in cutting the sample according to its strain rate since it was under discrete, static strain. However, in case of the polyurethane sac of the dynamic system, there is no borderline which

separates the areas of the sac having the same strain rate. Therefore, the sac had been cut roughly according to its deformation as shown in Fig 5.

The amount of deposited calcium and

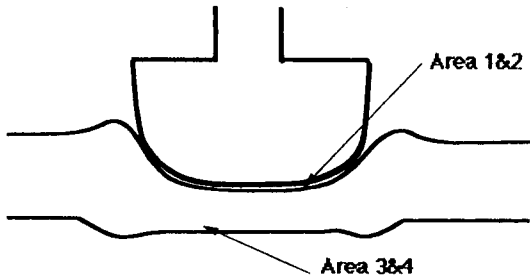


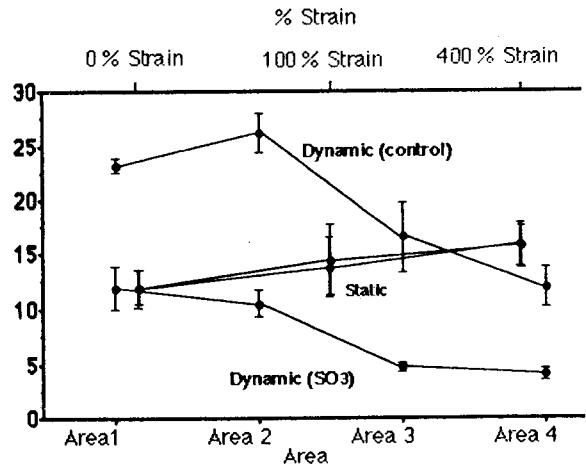
Fig 5 A schematics of the sac deformation

phosphorus is shown in table 1 and Fig 6. The sulfonated polyurethane sac exhibits less amount of calcium deposition than the unmodified one. It is already suggested by many researchers that the sulphonate group on a biomaterials surface prevents calcium deposition, although the mechanism is not discovered yet.

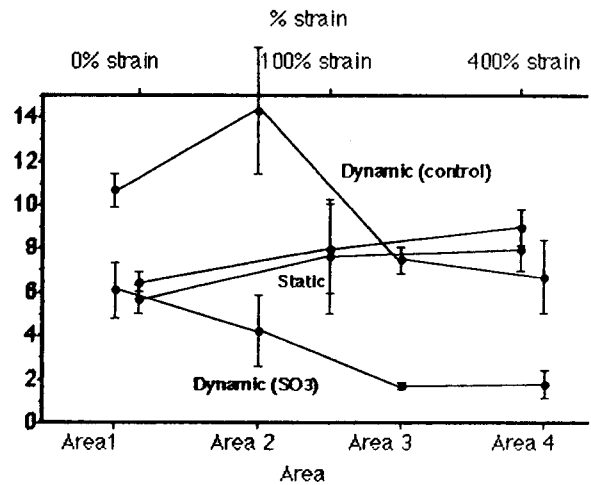
Table 1

The amount of calcium and phosphorus in the polyurethane samples

	% strain (area)	Calcium	Phosphorus
STATIC	0%	11.90±1.741	5.64±0.614
		12.17±1.576	6.43±0.492
	100%	13.04±2.604	7.62±2.610
		14.65±3.142	7.95±2.080
DYNAMIC	1	23.17±0.651	10.70±0.781
		11.97±1.960	6.13±1.273
	2	26.03±1.043	14.37±2.075
		10.57±1.542	4.23±1.629
3	16.53±3.108	7.53±0.611	
	4.73±0.404	1.73±0.153	
4	12.40±1.052	6.75±1.686	
	4.07±0.551	1.70±0.677	



(A) Calcium



(B) Phosphorus

Fig 6 Amount of calcium and phosphorus deposition

The ratio of deposited calcium and phosphorus is about 2:1. It means that the deposited material is similar to HAP. According to the data there is some relationship between the amount of calcium deposition and static strain rate. But, considering the large difference of strain rate, the difference of calcium deposition is too small to explain the large difference of calcium deposition between the regions of the polyurethane sac of the dynamic system. Therefore, it can be concluded that the mechanical stress itself does not play a

major role in the process of calcification of the polyurethane sac. It seems that there are other factors causing the heavy calcification of the severe flexing regions of the polyurethanes sac. After the calcification period, the length of the polyurethane strip was enlarged up to 290 %. It means that the total amount of calcium deposited onto the strip is about three times larger than the presented data. Therefore, it can be said that the change of surface area by the mechanical stress is one of the important factors causing heavy calcification of the severe flexing regions of the polyurethanes sac.

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