

Study of Morphology of Cardiac Valves (Human and Bovine Pericardium) after *In Vivo* Calcification

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Abstract. Pathologic calcification can lead to failure or deterioration of cardiac valves. Several researchers have tried alternatives to construct these devices, such as the incorporation or utilization of new biomaterials able to inhibit or decrease the calcification process. *In vitro* calcification tests can be used to screen new biomaterials regarding their potential to calcify *in vivo*. However, the mechanisms involved in both cases are not completely understood. In order to collect more information about the calcification process of implanted materials, morphology and elemental analyses of calcified cardiac valve fragments explanted from different patients were investigated and compared to previous reports of *in vitro* calcification tests. Scanning Electron Microscopy (SEM) and energy dispersive spectroscopy (EDS) analyses indicated that the calcium phosphate deposits from both bovine pericardium and human cardiac valves calcified *in vivo* were similar to the deposits obtained from *in vitro* calcification samples as previously reported in the literature.

Introduction

Cardiac valves are prone to undergo pathologic calcification that can cause its clinical failure and structural deterioration. The host metabolism, mechanical factors and implant structure and chemistry can be determinant to bioprosthetic valve calcification. This process is usually accelerated in young patients and can also be associated to high mechanical stress and glutaraldehyde fixation [1].

Several researchers [2-4] have been studying methods to prevent calcification in implanted biomaterials, such as therapy with anticalcification agents and biomaterials modification. The potential of new materials to calcify is generally evaluated by *in vitro* and *in vivo* calcification tests. *In vitro* calcification tests can be used as a screening method to predict the *in vivo* calcification on implanted materials [5-8], since the chemical composition of calcium deposits are comparable. However, some studies have shown that the morphologies of histological data are different and highly depended of the calcification site [4].

In previous reports we have shown the *in vitro* calcification of bovine pericardium after different treatments performed to avoid calcification [5]. In this study, we analyze the morphology of calcium phosphate deposits on bovine pericardium cardiac valves and human cardiac valves explanted from different patients. These results could be compared to the *in vitro* calcification test

results previously reported. However, further investigations need to be done to evaluate how precisely the *in vitro* tests can predict the *in vivo* calcification.

Materials and Methods

Four samples of human cardiac valves and three samples of bovine pericardium valves were supplied by the Heart Institute (InCor) of São Paulo Medical School, Brazil. The samples were fragments of *in vivo* calcified heart valves explanted from different patients and were stored in a 10% formaldehyde solution. The valve fragments were washed in a 0.9% NaCl solution and lyophilized prior to the morphological analyses. A scanning electron microscope (LEO 440i) was used to observe the morphology of sample surface. The elemental composition of deposits was analyzed using energy dispersive spectroscopy (EDS).

Results and Discussion

The SEM micrographs shown in Fig. 1 indicate that the morphology of the calcium phosphate deposits on bovine pericardium valves are similar to those observed for most of the analyzed human cardiac valves. For one sample of human cardiac valve, Fig. 1(d), a different morphology is observed: no spherical agglomerates of calcium phosphate crystals are noticed. However, the EDS analysis confirmed that all samples presented calcium phosphate deposits on their surface with Ca/P ratio of approximately 1.7, which is very close to hydroxyapatite Ca/P ratio. Therefore, there is no way to infer that different structures are being formed in each of the two types of cardiac valves that were analyzed in this study, since the Ca/P ratios were similar in both samples and the different morphology could be caused by the condition in which each sample surface was submitted before analyses.

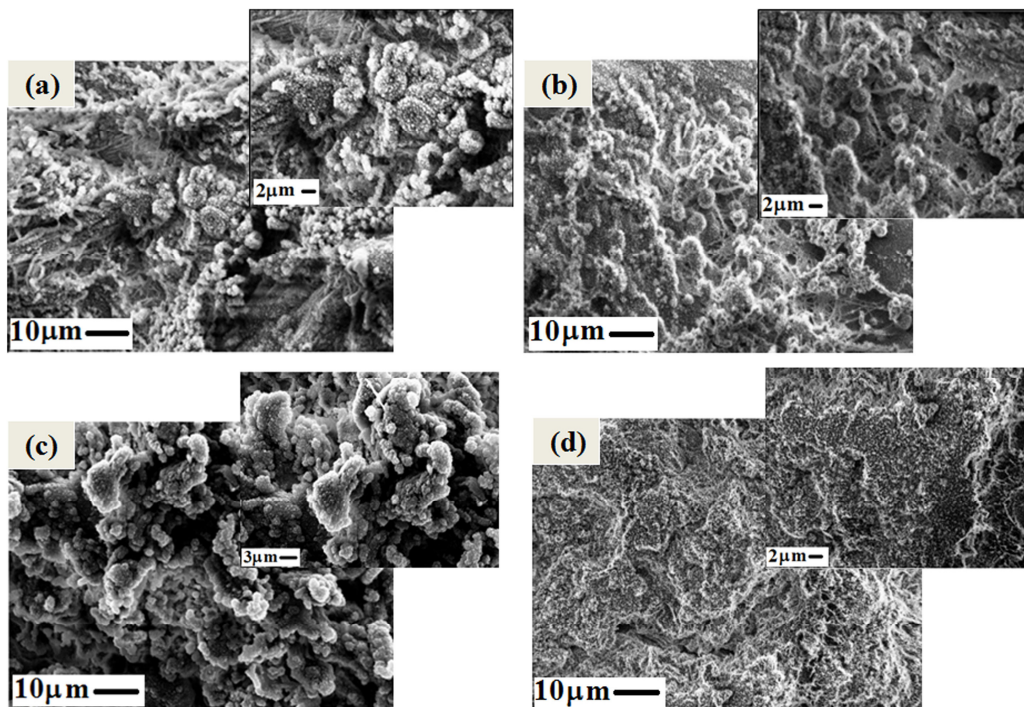


Fig. 1: SEM micrographs of bovine pericardium cardiac valves (a, b) and human cardiac valves (c,d) calcified *in vivo* explanted from different patients.

Elemental mapping for calcium, phosphorus and carbon can be observed in Figures 2 and 3 for bovine pericardium valves and human valves, respectively. Calcium, carbon and phosphorus are mapped in Fig. 2 (b), (c) and (d), respectively. The white spots indicate low concentration of the analyzed element. Image analysis indicates that calcium and phosphorus cover almost all sample surfaces even where no spherical crystals can be noticed, which could indicate that an early stage of calcium phosphate deposition is taking place or that intrinsic calcification (in the layers of tissue under the surface) occurred.

The spherical shape observed for calcium phosphate deposits on *in vivo* calcified samples is similar to that observed in our previous study of *in vitro* calcification of bovine pericardium [5]. This fact could indicate that *in vitro* calcification test results can be a good approach to the *in vivo* calcification of implanted bovine pericardium heart valves. Fine X-ray structure analyses are underway to investigate the crystal structure of the calcium phosphate deposits and therefore analyze the possible differences among the structure of these samples.

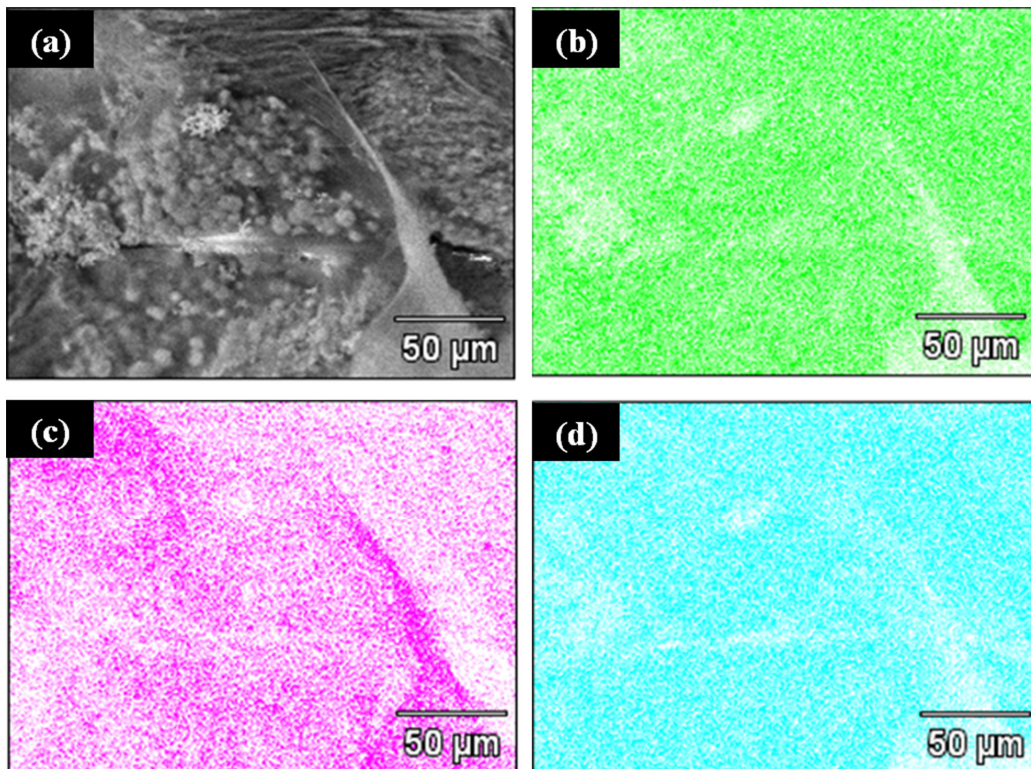


Fig. 2: SEM micrographs of bovine pericardium cardiac valve calcified *in vivo* (a) and its elemental mapping using EDS technique for (b) calcium, (c) carbon and (d) phosphorus.

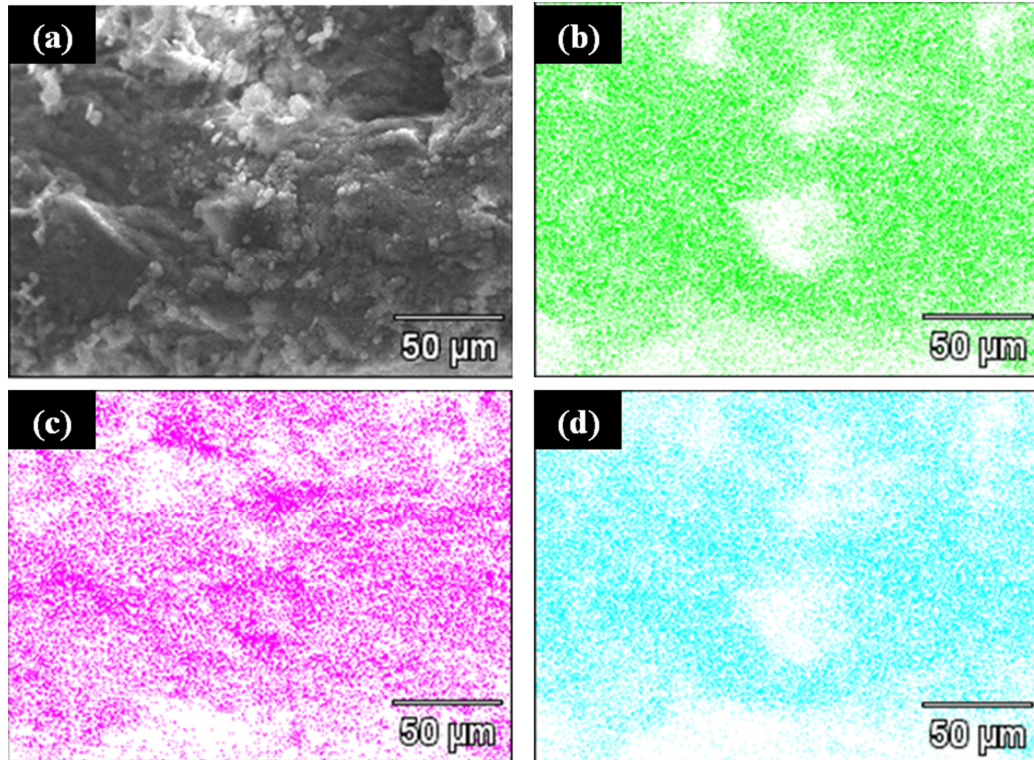


Fig. 3: SEM micrographs of human cardiac valve calcified *in vivo* (a) and its elemental mapping using EDS technique for (a) calcium, (b) carbon and (c) phosphorus.

Conclusion

The morphology and shape of calcium phosphate deposits observed on human cardiac valves were similar to those observed on bovine pericardium after *in vivo* calcification. The deposits gotten from *in vivo* process were also similar to those observed to *in vitro* calcification of valves. Further studies will indicate whether the formed compounds have also similar chemical and crystallographic structures.

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