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## Lyophilized Bovine Pericardium Coated with Silk Fibroin Solution as an Alternative to Prevent Calcification of Cardiovascular Bioprosthesis: Preliminary Calcification Results

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Calcification is the most frequent cause of the clinical failure of bovine pericardium bioprosthetic valves, preventing their widespread application for surgical treatment [1]. Previous studies from our group indicated that lyophilization may present an anticalcification effect on glutaraldehyde-treated bovine pericardium, besides the potential to promote less cytotoxicity and residual aldehydes [2].

Biological tissues used to prepare bioprosthetic heart valves are often treated to improve anticalcification properties, as well as their function and durability. Silk fibroin has been used to form a variety of biomaterials, such as films, for medical applications, due to its high cell biocompatibility, low inflammatory tissue reaction and mechanical properties.

This study investigated the calcification process with SBF solutions in bovine pericardium substrata submitted to lyophilization and treatment with silk fibroin solution.

Bovine pericardium samples were obtained from a commercial patch (Braile Biomédica, Brazil), 0.33 mm thick, pretreated with glutaraldehyde solution and stored in a 4% formaldehyde solution. The patch was extensively rinsed with 0.9% NaCl solution, cut into pieces of 4 cm<sup>2</sup> and lyophilized for 24h.

Calcification experiments were performed on three kinds of samples: non-treated control (fresh bovine pericardium rinsed with NaCl solution); lyophilized bovine pericardium and lyophilized bovine pericardium coated with silk fibroin solution.

For the preparation of fibroin coating solution, raw silk fibers of *Bombyx mori* (Bratac, Brazil) were processed three times (30 min each) in 0.5% Na<sub>2</sub>CO<sub>3</sub> solution at 85-90 °C to remove sericin (degumming), rinsed with deionized water, and dried at room temperature. The degummed fibroin (10g) was dissolved in 100 mL of the ternary solvent, CaCl<sub>2</sub>-ethanol-water, at 85 °C for 1h30min. The resulting fibroin solution was filtered and dialyzed in cellulose tubes against deionized water for 3 days at 8 °C. The final solution was diluted to 1%.

The coating of the lyophilized bovine pericardium was preformed by soaking in 1% silk fibroin solution for 20 min. After drying at room temperature, the samples were soaked in 70% ethanol solution for 1h to make silk fibroin film insoluble.

For the calcification experiments, simulated body fluid (SBF) with 1.5x the concentration of ions found in human serum was prepared by mixing solutions of NaCl, KCl, CaCl<sub>2</sub>, MgCl<sub>2</sub>, NaHCO<sub>3</sub>, K<sub>2</sub>HPO<sub>4</sub> and Na<sub>2</sub>SO<sub>4</sub> salts using "Tris" buffer, to accelerate the calcification process. "In vitro" calcification experiments were performed by immersing

the samples in SBF, changing the solutions for fresh ones in intervals of 2 days. The experiments were carried out in polyethylene flasks at 36.5±2 °C and agitation of 50 rpm. After 7 days, the samples were gently rinsed with Milli-Q water to remove SBF solution from their surface before lyophilization.

The morphological characterization and compositional analysis of the samples were performed by scanning electron microscopy (SNE) and energy dispersive X-ray spectroscopy (EDS), respectively, for observation and characterization of the possible mineral deposits formed during the calcification experiments.

In the micrographs obtained by SEM technique it was observed that none of the samples showed calcium phosphate deposits after carrying out calcification tests on 1.5x SBF solution. These results were confirmed by the EDS profiles, which confirmed the absence of calcium deposits on the samples.

Figure 1 exhibit the SEM micrographs of lyophilized bovine pericardium and lyophilized bovine pericardium coated with silk fibroin, respectively.

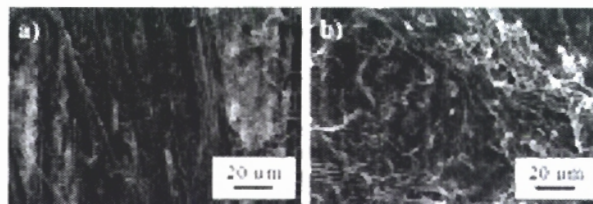


Figure 1: SEM micrographs of bovine pericardium in 1.5x SBF for 7 days (x1000) (a) lyophilized bovine pericardium, (b) lyophilized bovine pericardium coated with silk fibroin solution.

Despite its limitations, the immersion in SBF is a widely used technique that can be applied to disqualify some samples; however, it is not enough to assure that a material has really no tendency to calcify.

Based on the current results, a subsequent investigation will determine the behavior of lyophilized and fibroin coated bovine pericardium when submitted to dynamic "in vitro" calcification experiments.

1. Pettenazzo, E., *et al.* Dynamic in vitro calcification of bioprosthetic porcine valves: evidence of apatite crystallization. *The Journal of Thoracic and Cardiovascular Surgery*, 121(3), 500-509, 2001.
2. Aimoli, C. G., *et al.* Lyophilized bovine pericardium treated with a phenethylamine-diepoide as an alternative to preventing calcification of cardiovascular bioprosthesis: preliminary calcification results. *Artificial Organs*, 31(4), 278-283, 2007.

16435