Use of a novel polyvinyl alcohol membrane as a pericardial substitute reduces adhesion formation and inflammatory response after cardiac reoperation

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Background: Adhesions may increase the incidence of lethal complications of cardiac reoperations, which account for up to 20% of all open-heart surgeries. Herein, we describe the use of a polyvinyl alcohol membrane (PVAM) as a pericardial alternative and describe its performance during reoperation in a relevant animal model.

Methods: The PVAM samples were reticulated by electron beam radiation and manipulated into a tube shape. After thoracotomy, the pericardium of Wistar rats was opened to expose the heart. Rats were treated by pushing the heart back into the thoracic cavity (Sham group), sprinkling the epicardium with talcum powder (Talc group), encircling the heart with PVAM (PVAM group), or sprinkling the epicardium with talcum powder before placing the PVAM to encircle the heart (PVAM + Talc group). Animals were recovered for 8 weeks and then euthanized. Macroscopic findings (ie, extent and severity of adhesions) were classified according to a 4-grade adhesion scale. The PVAM was tested for direct and indirect cytotoxicity with Vero cells. The water absorption capability and in vivo calcification after 8 weeks of subcutaneous implantation of the membrane were examined. Data were analyzed by analysis of variance and Bonferroni post hoc tests.

Results: The PVAM group had lower adhesion scores than the Talc and Sham groups, as well as reduced epicardium thickness and inflammatory cell results, compared with the Talc and PVAM + Talc groups. The PVAM exhibited no direct or indirect cytotoxicity, good water absorption capability (42.4% $\pm$ 0.9%), and negligible calcification after 8 weeks ($4.42 \times 10^{-3} \pm 2.56 \times 10^{-3}$ percentage of the total mass).

Conclusions: The PVAM shows promising properties for its potential use as a novel pericardial substitute. (J Thorac Cardiovasc Surg 2014;147:1405-10)

Pericardial and mediastinal adhesions increase the mortality and morbidity rates after cardiac reoperations.[1,2] Many individuals with congenital heart defects will require multiple repeat sternotomies. Numerous strategies have been described for reducing postoperative adhesions, such as sprinkling solutions into the pericardial sac or using expanded polytetrafluoroethylene (ePTFE) or bioabsorbable gelatin sheets.[3-5] However, there is no accepted approach for reducing adhesion formation after cardiac surgery.[6] Herein, we describe a novel membrane assembled from 10% polyvinyl alcohol (PVA). We examined the use of this PVA hydrogel membrane (PVAM) as a pericardial substitute in an animal model of adhesion formation, focusing on its cytotoxic effects and inflammatory potential.

METHODS

Assembling the PVAM

The PVAM was made as described previously.[7] Briefly, the membrane was assembled from an aqueous solution of 10% PVA (Sigma Aldrich, São Paulo, Brazil), which was kept at room temperature for 7 days until dry. The dried membrane was 2-mm thick, and it was manipulated into a tube-like shape (1.5-cm height and 0.5-cm internal diameter).

The PVAM was subjected to a cross-linking step to stabilize the polymer and prevent its dissolution in contacted fluids. This step has prevented PVAM dissolution for at least 24 weeks after implantation in bone tissue.[7] Electron beam irradiation, which can simultaneously accomplish the reticulation and sterilization of the membranes, was performed for the cross-linking step. Before irradiation, the material was acetylated by immersion in a solution of 40% formaldehyde, 50% sulfur acid, and 300 g of anhydrous sodium sulfate at 60°C for 24 hours. The PVAMs were washed to remove any residual chemical and stored in distilled water. The membranes were cross-linked by electron beam irradiation with 25 kGy using a Radiation Dynamics electron beam accelerator (Instituto de Pesquisas Energéticas e Nucleares, São Paulo). After this process, the PVAMs were considered to be sterilized and ready for use.

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Abbreviations and Acronyms
CMC = carboxymethylcellulose
ePTFE = expanded polytetrafluoroethylene
PVAM = polyvinyl alcohol membrane

Surgical Protocol

Ethical approval was obtained from the local institutional review board. All animal handling and experiments were performed in accordance with the standards of the Brazilian Council for Animal Experimentation and the 1996 Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health. Animals were maintained under specific pathogen-free conditions, a 12-hour/12-hour light/dark cycle, and a room temperature of 21°C.

Four-week-old male Wistar rats (average weight, 300 g) were anesthetized with 2% isoflurane. A left thoracotomy was performed, the pericardium was opened, and the heart was exposed. Animals were randomized into 4 groups (10 animals per group). In the Sham group, the heart was pushed back to the thoracic cavity, and the incision was closed. In the Talc group, talcum powder (10 mg) was sprinkled over the epicardium. In the PVAM group, a PVAM tube was inserted into the thoracic cavity and used to encompass the heart from the surrounding tissue, except for where the major vessels exited/entered the heart. Finally, in the PVAM + Talc group, talcum powder (10 mg) was sprinkled over the epicardium, and then a PVAM tube was inserted into the thoracic cavity and used to encompass the heart completely. No sutures were needed to hold the PVAM tube in place, because of its flexibility and the limited nature of the thoracic space.

All animals were recovered from anesthesia for 8 weeks, during which time they were provided standard chow and water ad libitum. Eight weeks after the surgical protocol, the animals were euthanized with an intravenous dose of pentobarbital, the thoracic cavity was opened, and any adhesions were lysed. Two observers (P.P.M.O. and V.P.B.), blinded to each other, scored the adhesions from 0 to 3, according to an adaptation of the score described by Kajihara and colleagues[8]: 0, absence of adhesions; 1, weak adhesions that are easily lysed; 2, moderate adhesions that are lysed with dissection; and 3, severe adhesions that require sectioning by scissors for removal. In all animals, the PVAM tube was inspected for its correct position involving the heart.

Histologic Assessment

Cardiac tissue was fixed in 10% paraformaldehyde, embedded in paraffin, cut into slices, and stained with hematoxylin and eosin. For the inflammatory cell count and epicardial thickness assessments, a midportion of the left ventricle (ie, at the papillary muscle level) was used in all animals. A blinded observer randomly chose 3 optical fields (40× magnification) to evaluate the inflammatory cell count and the epicardial thickness.

Physical and Biological Characteristics of the PVAMs

Indirect and direct cytotoxicity assays were performed with an African green monkey epithelial kidney cell line (Vero cells; Instituto Adolfo Luftz, São Paulo). To assess the indirect cytotoxicity, PVAMs (0.2 g/mL) in the liquid state without cross-linking were mixed in HAM F-10 media (Sigma Aldrich) containing 10% fetal bovine serum (Nutricell, Campinas, Brazil) at 37°C. Different concentrations of the membrane solution were added to cell media containing 3×10^5 cells/mL. After 24 hours, the cell viability was assessed with the (dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide colorimetric method at 550 nm.[9] The negative control was an extract of 2% polystyrene in a solution of 10% bovine serum albumin and 10% phenol.

To assess the direct cytotoxicity, Vero cells were seeded and grown over reticulated and sterilized PVAMs. HAM F-10 media containing 10% fetal bovine serum was added, and the cells were grown at 37°C for 24 hours. Cell viability was assessed by the MMT (3-[4,5-dimethylthiazol-2-yl]-2,5- diphenyltetrazolium bromide; thiazolyl blue) colorimetric method.[9] Two controls were used: a positive control, containing 10% phenol in the media for 24 hours; and a negative control, containing only media.

The hydration capacity of the membranes was evaluated with dry samples of PVAM immersed in distilled water until a stable membrane weight was reached. Five measurements were made, and the hydration capacity was expressed as a percentage. The in vivo calcification index was determined by implanting a PVAM (1 cm^2) subcutaneously (s.c.) on

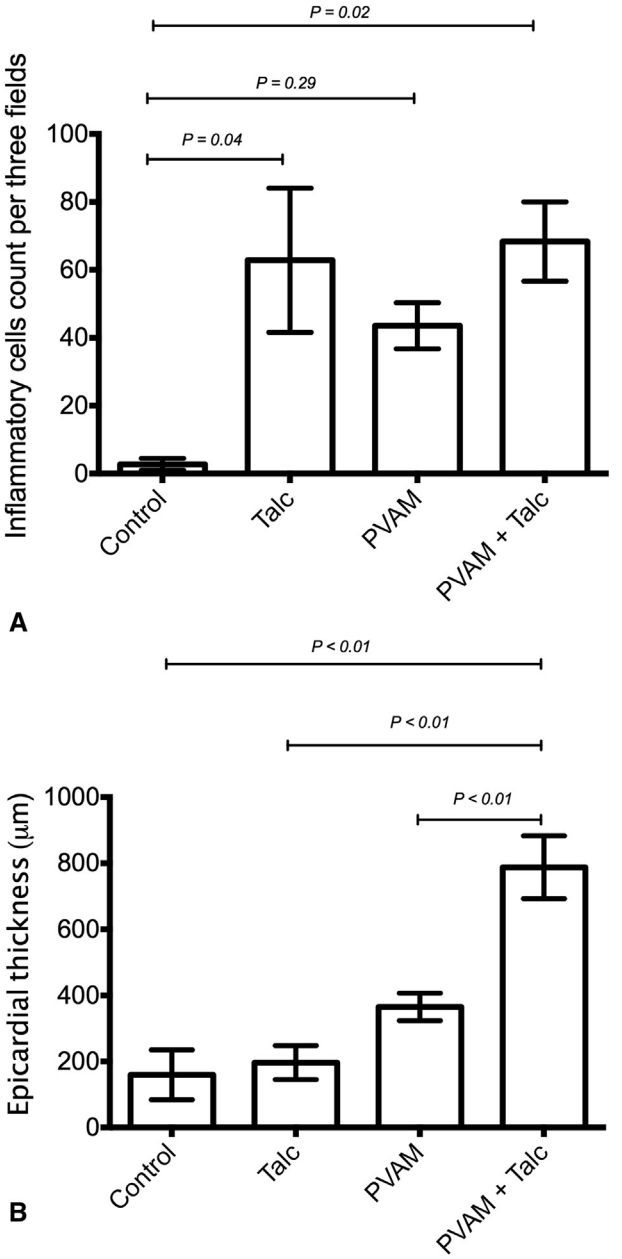

FIGURE 1. Inflammatory cell count (A) and epicardial thickness (B) in all 4 groups after 8 weeks of observation. *Bar height* indicates mean, with error bar showing standard error of mean. *P* values by analysis of variance with post hoc Bonferroni for multiple comparisons. *PVAM*, Polyvinyl alcohol membrane.

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