A Plasma-Based, Amiodarone-Impregnated Material Decreases Susceptibility to Atrial Fibrillation in a Post–Cardiac Surgery Model

David Schwartzman, MD, Vinay Badhwar, MD,*† Robert L. Kormos, MD,*† Jason D. Smith, PhD,‡ Phil G. Campbell, PhD,‡§|| and Lee E. Weiss, PhD||¶*

Objective: This study aimed to test the impact of a plasma-based, material (PBM) impregnated with amiodarone on atrial electrophysiology and atrial fibrillation susceptibility in a porcine post–cardiac surgery model.

Methods: Ten healthy pigs underwent implantation of transvenous pacing systems, after which sterile talc was infused into the pericardial sac via a pericardiotomy. In five animals, PBM was applied to the atrial epicardial surface just before talc infusion. Electrophysiologic evaluations were performed using the pacing system immediately after chest closure and 7 days later. Atrial histologic evaluations were performed.

Results: Immediately after chest closure, there were no significant differences in electrophysiologic parameters between talc-only and talc + PBM animals, and atrial fibrillation was largely noninducible. On postsurgical day 7, electrophysiologic evaluation revealed significantly shorter sinus cycle length and atrioventricular nodal refractoriness among talc-only animals relative to talc + PBM animals, possibly suggesting attenuated sympathetic nervous system activation in the latter. Atrial fibrillation inducibility and duration were significantly greater among talc-only animals. No significant differences in atrial refractoriness or conduction time between groups were apparent. Histologic evaluation revealed a relative reduction in epicardial inflammation and less myolysis among talc + PBM animals.

Conclusions: Epicardial application of a plasma-based, amiodarone-impregnated material was associated with a significant reduction in atrial inflammation and susceptibility to fibrillation.

Key Words: Atrial fibrillation, Amiodarone, Plasma-based material, Platelet-rich plasma, Pericardium.

(*Innovations* 2016;11:59–63)

Postoperative atrial fibrillation (AF) occurring early after cardiac surgery, a manifestation of atrial injury, continues to be an important problem, occurring in approximately 30% of patients after isolated coronary artery bypass grafting, 40% after valve repair/replacement, and 50% after combined procedures.[1,2] The occurrence of postoperative AF has important consequences, including increases in mortality, morbidity, length of stay, and cost of care.[2]

Amiodarone has proven effective in suppression of postoperative AF.[3] However, oral or intravenous delivery of amiodarone has multiple disadvantages, including the requirement of a loading period, drug-drug interactions, extracardiac toxicity, and unpleasant side effects. These are attributable to systemic drug delivery. The utility of direct amiodarone delivery to the atria, wherein systemic exposure is miniscule, has been previously demonstrated.[4,5]

Blood plasma is a complex material, composed of a multitude of growth factors and cytokines, each with roles in tissue healing.[6] Clinical use of plasma materials, including platelet-rich plasma, has been shown to accelerate healing after orthopedic, neurological, and cardiac surgery.[6,7] Thus far, the use in cardiac surgery has targeted noncardiac tissues (sternum, skin), although preliminary results hold promise for direct cardiac applications.[8]

Given the antiarrhythmic effect of amiodarone and accelerated healing associated with plasma-based materials, we hypothesized that direct atrial epicardial application of a combined material at the time of cardiac surgery would reduce susceptibility to AF. The present report summarizes a preliminary experience with this material in a porcine model.

Accepted for publication November 10, 2015.

From the *Heart, Lung and Vascular Medicine Institute and †Department of Cardiothoracic Surgery, University of Pittsburgh, Pittsburgh, PA USA; ‡Carmell Therapeutics Corporation, Pittsburgh, PA USA; and §Institute for Complex Engineered Systems, ||Robotics Institute, and ¶Department of Biomedical Engineering, Carnegie Mellon University, Pittsburgh, PA USA.

Supported by the Heinz Endowments, Pittsburgh, PA USA. Carmell Therapeutics Corporation, Pittsburgh, PA USA, had no input into the study design, data acquisition, data analysis, and manuscript preparation.

Disclosures: David Schwartzman, MD, Lee E. Weiss, PhD, and Phil G. Campbell, PhD, have disclosed an invention, to be assigned to Carnegie Mellon University, Pittsburgh, PA USA, and the University of Pittsburgh, Pittsburgh, PA USA, which covers PBM for the field of use of amiodarone delivery and postoperative atrial fibrillation prevention. Dr Schwartzman is a consultant for AtriCure, Inc, West Chester, OH USA; Avery-Dennison, Pasadena, CA USA; Biosense, Diamond Bar, CA USA; QuantMD, Pittsburgh, PA USA; and Medtronic, Inc, Minneapolis, MN USA. Dr Schwartzman receives a research grant support from Biosense, Diamond Bar, CA USA; Boston Scientific, Marlborough, MA USA; and Medtronic, Inc, Minneapolis, MN USA. Jason D. Smith, PhD, is an employee of Carmell Therapeutics Corporation, Pittsburgh, PA USA. Drs Campbell and Weiss are cofounders of Carmell Therapeutics Corporation, Pittsburgh, PA USA, and hold equity in the company. Vinay Badhwar, MD, and Robert L. Kormos, MD, declare no conflicts of interest.

Address correspondence and reprint requests to David Schwartzman, MD, University of Pittsburgh Medical Center, Presbyterian, B535, Pittsburgh, PA 15213–2582 USA. E-mail: schwartzmand@upmc.edu.

ISSN: 1556-9845/16/1101-0059

METHODS

Plasma-Based, Amiodarone-Impregnated Material

The material, hereinafter termed *PBM*, was obtained from Carmell Therapeutics Corporation (Pittsburgh, PA USA). The production of plasma-based materials has been detailed previously.[6] The PBM was derived from pooled units of frozen human blood plasma, including platelets, which were clotted with calcium chloride, freeze dried, ground into a powder, and subjected to viral inactivation. The powder was mixed with glycerol into a dough, into which amiodarone hydrochloride (3% wt/wt, Fisher Scientific, Pittsburgh, PA USA) was added. The dough was compression molded at 70°C and then cryogenically milled to form a powder with a particle diameter of less than 150 μm. Individual powder aliquots (1-cm^3 volume, containing 1.2 g of plasma and 36 mg of amiodarone) were packaged in foil pouches, terminally irradiated at 30 kGy (FTSI, Mulberry, FL USA), and stored at room temperature. The PBM, which is fully biodegradable, was prepared for use by sonicating with 5-mL of sterile water, forming a gel.

Experimental Techniques

This protocol was approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh and conformed to the position of the American Physiological Society on research animal use. All animals underwent identical surgical procedures. Ten large (40–60 kg) adult male mixed-breed pigs were studied.

Day 0

After endotracheal intubation, a surgical plane of anesthesia was initiated using isoflurane. A skin incision was made in the right cervical region, ventrally, and carried down to the internal jugular vein. This vein was cannulated, and two commercial active fixation pacing leads (Fineline model 4470; Boston Scientific Inc, St. Paul, MN USA) were implanted into the right atrium under multiplane fluoroscopic guidance. One lead was implanted into the right atrial free wall at the base of the appendage and the other into the right atrial septal wall adjacent to the fossa ovalis. After documentation of adequate pacing and sensing behavior, the leads were attached to a commercial pulse generator (Insync III; Medtronic, Minneapolis, MN USA), which was implanted subcutaneously in the ventral neck region.

After pacemaker system implantation, a left lateral thoracotomy was performed, and the lung was retracted to expose the pericardial sac. The sac was incised longitudinally (incision length, approximately 8 cm) along the lateral border of the left heart. Animals were then randomly assigned to one of two groups: (1) talc only (n = 5) where a slurry of sterile talcum powder (5 g suspended in 20-mL of sterile saline) was infused into the pericardial space favoring the base of the heart; (2) talc + PBM (n = 5) where approximately 5 minutes before the infusion of the same talc slurry as in the talc-only group, PBM gel was uniformly applied to accessible right and left atrial surfaces using a soft brush. In both groups, approximately 30 minutes after talc infusion, the pericardium and chest were closed.

Approximately 30 minutes after closure of the chest, an electrophysiologic evaluation was performed using the pacing system. Measured indices included (1) sinus cycle length; (2) right atrial (RA) free wall effective refractory period, defined as the longest atrial coupling interval, which achieved local capture after a 12-beat atrial drive (500-millisecond cycle length, unipolar pacing, three times threshold voltage, 0.5-millisecond pulse width); (3) RA septal effective refractory period; (4) atrioventricular (AV) nodal effective refractory period (defined using RA free wall pacing); (5) RA free wall to RA septal conduction time, defined during RA free wall pacing at a cycle length of 300 milliseconds; (6) AF inducibility, defined as the likelihood (success as a proportion of total attempts) of AF after a 30-second burst of atrial high rate/output pacing (cycle length, 150 milliseconds; output, 7.5 volts per 0.9 milliseconds); and (7) AF duration, defined from the moment of burst pacing cessation until spontaneous termination or until 5 minutes had elapsed, whichever came first. After 5 minutes, transthoracic direct current cardioversion was performed; in this situation, AF duration was defined as 5 minutes. Atrial fibrillation was defined by varying atrial morphology and irregularly irregular ventricular response on surface electrocardiogram, associated with atrial free wall bipolar electrogram, which had an average cycle length of less than 250 milliseconds (Fig. 1). Each index was measured 10 times, and the average value was calculated. After electrophysiologic evaluation, animals were recovered. Pacemakers were programmed so as not to pace but to detect any atrial tachyarrhythmia episode, defined by an atrial cycle length of less than 250 milliseconds.

Day 7

Animals were returned to the operating room, and a surgical plane of anesthesia was once again initiated. Transthoracic echocardiography was performed using a commercial system with 3.0-MHz transducer (Aplio, Toshiba Medical Systems, Tokyo, Japan). Apical four-chamber and short-axis windows were used to assay cardiac chamber dimensions and motion, valvular function, and the pericardial space. The electrophysiologic evaluation was then repeated. Animals were then killed, after which a midline sternotomy was performed, and the heart and lungs were removed en bloc. After visual inspection, transmural samples of the left atrial free wall were taken, with each stored in 4% paraformaldehyde. Microscopic assessment, performed by a single operator blinded to the tissue source, was performed on 10 separate sections (10-μm thickness) of these samples after staining with hematoxylin and eosin. When focusing on the myocardial compartment contiguous to the epicardial surface, inflammatory cell types were identified visually, and the degree of myolysis was estimated as mild (score, 1), moderate (score, 2), or severe (score, 3).

Analytical Methods

Data are reported as mean (SD). Comparisons of continuous variables within groups (day 0 vs day 7) were performed using Wilcoxon rank sum test and between groups using a Mann-Whitney *U* test. Comparisons of categorical

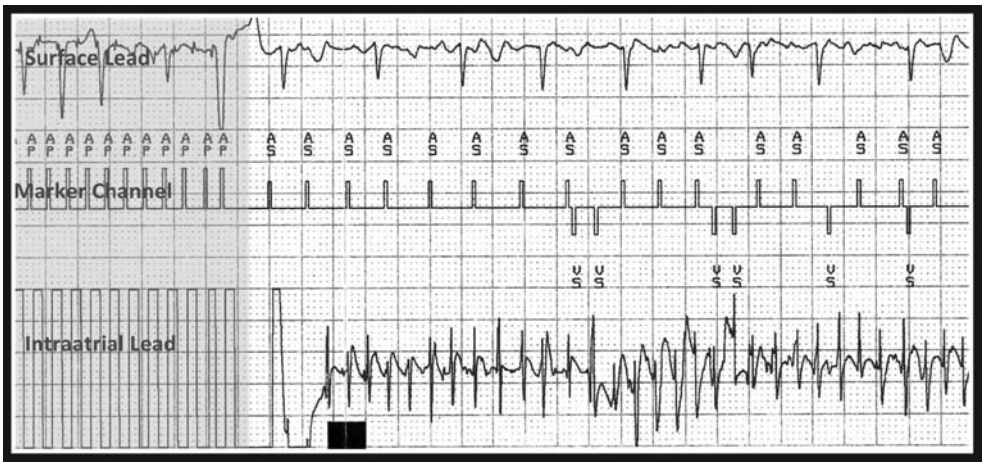

FIGURE 1. Representative recordings from pacemaker on day 7, demonstrating surface electrocardiographic lead (top), software marker channels, which provide insight into device sensing/pacing activities (middle; AS demarcates an atrial sensed event and VS demarcates a ventricular sensed event), and right atrial free wall bipolar intracardiac electrogram (bottom). The recording demonstrates induction of AF by atrial tachypacing (shaded region). The induced rhythm demonstrates varying atrial morphology and irregularly irregular ventricular response on the surface lead, associated with atrial free wall bipolar electrogram, which had an average cycle length of less than 250 milliseconds, AF, atrial fibrillation.

variables were performed using the Fisher exact test. For each test, a $P < 0.05$ was considered significant.

RESULTS

There were five animals in the talc-only group and five animals in the talc + PBM group. On day 0, electrophysiologic evaluation demonstrated no significant differences between talc-only and talc + PBM animals (Table 1). Atrial fibrillation, albeit brief, was inducible in a minority of attempts in two animals in each group. During the subsequent ambulatory evaluation period (7 days), no spontaneous atrial tachyarrhythmias were detected in any animal. Transthoracic echocardiographic evaluation on day 7 revealed normal chamber dimensions, systolic function, valve function, and filling pressures. No significant pericardial fluid collections were observed. Electrophysiologic evaluation on day 7 demonstrated a significant decrease in sinus cycle length and AV nodal effective refractory period relative to baseline among the talc-only animals but not among the talc + PBM animals (Table 1). Atrial fibrillation was inducible in all five talc-only animals versus two of five talc + PBM animals ($P = 0.16$). One or more cardioversions were required in three talc-only animals, whereas none was required in the talc + PBM animals ($P = 0.17$). A significantly higher proportion of induction attempts resulted in AF among talc-only animals than among talc + PBM animals (Table 1). Relative to day 0, the average duration of induced AF was significantly longer in both talc-only and talc + PBM groups, but the degree of prolongation was significantly greater among talc-only animals than among talc + PBM animals (Table 1).

Gross examination of the atria on day 7 revealed clear differences between the talc-only and talc + PBM animals, with the latter relatively free of fibrinous exudate and adhesions (Fig. 2). Histologically, the left atrial epimyocardium in the talc-only group demonstrated confluent granulomatous inflammation of the foreign body (histiocytic) type, with a myolysis score of 2.5 (0.5) (Fig. 3). By contrast, the inflammatory process in the talc + PBM group was patchy. In regions of active inflammation, a more mixed cell picture was apparent, including histiocytes, lymphocytes, and eosinophils, and the myolysis score was 1.7 (0.6) ($P = 0.03$ vs talc-only, Fig. 4).

DISCUSSION

Atrial fibrillation occurring early after cardiac surgery may be promoted by a variety of elements, including direct atrial trauma, atrial inflammation, and neurohormonal sequelae.[2,9]

TABLE 1. Electrophysiology Data

	Talc Only (Day 0)	Talc + PBM (Day 0)	Talc Only (Day 7)	Talc + PBM (Day 7)
Sinus cycle length, ms	821 (42)	893 (61)	627 (22)*	851 (54)
RA free wall ERP, ms	196 (23)	181 (12)	178 (17)	191 (14)
RA septum ERP, ms	187 (42)	179 (22)	190 (13)	183 (15)
AV node ERP, ms	263 (31)	287 (33)	211 (18)*	267 (29)
RA conduction time, ms	34 (18)	41 (20)	38 (14)	39 (11)
AF inducibility (per 10 attempts)	1.7 (0.9)	1.4 (1.6)	8.1 (1.6)*	2.2 (1.3)†
AF duration, s	6 (4)	4 (5)	228 (78)*	21 (9)*†

*$P < 0.05$ versus baseline within group.
†$P < 0.05$ between groups on the same day.
AF, atrial fibrillation; AV, atrioventricular; ERP, effective refractory period; RA, right atrium.

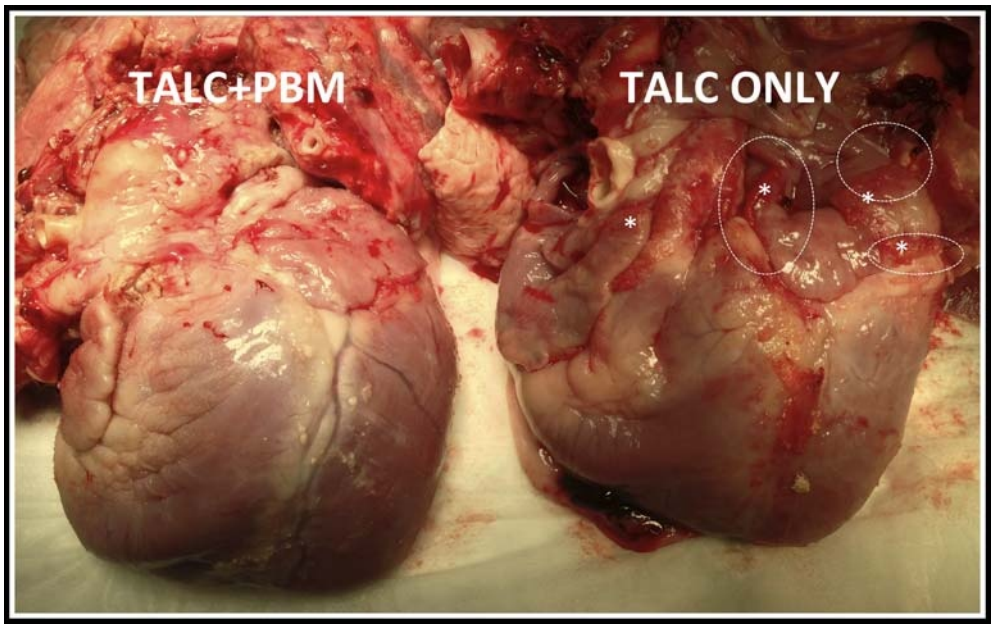

FIGURE 2. Representative gross whole heart specimens from talc-only (right) and talc + PBM (left) groups. The atria of the talc + PBM specimen have less fibrinous exudate (asterisks) and adhesions (circled).

Each of these may be expected after pericardiotomy and direct cardiac manipulation, with or without cardiopulmonary bypass. The peak arrhythmia incidence is between postoperative days 2 and 4; 70% of affected patients will manifest by day 4 and approximately 95% by day 6.[1,2] Herein, we applied an amiodarone-impregnated PBM paste to atrial epicardial surfaces at the time of surgery, with the intent of decreasing susceptibility to AF. Although no spontaneous AF was observed between days 0 and 7 in either group, animals in the talc-only group seemed more susceptible to induction than those in the talc + PBM group.

Several mechanisms may explain the relative decrease in AF susceptibility among the talc + PBM animals: (1) the antiarrhythmic effect of amiodarone. When added to pericardial fluid, which in health bathes a significant proportion of the atrial mass, amiodarone has been demonstrated to achieve significant atrial tissue levels.[10,11] Similar findings have attended application of amiodarone-containing hydrogel patches to the atrial surface.[4,5] Of note, to our knowledge, this is the first report of targeted atrial delivery of a drug in which active fixation (eg, suture or adhesive) was not required, which would be

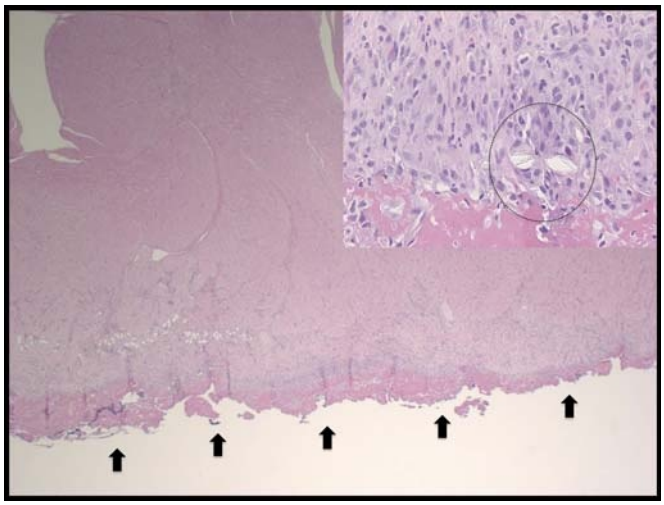

FIGURE 3. Low (2×) and high (inset, 40×) magnification photomicrographs of representative left atrial free wall cross-section from a talc-only animal, demonstrating confluent epimyocardial granulomatous inflammation with fibrinous exudate (arrows). Epithelioid histiocytes with engulfed foreign material are prominent (circled).

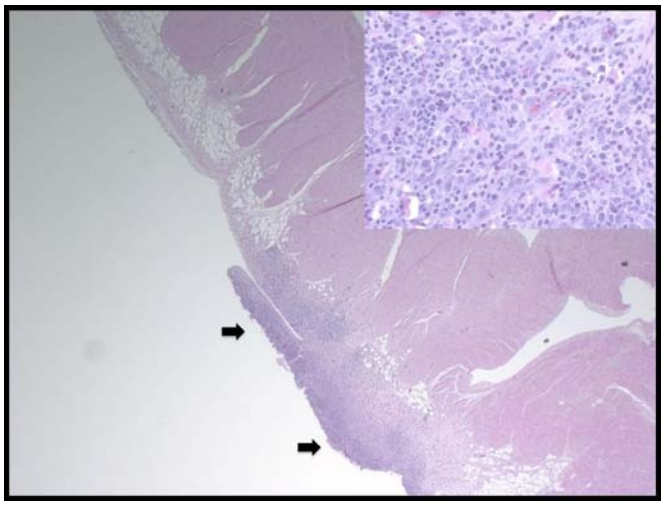

FIGURE 4. Low (2×) and high (inset, 40×) magnification photomicrographs of representative left atrial free wall cross-section from a talc + PBM animal, demonstrating patchy epimyocardial inflammation (arrows). A more mixed inflammatory cell picture is observed, including histiocytes, lymphocytes, and eosinophils.

advantageous for clinical use. (2) Growth factors or cytokines contained in the PBM material may have ameliorated atrial injury and/or accelerated healing, with an attendant antiarrhythmic effect. Evidence in favor of this effect included a less confluent epicardial inflammatory process attended by a reduced magnitude of myolysis. (3) The relative preservation of sinus cycle length and AV nodal refractoriness on day 7 among talc + PBM animals may suggest that there was less sympathetic nervous system activation, which could have been caused by less inflammation of cardiac autonomic nerve and/or cardiac tissues. This could have had an antiarrhythmic effect.

Our findings have several limitations. First, despite the observed significant differences among several electrophysiologic and histologic indices, the small number of animals in each group limits confidence in the findings. Second, although intrapericardial installation of talc is a well-reported technique for creating an atrial inflammatory milieu typical of cardiac surgery, in the present study, no direct cardiac surgery was performed. Third, during the observation period, no spontaneous AF was observed. Clinically, the use of atrial stimulation for assessing susceptibility to spontaneous AF is imperfect. Fourth, we did not study animals using PBM without amiodarone, which prohibits conclusions as to the relative roles of PBM and amiodarone in the observed reduction in AF susceptibility.

Fifth, we did not analyze amiodarone levels in pericardial fluid or in atrial tissue. Sixth, the pericardium was closed, which may have fostered retention of the PBM material relative to the open pericardium, which is typically practiced. Finally, we did not perform quantitative histologic analysis in which immune cell identities and proportions were clearly delineated.

In summary, in this preliminary animal evaluation, direct epicardial application of a plasma-based, amiodarone-impregnated material was associated with a reduction in atrial inflammation and susceptibility to fibrillation.

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CLINICAL PERSPECTIVE

This experimental study examined the impact of a plasma-based amiodarone-impregnated material (PBM) on atrial electrophysiology and atrial fibrillation (AF) susceptibility in a porcine model. In 10 animals, sterile talc was infused into the pericardial sac via a pericardiotomy. Five animals had talc only, and the other five had talc + PBM. On postsurgical day 7, electrophysiological evaluation revealed a significantly shorter sinus cycle length and atrioventricular nodal refractoriness in the talc-only animals compared with the talc + PBM group. Atrial fibrillation inducibility and duration were significantly higher in the talc-only animals.

This is an interesting study that suggests a novel approach to preventing postoperative AF. However, this study has a number of limitations. The talc model may not be directly applicable to the clinical situation. On top of this, there were relatively few animals in each group. They also did not include a group that included just the plasma-based material without amiodarone to evaluate the particular benefits of this antiarrhythmic drug added to the PBM. Finally, the authors did not analyze amiodarone levels in the pericardial fluid or atrial tissue, and they only examined inducibility of AF since animals had no spontaneous AF. With these limitations in mind, however, this preliminary study does suggest that direct epicardial application of a PBM reduces atrial inflammation and susceptibility to AF in a porcine model.

