A new absorbable collagen membrane to reduce adhesions in cardiac surgery

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Abstract

Reduction of sternal adhesions is still an issue in cardiac surgery. To evaluate a new fibrillar porcine collagen absorbable membrane (Cova™ CARD), 16 sheep underwent a sternotomy followed by scratching of surface of the heart. They were then divided into three groups: pericardium left opened (n = 4), placement of Seprafilm®, the reference absorbable substitute (hyaluronic acid and carboxymethylcellulose, n = 6) or of Cova™ CARD membrane (n = 6). Four months thereafter, the animals underwent repeat sternotomy and were macroscopically assessed for the degree of resorption of the material and the intensity of adhesions. Explanted hearts were blindly evaluated for the magnitude of the inflammatory response and fibrosis. The Cova™ CARD membrane was almost totally absorbed by four months and replaced by a loosely adherent tissue. There was no inflammatory reaction and both the extent and density of fibrosis were minimal. The composite score (median [min;max]) integrating tightness of adhesions and histological findings of inflammation and fibrosis was two-fold lower in the Cova™ CARD than in the Seprafilm® group (2.0 [0;3.5] vs. 5.5 [3;7], P = 0.01 by Wilcoxon test). The Cova™ CARD membrane might represent an attractive pericardial substitute for preventing postoperative adhesions in cardiac surgery.

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1. Introduction

Despite continuous improvements in preoperative imaging modalities and surgical techniques, reoperations in cardiac surgery remain challenging because injury to the heart, great vessels or bypass conduits can be life-threatening. Over the past decades, several pericardial substitutes have been developed that broadly fall into two categories: non-absorbable, exemplified by the e-polytetrafluoroethylene (ePTFE) membrane and absorbable. The latter are expected to provide superior healing by guiding tissue regeneration as shown in visceral and periodontal surgeries [1]. However, they also raise technical challenges as they have to combine mechanical strength, sutureability and resorption time matching the kinetics of the natural healing process. The present study was designed to test a new collagen-based pericardial substitute (Cova™ CARD, Biom’Up, Lyon, France).

2. Materials and methods

Sixteen pré-Alpes sheep, weighing ~60 kg, were used in this study which was approved by our Institutional Review Board and conducted in compliance with the European Convention on Animal Care.

2.1. Preparation of pericardial membranes

Cova™ CARD is a CE marked patented membrane made of purified porcine type I collagen. Briefly, porcine tendons are washed and incubated in an acid solution. Then, the swollen tendons are ground and mechanically treated for the collagen extraction. Purification of the collagen is performed according to the classical steps of solubilisation and precipitation in acidic conditions with sodium chloride. A viral inactivation step, shown to inactive viruses and bacteria is performed at the end of the purification process [2]. The collagen obtained is processed to yield an elastic,
resorbable and sutureable membrane and cross-linked with an oxidised polysaccharide. This cross-linking agent provides reproducible and biocompatible medical devices with a controlled resorption time [3] without cellular toxicity. Under dry shape, the membrane has a thickness of approximately 100 μm.

2.2. Surgical protocol

After isoflurane-based anaesthesia and endotracheal intubation, a median sternotomy was performed, the pericardium longitudinally opened and a 4-cm wide, 20 cm long strip of pericardium removed. The epicardium was smoothly scratched with a gauze to create local inflammation and the sheep were then randomly allocated to one of the following three groups. In four animals, the pericardial edges were not reapproximated, thus, leaving a central defect similar to what commonly occurs after cardiac operations (Controls). In six sheep, the defect was closed by the epicardial application of a Seprafilm® membrane (Genzyme, Cambridge, MA, USA) which currently represents the most commonly used pericardial substitute. In six other animals, the defect was closed with the Cova™ CARD membrane which was anchored to the pericardial edges by a few 4/0 polypropylene sutures. The chest was then closed conventionally with steel wires.

Four months later, the animals underwent a repeat sternotomy under a similar anaesthesia protocol. The surgeon, blinded to the treatment group, graded adhesion formation according to the score defined by Heydorn et al. [4]: grade 0: no adhesions; grade 1: light adhesions easily lysed by digital dissection; grade 2: stronger adhesions; and grade 3: dense adhesions requiring sharp dissection. One score was established for each animal. The sheep were then euthanized with an overdose of IV Dolethal. An in-block fragment was obtained of the anterior surface of the heart and formed, from the inside to the outside, by the right ventricular wall, the adhesion tissue and sometimes bone fragments. Specimens were fixed in 10% formaldehyde, embedded in paraffin and sectioned into 5 μm slices which were stained with hematoxylin-eosin (H&E) and Masson’s trichrome to assess the inflammatory reaction and check for remnants of the pericardial substitute in the two treated groups.

2.3. Light microscopic examination

Sections were examined using a 5× magnification objective lens by a pathologist blinded to the treatment group. The severity of the inflammatory reaction was based on the quantification of inflammatory cells (i.e., neutrophils, plasma cells, lymphocytes) and inflammatory foci and classified from 0 to 3, using an inflammatory reaction score derived from the one described by Lu et al. [5]: grade 0 = no cell infiltration; 1 = sparse inflammatory cell infiltration; 2 = focal inflammatory cell infiltration; 3 = diffuse inflammatory cell infiltration. Fibrosis was assessed with regard to its extent (0 = limited; 1 = great), density (0 = loose; 1 = dense) and thickness (0 = thin; 1 = thick). A composite end-point was then created for each heart by summing the gross adhesion score (from 0 to 3) and the histological indices (inflammatory reaction score: from 0 to 3, and fibrosis score: from 0 to 3).

2.4. Statistical analysis

Percentages were compared by the Fisher’s exact test and a Wilcoxon test was used for comparing scores. Statistical significance was set at the 0.05 level without adjustment for P-values.

3. Results

3.1. Macroscopic findings

At four months, the Cova™ CARD sheets were almost completely absorbed and replaced by a loose regenerated tissue allowing the surgeon to find a smooth cleavage plane. In most cases, adhesions were split by blunt dissection. The surface of the heart was smooth and the coronary vessels were clearly identifiable. At the opposite, the Seprafilm® membrane was consistently absorbed and associated with tight adherences to the sternum and the rest of the pericardium requiring sharp dissection. The control group animals split between no or moderate adhesions.

3.2. Pathology findings

The Cova™ CARD group sheep never demonstrated an inflammatory reaction, in contrast to one sham-operated sheep and two sheep of the Seprafilm® group.

In keeping with these data, the extent and density of fibrosis were minimal in the Cova™ CARD group, and its thickness was moderate. The Seprafilm® group had the worst results for these three criteria, with a wide and harsh fibrosis of moderate thickness (Fig. 1). There were also some foci of neovascularization and of calcifications. In the two control sheep that experienced adhesions, the fibrosis was of limited extent, density and thickness.

Adhesion and histological scores are summarized in the Table 1.

4. Discussion

Complete closure of the pericardium after cardiac surgery, although theoretically ideal, is not always possible or recommended because of the risk of compression of bypass conduits or enlarged cardiac cavities. Furthermore, it does not prevent from tight pericardial adhesions. Local delivery of drugs or biologics have been proposed to prevent adhesions [6–8]. The keratinocyte growth factor topically applied experimentally [8] presumed to act through a resumption of the pericardial fibrinolytic function by mesothelial cells. In a clinical setting, however, this approach would probably not be so effective because the pericardium has to be left open most of the time at the end of the procedure and drained. Indeed, most studies have rather focused on biologic or synthetic sheets designed as pericardial substitutes [9–13], among which the ePTFE patch is the most widely used. Unfortunately, it has a number of significant disadvantages as it remains in situ as a permanent foreign body: it may form a fibrous capsule that may negatively interfere with visualization of the cardiac archi-
an ePTFE sheet and was ultimately replaced with regenerated tissue. However, a gelatin sheet has two substantial disadvantages. First, due to the absence of structure of the gelatin, the sheet swells once wet, which leads to a very malleable, hard to handle material. Second for the same reason, its tensile strength is not high enough to steadily hold sutures to the native pericardium, hence, the proposal to lattice the sheet with polyglycolic acid to improve its mechanical properties [13].

In human cardiac surgery, two resorbable membranes are currently available: the Repel CV\textsuperscript{w} membrane (SyntheMed, Iselin, NJ, USA) made out of polylactic acid and Seprafilm\textsuperscript{w}, composed of carboxymethyl cellulose and hyaluronic acid. The two main disadvantages of these membranes are their composition and lack of mechanical resistance. In a 142-patient study, Repel CV\textsuperscript{w} has been shown effective in the prevention of pericardial adhesions [10] but inflammatory reactions have been reported with polylactic acid [15]. Seprafilm\textsuperscript{w} has been widely used in abdominal surgery and was more recently introduced for cardiac operations with encouraging results based on reduced tightness and extent of adhesions seen in reoperated patients compared with their untreated counterparts [9]. Technically speaking, Seprafilm\textsuperscript{w} becomes a gel which sticks to organs preventing from repositioning or suturing. Furthermore, although carboxymethylcellulose and hyaluronic acid are biocompatible, they do not have specific effects on cell adhesion, wound healing and tissue modelling. Finally, resorption of carboxymethylcellulose does not involve naturally present endogenous enzymes (like collagenases) but occurs by hydrolysis, which causes the release of non-metabolized fragments.

To try overcoming these hurdles, the Cova\textsuperscript{w} CARD membrane, made out of purified porcine type I collagen, was tested in the present study. Collagen has an extensive safety record in cardiovascular applications where it has been mainly used as a topical adjunct to hemostasis or as a coating of vascular prostheses to make them blood-proof. A major advantage of collagen, particularly of porcine origin, is that its resorption is a naturally occurring event mediated by macrophage-released collagenases. Cleavage of the triple-helix structure leads to the liberation of biocompatible fragments which after denaturation and proteolysis are eliminated through kidney clearance without

Table 1
Comparison of macroscopic and histological data across groups

<table>
<thead>
<tr>
<th></th>
<th>Controls n=4</th>
<th>Seprafilm\textsuperscript{w} n=6</th>
<th>Cova\textsuperscript{w} CARD n=6</th>
<th>Statistics</th>
<th>P-values\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macroscopic findings</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Complete resorption, n (%)</td>
<td>–</td>
<td>6/6 (100%)</td>
<td>3/6 (50%)</td>
<td>–/–/0.18</td>
<td></td>
</tr>
<tr>
<td>Adhesions, median [min;max]</td>
<td>0.75 [0;3]</td>
<td>3 [3;3]</td>
<td>1 [0;2]</td>
<td>0.03/0.91/0.01</td>
<td></td>
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<tr>
<td>Histological findings</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammatory reaction, n (%)</td>
<td>1/4 (25%)</td>
<td>2/6 (33%)</td>
<td>0/6 (0%)</td>
<td>1.00/0.40/0.46</td>
<td></td>
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<tr>
<td>Extent of fibrosis, n (%)</td>
<td>5/6 (83%)</td>
<td>4/6 (67%)</td>
<td>2/6 (33%)</td>
<td>1.00/0.78/0.01</td>
<td></td>
</tr>
<tr>
<td>Density of fibrosis, n (%)</td>
<td>2/4 (50%)</td>
<td>2/4 (50%)</td>
<td>2/4 (50%)</td>
<td>0.50/0.13/0.02</td>
<td></td>
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<tr>
<td>Thickness of fibrosis, n (%)</td>
<td>3/6 (50%)</td>
<td>3/6 (50%)</td>
<td>3/6 (50%)</td>
<td>0.01/0.57/1.00</td>
<td></td>
</tr>
<tr>
<td>Composite score, median [min;max]\textsuperscript{b}</td>
<td>2.25 [0;6]</td>
<td>5.5 [3;7]</td>
<td>2 [0;3.5]</td>
<td>0.15/0.78/0.01</td>
<td></td>
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</tbody>
</table>

\textsuperscript{a}Seprafilm\textsuperscript{w} vs. Controls/Cova\textsuperscript{w} CARD vs. Controls/Seprafilm\textsuperscript{w} vs. Cova\textsuperscript{w} CARD.

\textsuperscript{b}Composite score = [adhesion score (from 0, absence to 3, tight)] + [inflammatory reaction score: from 0, absence to 3, diffuse] + [fibrosis score: for each component (extension, density and thickness) 1 if present, 0 if absent].
the induction of a pathological inflammatory reaction. In the particular setting of adhesion prevention, collagen has the additional advantage of providing a substrate for cell adhesion and growth, thereby promoting wound healing. Finally, shaping of the collagen is relatively easy and films, gel, foam or membranes can be produced to accommodate the final indication.

The present results document the superiority of the Cova™ CARD membrane under our experimental conditions. During implantation, this membrane could be easily manipulated, repositioned if necessary and sutured. The dissection at reoperation was then the easiest in this group, with very loose adhesions easily cleavable, except for one case. Expectedly, these macroscopic observations translated histologically into a loose and limited fibrosis and the lack of inflammatory cell invasion, neovascularization and calcifications, and the membrane could no longer be identified. They sharply contrasted with the findings made in the Seprafilm™ group which featured a dense tissue, some foci of inflammatory cells around neovessels, or foci of calcifications. The thickness of the fibrosis was noted similar in the two treated groups, probably because of the preset thickness of the Cova™ CARD membrane (150 μm when wet). From a surgical point of view, the thickness of the healing tissue is not an issue, in contrast to the density of fibrosis which may increase the difficulties and risks of dissection.

4.1. Study limitations

We did not include a Repel CV® treated group because of the limited clinical use of this product or a ePTFE membrane-treated group as the study focused on absorbable membranes expected to provide superior healing patterns through enhanced tissue regeneration and reduced inflammatory responses as well as avoidance of the risk of infection inherent in the introduction of durable foreign materials. A sheep model was used (sheep experiencing postoperative adhesions as in humans) to eliminate the possible biases associated with scale discrepancies inherent in rodent studies. Reoperation entailed a new sternotomy, which is more accurate than a lateral thoracotomy to induce tight adhesions between the heart and the sternum but we acknowledge that the tested material is designed to make dissection of adhesions easier and not to constitute a safety barrier for avoiding cardiac injury at the time of re-entry into the chest cavity. A 4-month reoperation delay was chosen to assess outcomes at the expected time of maximal inflammatory reactions and check for the biological membrane resorption. The characteristics of the membrane (thickness and reticulation) were chosen to achieve a full resorption by six months, although, in a rat model, the subcutaneously implanted Cova™ CARD mem-

brane had completely disappeared by 13 weeks (unpublished observations). The absence of cardiopulmonary bypass may have minimized the extent of the whole-body inflammatory reaction and consequently reduced the magnitude of postoperative adhesions but this limitation applies equally to all groups.

In conclusion, the present data support the effectiveness of the collagen-based Cova™ CARD membrane in reducing postoperative adhesions following sternotomy and may warrant its clinical evaluation.

References

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