

Evaluation of a Bioabsorbable Polylactide Film in a Large Animal Model for the Reduction of Retrosternal Adhesions

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Objectives. An adult pig model of retrosternal adhesion formation via an inferior hemisternotomy was used to evaluate the formation and development of pericardial and retrosternal adhesions, as well as adhesion reduction using two thicknesses of a bioabsorbable polylactide film.

Materials and methods. Twenty-five adult female pigs (70 kg) were allocated to either a control group or four different treatments using two thicknesses (0.02 or 0.05 mm) of a polylactide film. In each animal, the film was placed either inside the pericardium or inside and outside the pericardium.

Results. All animals demonstrated adhesions between the posterior and lateral surfaces of the heart and pericardium. Thick fibrous retrosternal adhesions and pericardial adhesions were noted in the control animals with complete obliteration of the anatomical plane. The polylactide films preserved the anatomical planes and reduced the adhesion response.

Conclusions. A reproducible animal model was used to examine the formation and reduction of retrosternal and pericardial adhesions. A polylactide film placed inside the pericardium or between the heart and sternum was able to limit adhesion formation and maintain the anatomical planes, which would facilitate reentry. © 2004 Elsevier Inc. All rights reserved.

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INTRODUCTION

Cardiac reoperations, particularly for coronary revascularization, carry increased risk of damage to the

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heart during reoperation [1]. Retrosternal and intrapericardial adhesions following median sternotomy are a widely known phenomenon and are a significant cause of morbidity and mortality [2–4]. The presence of pericardial adhesions at reoperation is associated with increased risk of injury to the heart and the great vessels due to lack of visibility during reentry. Dissection through the adhesions increases the operative time [5, 6] and bleeding [6] and can significantly impact the clinical outcome and physiological function [7]. A long-term and unpredictable problem is that postoperative adhesions impact the surgical workload and hospital resources, resulting in considerable health care expenditures [8].

Adhesions form between the epicardium and surrounding structures such as the pericardium, mediastinal fat, pleura, and sternum [9] and form in the initial postoperative period [8]. Epicardial adhesions are believed to form secondarily to impaired pericardial fibrinolytic activity [10] that is decreased following surgical injury [11]. Research in postsurgical adhesion formation and prevention to date has focused on (1) identification on a molecular level of the components involved in adhesiogenesis and their interactions; (2) clarification of the role of fibrin and fibrinolysis in adhesion formation; (3) standardization of design in preclinical and clinical studies of adhesion formation and prevention; (4) delineation of the relationships among adhesion formation and adhesive complications; and (5) elucidation of efficient, site-specific methods of prophylactic drug delivery [8].

A greater understanding of the surgical causes of adhesions including tissue trauma, ischemia, exposure, introduction of foreign bodies (i.e., talc or starch in gloves), as well as the role of prophylactic or adjunctive therapy may decrease the prevalence of adhe-

sions. Control or prevention of adhesions can theoretically be achieved through pharmacologic intervention [10, 12] or the so-called barrier method. Prophylactic use of biomaterial barriers including hydrophilic polymer solutions [2], hydrogels [13], fibrin sealants [14], and polymeric membranes [15, 16] have been evaluated to prevent or minimize the development of postoperative adhesions after cardiac surgery with varied success. While nonbiodegradable materials, such as polytetrafluoroethylene (PTFE) [17], silicone rubber [18], and xenograft pericardium [19], have been used in adhesion reduction, their permanent nature, chemical fixatives, and formation of a fibrous capsule is a limiting factor. Poly lactides are bioabsorbable and their hydrolytic degradation products are well handled by the body and considered to have excellent overall biocompatibility [20]. Poly lactides can be formed into sheets for simple and safe surgical placement following cardiac surgery. Bioabsorbable films may provide the necessary barrier to prevent adhesion formation in the early postoperative period as well as serve as a potential scaffold for new tissue ongrowth [15].

A variety of animal models has been used in these studies including rabbits [13, 16, 23], dogs [15], sheep [6, 21], and pigs [14, 22]. This study used an adult pig model of retrosternal adhesion formation via an inferior hemisternotomy to evaluate the development and formation of pericardial and retrosternal adhesions as well as their prevention using two different thicknesses of a bioabsorbable poly lactide film.

MATERIALS AND METHODS

Twenty-five adult female pigs (70 kg) were used and treated in accordance with institutional ethics approval in compliance with the European Convention on Animal Care. All animals had a uniform survival time of 4 weeks and were allocated to either a control group or one of four treatment groups with five animals per group. Group 1 is nontreated control; Group 2 had a 0.02-mm-thick poly lactide film placed inside of the pericardium; Group 3 had a 0.02-mm-thick poly lactide film placed both inside and outside of the pericardium; Group 4 had a 0.05-mm-thick poly lactide film placed inside of the pericardium; and Group 5 had a 0.05-mm-thick poly lactide film placed both inside and outside of the pericardium. The dimensions of the films used were 75 × 100 mm. The poly lactide film (Surgi-Wrap™, Macropore Biosurgery, San Diego, CA) is an amorphous copolymer of 70:30 poly(L-lactide-co-D,L-lactide) sterilized by electron-beam irradiation.

Surgical Procedure

Anesthesia was induced with intramuscular injection of Ketamine (Parnell Laboratories, Sydney, Australia) followed by spraying of the vocal chords with lignocaine and endotracheal intubation. Anesthesia was maintained with halothane and oxygen. Temgesic (0.324 mg Buprenorphine IM, Schering Plough Pty. Ltd., North Ryde, Australia) was used for analgesia prior to start of the surgical procedure. Intravenous Keflin (1 g Cephalozin IV, Eli Lilly, Rome, Italy) was used for antibiotic prophylaxis. The animal was positioned supine; the operative site was marked, and the skin prepped and draped in a sterile manner. All surgeons used powder-free gloves. A skin inci-

sion was made in the midline extending from the middle of the sternum to just below the xiphisternum, inferiorly. The soft tissues were divided using electrocautery, and the xiphisternum was divided using a scissors. The retrosternal space was dissected inferiorly using blunt finger dissection. The periosteum was marked using electrocautery and the inferior half of the sternum was divided in the midline using an oscillating saw (Microaire, Smith & Nephew Surgical, North Ryde, Australia). The cut sternal edges were gently displaced using a sternal retractor. The pericardium was divided in the midline, longitudinally. The anterior pericardium was opened with a longitudinal incision (superior to inferior) measuring 8 cm. The anterior surface of the heart was abraded using 30 controlled firm strokes with surgical 4 × 4 gauze [23]. Fifty millimeters of sterile saline was then infused into the pericardial space. The pericardium was loosely apposed using two interrupted 3-0 Dexon sutures (Davis and Geck, North Ryde, Australia) to form a pericardial window of 2 cm by approximately 6 cm in all animals. The inferior hemisternotomy was closed using two interrupted sternal wires (Ethicon Surgical, North Ryde, Australia) followed by closure of the soft tissue and skin in layers (3-0 Dexon, Davis and Geck, North Ryde, Australia). Chest drains were not used.

Treatment

No material was used in the control group of animals. In Groups 2 and 4, the 0.02- or 0.05-mm-thick poly lactide film was placed underneath the pericardium adjacent to the anterior surface of the heart prior to apposition of the pericardium. No sutures were used to fix the film in place. In Groups 3 and 5, the 0.02- or 0.05-mm-thick poly lactide film was placed underneath the pericardium adjacent to the anterior surface of the heart prior to apposition of the pericardium. A second layer of the same thickness of film was placed inferior to the sternum and superior to the pericardium.

Recovery

Animals were recovered and monitored daily for signs of pain, lethargy, changes in demeanor, appetite, and wound integrity. All pigs received postoperative analgesia (0.324 mg Buprenorphine IM, Schering Plough Pty. Ltd.) as required following clinical assessment. Pigs were housed in individual pens and euthanized at 4 weeks postoperatively with a lethal dose of sodium pentothal (Lethobarb, Virbac Australia Pty. Ltd., Sydney, Australia) administered via an ear vein.

Evaluation of Adhesion Formation

Macroscopic Grading

The costal cartilages and soft-tissue attachments of the sternum were sectioned around its perimeter and the sternum and thoracic contents were lifted from the thorax en masse following sacrifice. Careful dissection was performed to allow assessment of the retrosternal and intrapericardial adhesions. In all animals, the regions of the heart were graded in the same order starting with the posterior surface, right and left (lateral) surfaces, the superior aspect of the anterior surface (where pericardium and sternum were not incised), as well as the inferior aspect of the anterior surface of the heart (exposed to the operative site). A semiquantitative grading scale from 0 to 5 was used in the grading process as outlined in Table 1. The ability to delineate an anatomical dissection plane between the anterior surface of the heart and the inferior portion of the sternum in the region where the surgery was performed was assessed and graded using a scale ranging from 0 to 3 (Table 2). The total area of retrosternal adhesions (as a percentage of the operative site) was estimated for each animal. Finally, the sternum was carefully examined for evidence of any wound breakdown and general integrity.

Four trained observers graded the retrosternal and intrapericar-

TABLE 1
Adhesion Grading Scale

Locations evaluated between the pericardium and the myocardium
 Region A: posterior aspect of the heart
 Region B: lateral, left side of the heart
 Region C: lateral, right side of the heart
 Region D: anterior aspect of the heart, inferior (surgical site)
 Location evaluated between the pericardium and the sternum
 Region E: anterior aspect of the heart, retrosternal

Scale used for all regions
 0 = no adhesions
 1 = sparse, can easily dissect manually, focal
 2 = infrequent, can be easily dissected manually, focal
 3 = frequent, requires sharp dissection, but easily dissected, focal
 4 = numerous, requires sharp dissection, moderately difficult to dissect, not focal
 5 = numerous, requires sharp dissection, very difficult to dissect, not focal

dial adhesions in a blinded fashion during the dissection. However, at the 4-week time period, it was possible to discern the resorbable polylactide film at the operative site, which was evaluated last in the dissection process. Additional observations noted at the time of dissection and grading were as follows: the appearance of the pericardium (transparent, translucent, or opaque); visibility of the coronary vessels; and the macroscopic tissue response to the presence of the film. Data were analyzed using Kruskal–Wallis test (adhesion scores Regions A–E, and dissection plane scores) or analysis of variance (total area or retrosternal adhesions). Specific differences were determined post-hoc using Dunn's procedure or multiple comparisons, adjusting for the number of treatment groups. SPSS Inc. (Chicago, IL, USA) was used to perform the statistical analysis.

Histology

The heart and sternum were fixed in phosphate-buffered formalin for a minimum of 72 h following dissection. Full-thickness biopsy samples were taken from the posterior, lateral, and anterior aspect of the heart and embedded in paraffin. The sternum was decalcified in formic acid–formalin solution; wires were removed, cut into blocks, and embedded in paraffin. Five-micron sections were cut using a Leica Microtome (USA) and stained with Hematoxylin and Eosin. Sections were evaluated under transmitted light and polarized microscopy using an Olympus microscope by three trained observers. The histological appearances of the adhesions and tissue response to the polymeric film were evaluated in the sections from the heart. The healing of the sternum was assessed based on histology of the decalcified sections.

Mechanical Properties of the Polylactide Film

The tensile properties of the two-thickness polylactide films were determined using a Mach 1 Micromechanical Testing Machine (Biosyntech, Montreal, Canada). Stress relaxation experiments were performed on uniform testing samples cut from the 0.02- and 0.05-mm-thick polylactide films. Eight samples were cut of the 0.02- and 0.05-mm-thick polylactide films having a gauge length of 10 mm and width of 10 mm. Samples were tested in PBS at room temperature with a 2-mm displacement at 0.1 mm per second and held at this level for 5 min. The stiffness and stress relaxation of the films were determined for all samples. Data were analyzed using a two-way analysis of variance followed by a Tukey Honest significant difference post-hoc test using SPSS for Windows.

RESULTS

Surgery

There were no operative deaths or postoperative complications in the study. Specifically, there was no evidence of wound infection, wound dehiscence, hemodynamic compromise, and respiratory or gastrointestinal complications. Placement of the films at the time of surgery was a simple procedure with no evidence of change in gross cardiac function. All animals recovered well from the procedure and were standing and drinking within a few hours, and mobilized and eating by the first postoperative day. Postoperative analgesia was not required beyond the first postoperative day. The skin incisions were macroscopically healed at 4 weeks postoperative with no evidence of infection or dehiscence in all animals.

Adhesion Formation

The summary of results from the grading scales outlined in Tables 1 and 2 for the macroscopic dissection and ability to identify and enter anatomical planes, respectively, are presented in Table 3 and graphically in Fig. 1. Interobserver variability for grading was minimal in the study. For all regions, there were highly significant differences among the scores for the various treatment groups (Kruskal–Wallis test, all $P < 0.5 \times 10^{-5}$). In Region A (posterior), Group 4 and Group 5 were significantly different from the control group (all $P < 0.025$). In Region B and Region C (lateral regions), Groups 3, 4, and 5 were all significantly different from the control group (all $P < 0.005$). In Region D (anterior), all treatment groups were significantly different from the control group (all $P < 0.0001$). In Region E (retrosternum), Groups 3, 4, and 5 were all significantly different from the control group (all $P < 0.0001$). For the dissection plane scores, again all treatment groups were significantly different from the control group (all $P < 0.0001$). Similarly, the percentage area of the retrosternal adhesions was significantly different among the treatments (analysis of variance, $P < 0.5 \times 10^{-5}$), and all treatment groups were significantly different from the control group (all $P < 0.05$).

TABLE 2

Dissection Plane Grading Scale

Scale
0 = no adhesions, preservation of dissection plane
1 = thin adhesions, can easily dissect manually, preservation of dissection plane
2 = moderate adhesions, can be dissected, preservation of dissection plane
3 = thick adhesions, can not be dissected, obliteration of dissection plane

TABLE 3
Summary of Adhesion Score and Dissection Plane Grading Scale Results

Groups	Region A posterior	Region B lateral-left	Region C lateral-right	Region D anterior	Region E sternum	Area % of surgical site	Dissection plane
Control	2.4 (1–5)	2.9 (1–4)	3.0 (1–4)	4.8 (4–5)	5.0 (4–5)	91% (60–100%)	3.0 (2–3)
0.02 mm film inside pericardium	1.5 (1–2)	1.6 (1–3)	1.6 (1–3)	1.9 (1–3)	3.8 (2–5)	18% (10–33%)	1.1 (1–2)
0.02 mm film inside and outside pericardium	1.7 (1–4)	1.4 (1–3)	1.4 (1–3)	1.4 (1–3)	2.3 (1–5)	17.5% (10–40%)	1.1 (1–2)
0.05 mm film inside pericardium	1.1 (1–2)	1.3 (1–2)	1.3 (1–2)	1.3 (1–3)	2.1 (1–5)	12% (10–20%)	1.0 (All = 1)
0.05 mm film inside and outside pericardium	1.0 (All = 1)	1.1 (1–2)	1.1 (1–2)	1.3 (1–3)	1.4 (1–2)	13% (10–25%)	1.0 (All = 1)

Note. Data shown are the average of all scores (range). Averages shown are from 20 scores each (5 animals in each group \times 4 observers).

The following descriptions summarize the macroscopic findings in each region.

Posterior and Lateral Regions

All animals demonstrated adhesions between the posterior and lateral surfaces of the heart and pericardium. In Groups 1, 2, and 3 the posterior and lateral surface adhesions were thin and easily dissectable with blunt dissection and did not require cutting to separate (Fig. 2). The anatomical plane was easily accessible (grade 0–1). In Groups 4 and 5 (0.05 film) the posterior adhesions were less frequent, more translucent, and not as well developed as in Groups 1, 2, and 3. These adhesions failed easily during the dissection, requiring only finger dissection with a clearly preserved anatomical plane (grade 0–1).

Anterior Region

Group 1 (Controls)

The retrosternal space in Group 1 was completely obliterated by the presence of adhesions in all animals. The cut pericardial edges were intimately adherent to the posterior surface of the sternum and not dissectable from it. The area of the heart exposed to the sternum between the cut pericardial edges (Region D) was adherent to the sternum by thick tenacious adhesions that obliterated the space with a mean grade of 4.8 (range 4–5). The cut pericardial edges were intimately adherent to the posterior surface of the sternum (Region E) and could not be dissected and received a mean grade of 5. No dissection plane was identifiable in any of the control animals and the retrosternal adhesions occupied an average of 91% of the operative site (range 60–100%, Table 3). The adhesions in this region were so tenuous that the tissue was not dissectable from the sternum without risk of damaging the myocardial tis-

sue. The coronary vessels were not identifiable, nor accessible in this region of the heart in Group 1.

Group 2 (0.02-mm-thick polylactide film inside of the pericardium)

Animals in Group 2 demonstrated thin focal adhesions between the exposed anterior region of the heart and posterior surface of the sternum between the cut pericardial edges. These adhesions were easily dissectable with blunt dissection and a clearly definable anatomical plane was present between the exposed region of heart and posterior surface of the sternum (Region D, mean grade 1.9, range 1–3). The cut edges of pericardium were intimately adherent to the posterior surface of the sternum (Region E, mean grade 3.8, range 2–5). In contrast to the control animals (Group 1), a clearly definable anatomical plane was preserved between the exposed anterior region of the heart and the posterior surface of the sternum (mean grade 1.1, range 1–2). The retrosternal adhesions occupied an average of 18% of the operative site (range 10–33%). The coronary vessels were easily identifiable and accessible. In three of five animals, the defect between the pericardial edges adherent to the sternum was variably filled by the formation of a connective tissue layer. This tissue was not dissectable from the sternum. The 0.02-mm-thick film was not visible on macroscopic dissection. There were, however, small remnants of the material present on the anterior surface of the heart that could be detected upon palpation.

Group 3 (0.02-mm-thick polylactide film inside and outside the pericardium)

In Group 3, thin focal adhesions were present between the anterior surface of the heart and pericardium (Region D, mean grade 1.4, range 1–3). The cut pericardial edges were adherent to the posterior sur-

Cardiac Adhesions - 4 weeks

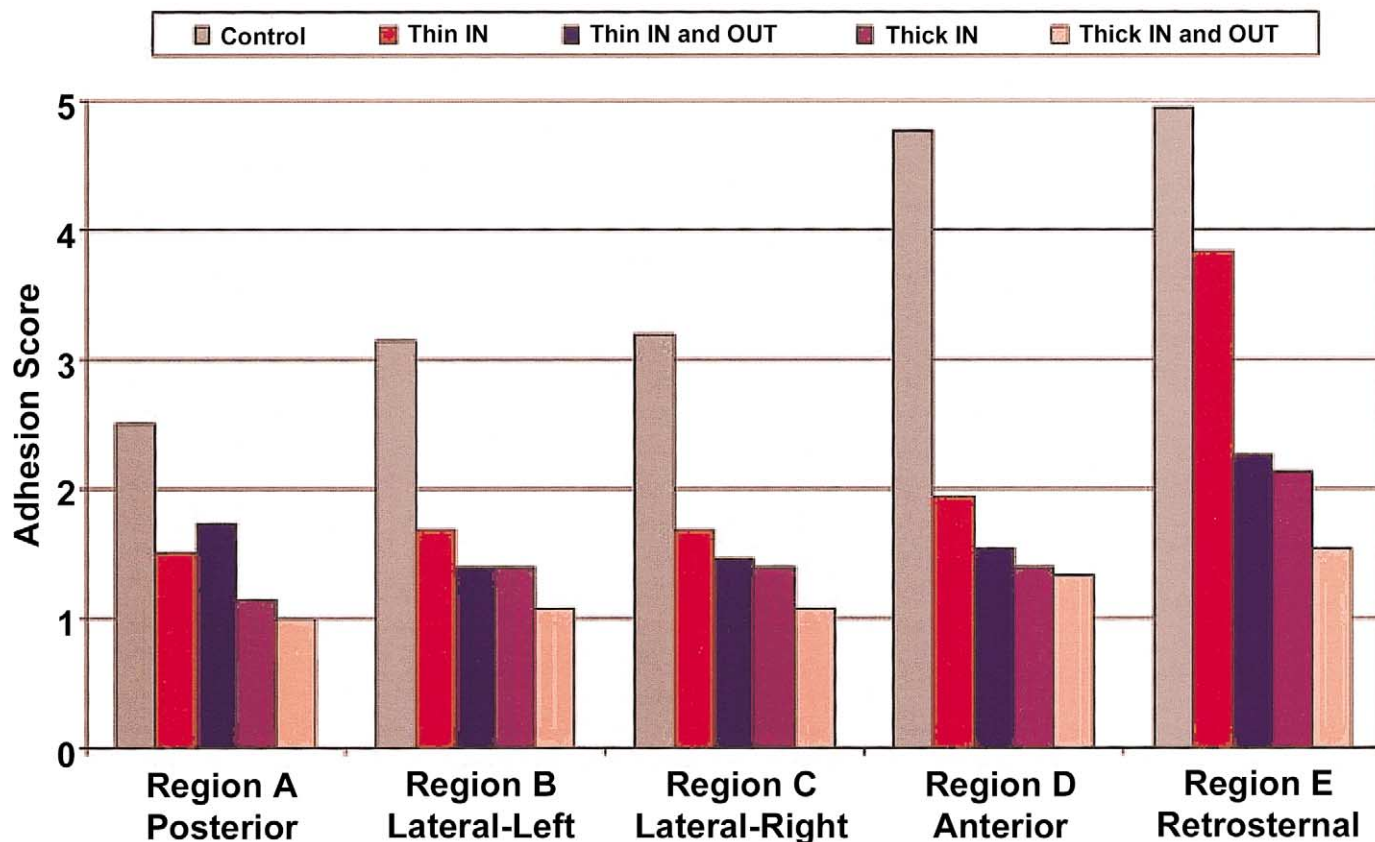


FIG. 1. Summary of adhesion scores (range from 0 to 5) in the different anatomical regions.

face of the sternum but were dissectible from the sternum (Region E, mean grade 2.3, range 1–5). Similar to Group 2, the region between the cut edges of



FIG. 2. An example of macroscopic adhesions encountered in all animals during the dissection. The adhesions were fibrous and were easily dissected and the anatomical plane was easily accessed laterally as well as posteriorly.

the pericardium showed formation of a new tissue layer, which variably closed the defect in three of five animals. This tissue layer was macroscopically continuous with the cut edges of pericardium and was easily dissectible from the posterior surface of the sternum. Thin focal adhesions were present between anterior surface of the heart and this new tissue layer. A clearly definable anatomical plane was present between the anterior surface of the heart and new tissue layer, as well as between the heart and pericardium (mean grade 1.1, range 1–2). The retrosternal adhesions occupied an average of 17.5% of the operative site (range 10–40%). The coronary vessels were easily identifiable and accessible. The 0.02-mm-thick film was not visible on macroscopic dissection. There were small remnants of the material present palpable on the anterior surface of the heart as found in Group 2, as well as posterior surface of the sternum.

Group 4 (0.05-mm-thick polylactide film inside of the pericardium)

Animals in Group 4 demonstrated thin focal adhesions between the exposed anterior region of the heart

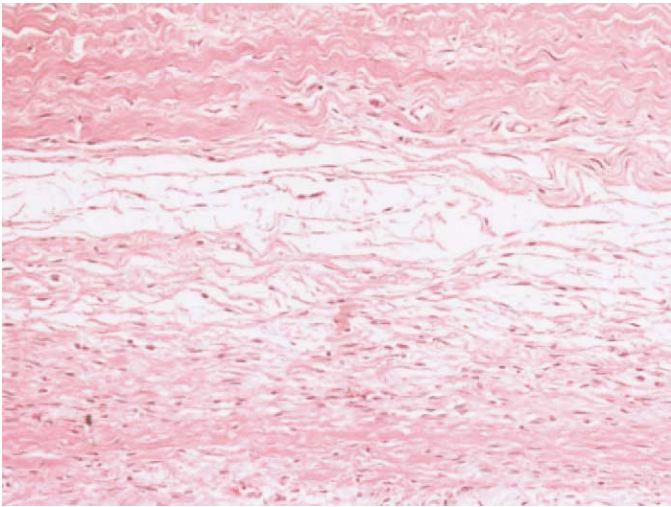


FIG. 3. H&E section of a posterior adhesion viewed under transmitted light ($\times 20$ objective) revealed the presence of loose connective tissue, creating an adhesion between the pericardium and heart.

and posterior surface of the sternum between the cut pericardial edges (Region D) similar to Groups 2 and 3. These adhesions were easily divided by blunt dissection (Region D, mean grade 1.3, range 1–3). The adhesions between the cut edges of pericardium and the posterior surface of the sternum were sparse to infrequent with the majority released with blunt dissection (Region E, mean grade 2.1, range 1–5). Similar to Groups 2 and 3, a clearly definable anatomical plane was present between the exposed region of the heart and posterior surface of the sternum (all scores 1). The retrosternal adhesions occupied an average of 12% of the operative site (range 10–20%). In all five animals the defect between the pericardial edges adherent to the sternum was again variably filled with a new connective tissue layer, greater in magnitude than that observed in Groups 2 and 3. The coronary vessels of the heart were easily identifiable as observed in Groups 2 and 3. The 0.05-mm film was visible on macroscopic dissection with new tissue formed on the anterior and posterior surfaces.

Group 5 (0.05-mm-thick polylactide film inside and outside of the pericardium)

Animals in Group 5 demonstrated sparse thin focal adhesions between the exposed anterior region of the heart and the pericardium (Region D, mean grade 1.3, range 1–3) similar to Groups 2 through 4. The cut edges of pericardium were adherent to the sternum, but were infrequent and could be dissected from the posterior surface of the sternum (Region E, mean grade 1.4, range 1–2). A clearly definable anatomical plane was present between the exposed region of heart and posterior surface of the sternum, which was more evident than all other groups. All animals received a

grade of 1. The retrosternal adhesions occupied an average of 13% of the original operative site (range 10–25%). Similar to Group 4, the defect between the cut edges of pericardium demonstrated a new connective tissue layer that variably closed the defect for all animals in Group 5. New tissue was noted on the anterior and superior surfaces of the material placed inside as well as outside the pericardium. The coronary vessels of the heart were easily identifiable as similar to Groups 2–4. The 0.05-mm film was visible on macroscopic dissection.

Sternum

The sternum appeared well healed in all animals with no evidence of wound breakdown. The presence of the polylactide film in Groups 2–5 did not appear to have any adverse effect on healing of the sternal osteotomy.

Histology

Histology from the posterior and lateral regions of the heart was similar in all animals, demonstrating a thin band of adhesions in the pericardial space (Figs. 2 and 3). These findings were in direct agreement with those observed at the time of macroscopic grading. The adhesions were characterized by a fibrous layer and fibroblastic cells and new collagen (Fig. 3). No evidence of an inflammatory response was noted in the control

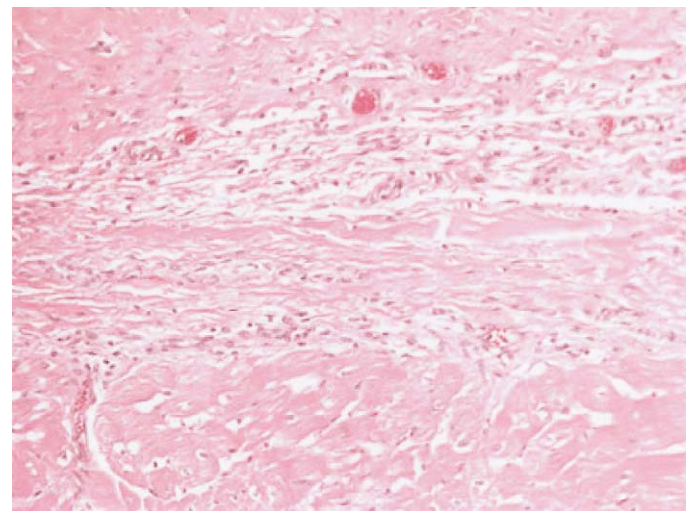


FIG. 4. (A) H&E section of anterior adhesions in a control animal (Group 1, image A) under transmitted light ($\times 20$ objective) revealed the presence of denser connective tissue compared to the posterior adhesions (Fig. 3), creating an adhesion between the pericardium and heart with obliteration of the anatomical plane. (B) H&E section of anterior adhesions with the 0.02-mm film (Group 2, image B) and 0.05-mm film (Group 4, image C) viewed under transmitted ($\times 20$ objective) revealed the presence of some loose connective tissue similar in appearance to the posterior adhesions (Fig. 3). These adhesions between the pericardium and heart were easily dissectible and allowed entry to the anatomical plane.

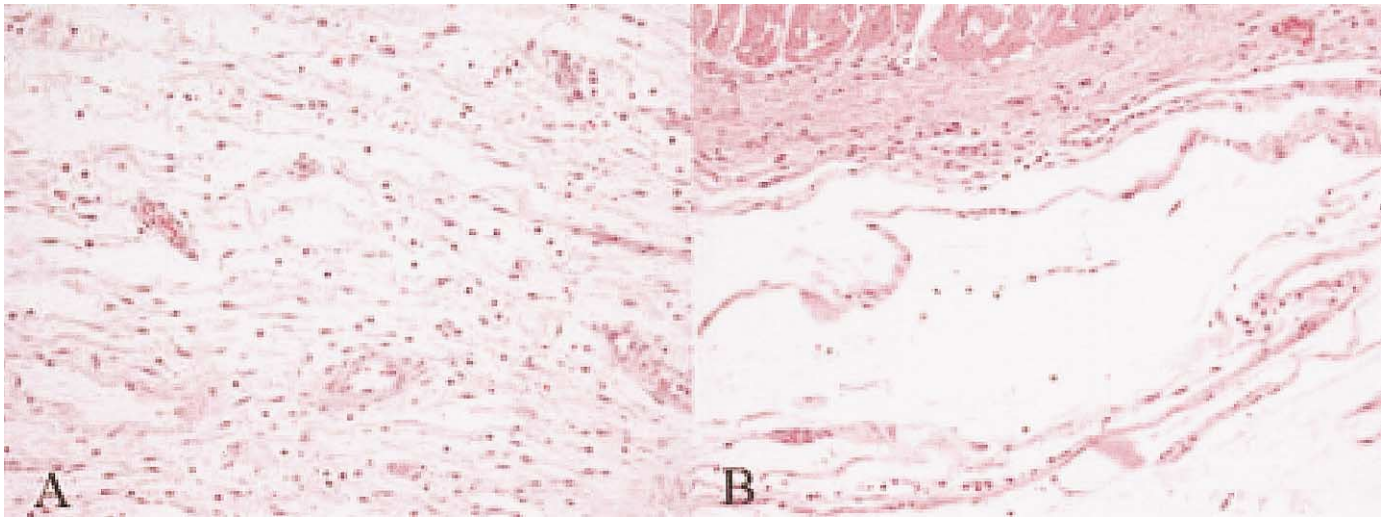


FIG. 5. Histological reaction to the 0.02-mm ($\times 20$ objective, image A) and the 0.05-mm film ($\times 10$ objective, image B) revealed a mild inflammatory reaction at 4 weeks with the presence of some lymphocytes and giant cells. The reaction to the 0.02-mm film was greater than that observed with the 0.05-mm film and could be related, in part, to the mechanical breakdown of the 0.02-mm film.

animals or the animals that had a film placed anteriorly. The overall amount of adhesions observed macroscopically in Groups 2–5 in the posterior and lateral regions of the heart also appeared to be less well developed compared to controls.

Histology sections of tissues from the anterior region of the heart, where the surgical procedure was performed (Region D), were the most developed and revealed significant differences between the control animals (Group 1) as compared to animals where the polylactide film was placed (Groups 2–5). Histology from Region D in Group 1 revealed obliteration of the normal anatomical plane between the heart and pericardium. The pericardium and heart were in continuity in these sections connected through the fibrous adhe-

sions. These adhesions were composed of a thick layer of dense connective tissue with a population of plump fibroblastic cells (Fig. 4). No evidence of an inflammatory reaction was noted.

The polylactide film was observed histologically in all animals in Groups 2–5 between the anterior aspect of pericardium and heart where it was originally placed when viewed under polarized light. In general, a quiescent fibrous tissue layer was observed surrounding the film adjacent to a clearly definable anatomical plane between the heart and pericardium. Considering that some shrinkage could have occurred during histology processing, this layer was difficult to quantify.

A distinct difference was noted between the histology of control animals (Group 1) and the treated animals

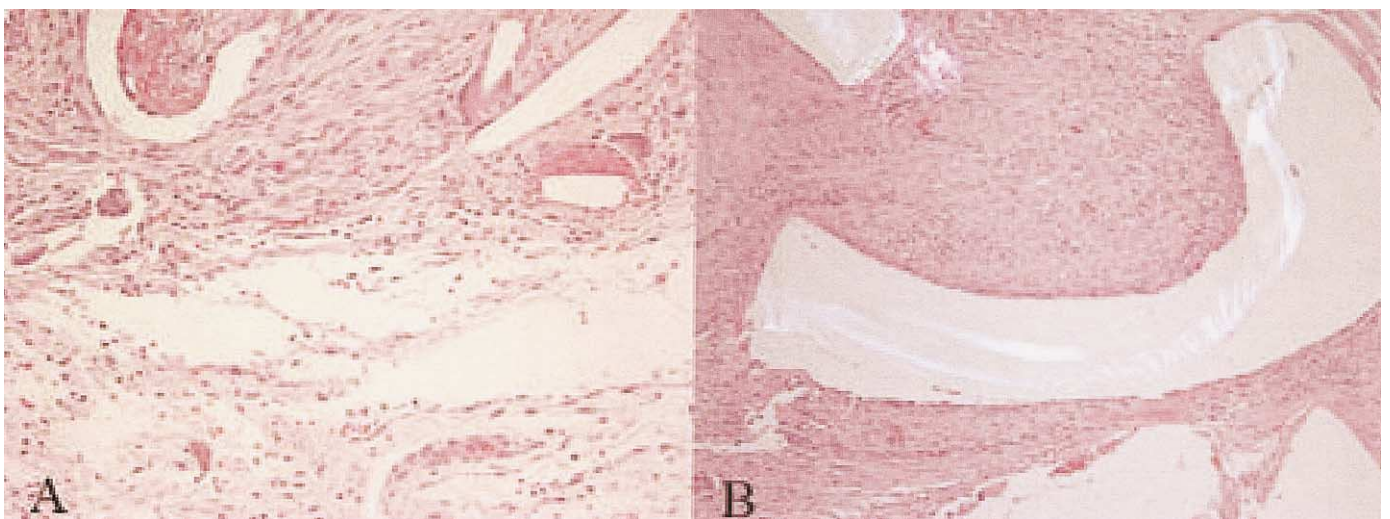


FIG. 6. Histological reaction to the 0.05 mm ($20\times$ objective, image A) and the 0.05 mm film ($10\times$ objective, image B) revealed a mild inflammatory reaction at 4 weeks with the presence of some lymphocytes and giant cells.

(Groups 2 through 5), which correlated with the scores assessed in gross observations. The control sections demonstrated a fibrous band of adhesions from the anterior surface of the heart (Region D) essentially connecting the pericardium and heart. In contrast, animals where the film had been placed between the pericardium and the heart (Groups 2–5) demonstrated preservation or partial preservation of the normal anatomical plane present between the pericardium and heart (Fig. 4), which again correlated with gross observations. A mild inflammatory response with isolated lymphocytes and giant cells was present adjacent to the material and appeared to be more prevalent in the thinner 0.02-mm film compared to the 0.05-mm film (Fig. 5). A chronic inflammatory response was not observed at 4 weeks. The film has some partial folds in some sections rather than a single plane of material and was surrounded by a layer of new connective tissue, which appeared thicker in the 0.05-mm film than in the 0.02-mm film.

Tensile Properties

The 0.05-mm-thick film was slightly stiffer than the 0.02-mm film (34.3 N/mm *versus* 28.1 N/mm) but no statistically significant differences were found. Both thicknesses demonstrated a viscoelastic response with relaxation of 38.8 and 30.3% of the load over the 5-min testing period but again no statistically significant differences were found.

DISCUSSION

The development of postoperative pericardial adhesions is well known to increase the risk of cardiac reoperations because of the danger of damaging the heart, great vessels, or grafts as well as general problems related to increased operating time, potential for blood loss, and infection [16, 24]. The current study explored the use of a bioresorbable polylactide film placed between the pericardium and heart as well as the pericardium and sternum in a pig model. The macroscopic grading and histology demonstrated the consistent development of thick tenacious adhesions in all control animals between the pericardium and heart as well as the pericardium and posterior surface of the sternum. In contrast, placement of the polylactide film between these anatomical structures resulted in the preservation of the anatomical plane as well as the development of new layer of connective tissue on the material.

The pig model used in the current study provided a reproducible method to evaluate adhesion formation. The mini-sternotomy described in this model was a useful surgical procedure to minimize excessive trauma to the animal, which was extremely well tolerated, while still allowing for a reproducible develop-

ment of adhesions. An adult pig model was chosen considering placement of the film in these animals would be similar to that encountered in the human scenario. In contrast to other models, such as the rabbit or dog where the development of retrosternal adhesions is difficult [15], the pig model did not appear to have this limitation. The retrosternal adhesions in all control animals were well developed with the heart found to be completely adherent to the sternum and no anatomical plane present. The pericardium was not sutured to the sternum in this study, as reported by Okuyama *et al.* [15]. The presence of a polylactide film at this interface provided a barrier, which allowed preservation of this anatomical space and would facilitate reentry.

A macroscopic scoring system and routine histology were used to evaluate the presence of adhesions at 4 weeks. A qualitative subjective grading scale was developed to examine not only adhesions in the anterior region of the heart, where the original surgery was performed, but to assess the effect of the surgical procedure on the posterior and lateral aspects of the heart as well as the retrosternal space. A 4-week time period was chosen after considering that the initial inflammatory reaction following surgery would have subsided; the healing would have substantially occurred allowing the ability to assess the preservation of the anatomical plane, and any subsequent biocompatibility issues could all be assessed.

Both thicknesses of polylactide films examined in this study were easy to handle and manipulate during surgery. The films did not crinkle or break during placement and were easily manipulated in a blood-filled site. These handling properties may allow for intraoperative correction of placement in contrast to other materials [13, 25], whose physical properties may differ in the dry and wet states. The stiffness of the films determined in uniaxial tension did not vary with sample orientation, confirming the uniformity of the manufacturing technique. As expected, the 0.05-mm-thick film was significantly stiffer and less relaxed than the 0.02-mm film. No failure of the films was noted during the tensile loading or stress relaxation. The failure loads over time have been characterized and found to maintain 100% of the initial strength at up to 12 weeks when aged statically *in vitro* [26]. The stiffness data reflect the material's ability to resist deformation and were great with the thicker, 0.05-mm film, compared to 0.02-mm-thick film. The stress relaxation data confirm the viscoelastic nature of the amorphous polylactide film.

In vivo, the 0.02-mm-thick polylactide films used were no longer contiguous as a film at 4 weeks, but rather reduced to fragments or particles. This did not have an adverse effect on the ability of the 0.02-mm film to prevent or minimize adhesion and supports a

barrier mechanism for this material. The placement of a film between the beating heart and pericardium likely imposed a multiaxial loading condition (beating heart at 80 beats per minute would result in 3.2 million cycles at 4 weeks) as well as a unique biological environment that could accelerate degradation or breakdown. A chronic inflammatory reaction was not seen at 4 weeks. The thicker 0.05-mm film was still present in film form at 4 weeks and provided similar results to the 0.02-mm film with regards to adhesion prevention between the pericardium and heart and pericardium and sternum. As discussed by Okuyama [15], a bioabsorbable film may only be required to limit adhesion formation during the initial formation of adhesions reported to occur during the initial postoperative period [8]. Neither film elicited an acute inflammatory reaction *in vivo* when examined histologically. The gross examination of the anterior surface of the heart (Region A) did not reveal any gross erythema with either thickness film. The polylactide films supported the growth of a new connective tissue layer and may have served as a scaffold for regeneration or use a pericardial patch.

An important limitation to consider in this, as in all animal models of retrosternal adhesions, is that the dissections are performed postmortem. This may introduce an artificial environment in the grading process considering the beating heart, bleeding, and surgical access may make reentry more difficult in the human scenario. The current results support that a bioabsorbable film composed of polylactide does indeed provides a barrier to adhesion formation and preservation anatomical planes and could play a role as a supporting scaffold for the regeneration of pericardium. Whether or not this new tissue layer functions as neopericardium is beyond the scope the current study.

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