

Evaluation of a Novel Synthetic Sealant for Inhibition of Cardiac Adhesions and Clinical Experience in Cardiac Surgery Procedures

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ABSTRACT

Background: Pericardial adhesions subject patients requiring reoperation to potential injuries to the heart, great vessels, and cardiac grafts during the re-sternotomy. These adhesions can severely complicate re-operations by making re-entry hazardous, impeding orientation and visibility, increasing the amount of blood loss, and prolonging the operation time. The efficacy of an in situ-forming polyethylene glycol (PEG) material, CoSeal[®] surgical sealant (CoSeal[®]), for inhibiting cardiac adhesions in an animal model is reported. It is currently estimated that 10-20% of patients undergoing aortic valve replacement and coronary artery bypass grafting (CABG) will require a second operation later in their lives. Successful clinical experience using CoSeal[®] for sealing suture lines of the aorta and CABGs with the data reported here suggest that CoSeal[®] may have multiple applications in cardiac surgery.

Methods: In rabbits, a sternotomy and pericardiotomy were performed to expose the heart and the epicardium of the left ventricular surface. The epicardium was abraded for five minutes with dry gauze and cotton to develop punctate bleeding. In treated animals, CoSeal[®] or Tissucol[®] was applied directly to the abraded bleeding epicardium while retracting the pericardium. The pericardium was released, and the material over-sprayed to the cut edges of the pericardium. No material was applied in control animals.

Results: At necropsy, CoSeal[®] was found to significantly reduce the formation of adhesions, the tenacity of the adhesions, and the percent of the abraded site with adhesions as compared to surgical control and Tissucol[®]. Tissucol[®] showed no significant difference from the surgical control in any adhesion parameter. CoSeal[®] treated hearts showed re-establishment of the mesothelial layer and tissue morphology similar to a normal un-operated heart. During the clinical

cardiac procedures, CoSeal[®] was easily mixed and applied to the suture lines of the aorta and coronary artery grafts. No bleeding was found at the suture lines.

Conclusions: In the rabbit cardiac adhesion model, CoSeal[®] significantly reduced the formation of adhesions as compared to surgical control and Tissucol[®], and demonstrated good biocompatibility. In CoSeal[®] treated patients undergoing cardiopulmonary bypass or vessel repair, sealing was achieved comparable to previous cases using Tissucol[®] fibrin sealant. CoSeal[®] effectively sealed the suture lines of the aorta and coronary artery bypass grafts.

INTRODUCTION

Adhesion formation following primary median sternotomy can obscure the surgeon's view upon re-operation, potentially leading to injuries of the heart and the great vessels and significantly increasing surgery time [Dobelle 1984, Loop 1984, Segesser 1987]. The formation of adhesions is a normal physiological response of the healing process that occurs when mesothelial or cardiac muscle cells are damaged or become necrotic as a result of drying, suturing, infection, or inflammation and deposition of fibrin from blood or exudate [Cliff 1973, Tomizawa 1992, Holmdahl 1999]. Blood and wound exudate contain fibrinogen, which rapidly converts to fibrin at the surgical site. Because fibrin is sticky, the fibrinous exudate or clot can attach to adjacent tissues. During healing, fibroblasts invade the fibrin network and produce collagen fibers to form the fibrous connective tissue, commonly called adhesive tissue. As a result, adhesions are frequently encountered in cases of repeat cardiac surgery (i.e., coronary artery bypass graft [CABG] and valve repair operations). Therefore, re-operations carry incremental risks of morbidity and mortality, due in part to retrosternal and pericardial adhesions.

Barrier materials may inhibit adhesion formation between the sternum and heart; however, they are often difficult to put in place. Most barrier materials must be sutured into place, and sutures themselves are known to elicit adhesion formation. Permanent synthetic materials may induce the formation of a tissue capsule, which in some instances can lead to

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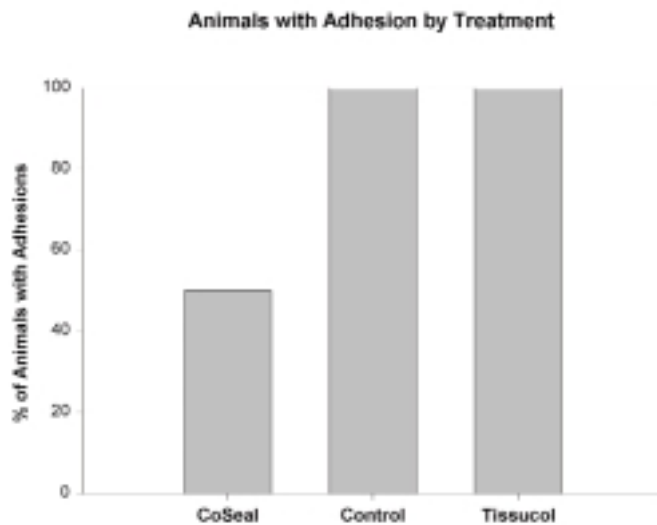


Figure 1. CoSeal® significantly reduced the formation of adhesions in the rabbit cardiac adhesion model as compared to controls and Tissucol®.

contraction of the synthetic barrier and fibrosis of the surrounding tissue. In other instances, barrier materials have been reported to cause local tissue inflammation.

The use of fibrin sealants to control leaks from anastomoses has become standard practice in some cardiac centers. Some reports indicate that fibrin sealant application results in adhesion formation similar to untreated controls [Joyce 1991, Arnold 2000]; others indicate an inhibition of adhesion formation [Boris 1996, de Virgillio 2000, Toosie 2000]. CoSeal®, a new synthetic vascular sealant, can be used in cardiac procedures in place of Tissucol®. The potential of these materials to inhibit or increase the formation of adhesions is a clinically relevant issue. The objective of this study is to evaluate the cardiac adhesion formation in an in vivo animal model. CoSeal® was compared to Tissucol®, a commercial fibrin sealant, and to a surgical control. The cardiac adhesion model was based on research in the literature using similar techniques to produce cardiac adhesions in rabbits [Cliff 1973].

MATERIALS AND METHODS

In this study, CoSeal® and Tissucol® treatments were compared to surgical control. CoSeal® is designed as an in situ polymerizing tissue sealant and is approved for use as a vascular sealant in the European Community [Hill 2000, Mersman 2000]. CoSeal® is comprised of two synthetic polymers of polyethylene glycol. CoSeal® is prepared in about three minutes and is easy to use. It gels within five seconds and is set within 60 seconds. Tissucol® is a commercial fibrin sealant manufactured by Immuno and distributed by Baxter. In the United States, Tissucol® is distributed as Tisseel® FH Fibrin Sealant. Haemacure distributes the same product as Hemaseal™ APR in the United States. Tissucol®/Tisseel®/Hemaseal™ contains fibrinogen and thrombin from pooled human plasma, bovine aprotinin, and calcium chloride solution. Preparation of Tissucol® is complex requiring several

steps, including two warming steps and the use of ancillary equipment for preparation. It takes 10-20 minutes to prepare.

Twenty-two female New Zealand White rabbits were assigned to three groups: CoSeal®, Tissucol®, and control. All animals were explanted at 14 to 21 days post-operatively.

Surgery

All procedures were carried out according to the principles set forth in the *Guide for the Care and Use of Laboratory Animals* (U.S. Department of Health and Human Services). All rabbits were anesthetized intravenously, intubated, and placed on a respirator. A sternotomy was performed and the sternum was gently spread with a pediatric retractor. A pericardiotomy approximately 4-5 cm in length was performed at the anterior pericardium. The epicardium of the left ventricular surface of the heart was then abraded for five minutes with dry gauze and cotton to develop punctate bleeding. The pericardium was not closed. In treated rabbits, the material (2-3 mL) was applied directly to the abraded epicardium and sprayed over the pericardial edge. Control animals were treated in the same manner, except that no material was applied. Marcaine was given as an intercostal nerve block. The sternum and muscle were closed using interrupted non-degradable sutures (2-0 Ti-Cron, DG), while the subcutaneous tissue was closed with continuous sutures. The skin was closed with interrupted sutures (2-0 Dermalon). Morphine or bupronex containing gentamycin and penicillin was administered intramuscularly twice daily for 3 days post-operatively. Animals were observed daily for general health and sacrificed 14-21 days after surgery.

Evaluation

An individual blinded to the treatment received by the animal performed the in situ evaluations. Epicardial adhesions were evaluated on presence or absence of adhesions, and percent of abraded site with adhesions. Percentages were measured as: 0% = no adhesions; 25% = adhesions covering 1-25% of site; 50% = adhesions cover 26-50% of site; 75% = adhesions cover 51-75% of site; and 100% = adhesions cover 76-100% of site. The following qualitative grading system was used to evaluate tenacity of the adhesions: 0 = no adhesions; 1 = mild adhesions (filmy); 2 = moderate adhesions (blunt dissection); and 3 = severe adhesions (sharp dissection). The data collected for the formation of adhesions, tenacity of adhesions, and percent of abraded site with adhesions are rank order data and were analyzed using the Kruskal-Wallis test with StatXact (Cytel Software Corporation, v. 4).

All adhesions were lysed and the heart was dissected out. India ink was applied to the surface to mark the area of application of materials, and samples were placed in 10% formalin. Explant samples were bisected; one section was embedded in plastic and the other in paraffin. Tissue blocks were sectioned, and stained with H&E and trichrome. Histologic evaluation included fibrosis, necrosis, and inflammation at the surgical site. The type of inflammatory cells, the relative number of each cell type, and the distribution of the cells in relation to the surgical site/implant site were recorded. The presence of residual material was noted, as well as any

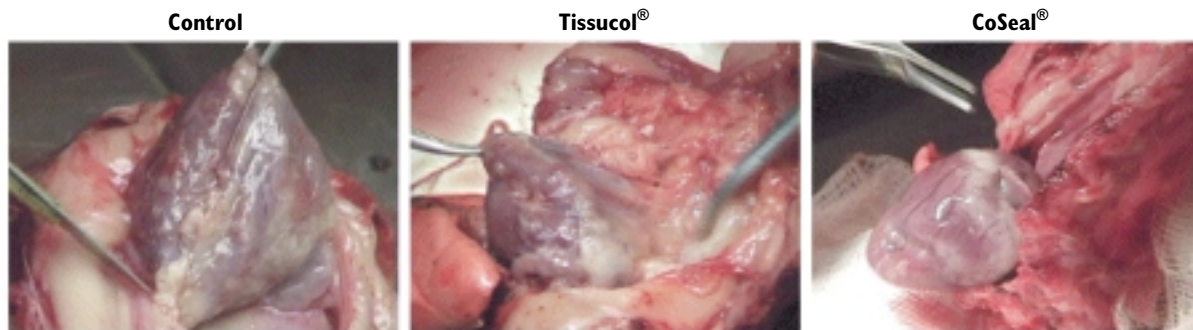


Figure 2. Gross observation at necropsy showed 100% of the Control and Tissucol® animals with adhesions of a tenacity grade 2-3.

changes in surrounding tissue morphology. The presence of Tissucol® was confirmed using an antibody stain for human fibrin, which is present in Tissucol®.

RESULTS

Analysis using the Kruskal-Wallis exact test showed that CoSeal® significantly reduced formation of adhesions as compared to controls ($p=0.002$) and Tissucol® ($p=0.007$) (see Figure 1, ©). (See Movie 1, gross observations of Controls with adhesions, Movie 2, gross observations of Tissucol® with adhesions, and Movie 3, gross observations of CoSeal® with no adhesions.) Fifty percent of CoSeal® treated animals were adhesion free, while no control or Tissucol® treated animal was adhesion free (see Figure 2, ©). Tissucol® did not inhibit the formation of adhesions, with 100% of the animals in this group forming adhesions. Eighty-seven percent of CoSeal® animals had grade 1 or no adhesions. CoSeal® significantly reduced the tenacity of adhesions compared to controls ($p=0.02$) and Tissucol® ($p=0.004$) (see Table 1, ©). There was no significant difference in tenacity between controls and Tissucol® treated animals. Analysis of the percent of the abraded area with adhesions showed that CoSeal® significantly reduced the area with adhesions as compared to controls ($p=0.002$) and Tissucol® ($p=0.005$) (see Figure 3, ©).

Gross observations at necropsy, other than the formation of adhesions, were minimal. The only difference between treatment groups was that the Tissucol® treated animals consistently had clear reddish tinted fluid within the surgical site.

Inflammation in all CoSeal® and control animals ranged from very mild to moderate (see Figure 4, ©). The Tissucol® animals had moderate to marked inflammation. The predominant inflammatory cells present in all groups were lymphocytes and macrophages with a small number of neutrophils and multinucleated giant cells. The Tissucol® sites had moderate numbers of plasma cells and eosinophils, more than the amount seen in CoSeal® and the control animals. No implant material was detectable in any CoSeal® treated animal. Half of the Tissucol® treated sites had residual implant material.

The myocardial tissue was well preserved in all groups, except in areas of apparent surface abrasion. As would be expected from the surgical injury, fibrous tissue increased in the abraded area (see Figure 5, ©). CoSeal® treated hearts showed re-establishment of the mesothelial layer with only

mild fibrosis and a small amount of new collagen deposition at the abraded site. The resulting overall tissue morphology was similar to a normal un-operated heart. Tissucol® treated and Control hearts showed marked fibrosis at the abraded sites with increased collagen deposition.

DISCUSSION

Ten to twenty percent of CABG or valve replacement patients will undergo a second cardiac procedure [Lytle 1986, Higgins 1997, Loop 1990]. At least 3% are expected to have catastrophic hemorrhage during a repeat median sternotomy. This complication is associated with 36% mortality. The lack of discernible pericardial planes makes dissection difficult and only becomes more complicated with each subsequent operation. Therefore adhesions are potentially life threatening because they impede the ability of the surgeon to visualize tissue planes and to orient the heart and great vessels [Dobelle 1984, Loop 1984, Segesser 1987].

No treatment for adhesions exists. Therefore, prevention is important in all cardiac procedures. Many agents have been evaluated for their efficacy in preventing, reducing, or inhibiting adhesions in animal models, including low molecular weight dextran, hyperosmolar saline, hyaluronic acid solutions, and recombinant tissue plasminogen activator (rt-PA) [Wiseman 1994]. Investigators have shown impaired wound healing as well as increased bleeding with the use of fibrinolytic agents for the prevention of adhesions [Wiseman 1994, Okuyama 1998, Holmdahl, 1999].

Pericardial substitutes have been employed in an effort to reduce adhesions. Pericardial xenografts and synthetic membranes have been used [Mitchell 1994a, Chanda 1996]. The use of xenogeneic material (most often bovine or equine peri-

Table 1. Tenacity of Adhesions

Treatment	Tenacity Scores			
	0	1	2	3
CoSeal®	50%	37.5%	12.5%	
Control		12.5%	25%	62.5%
Tissucol®			16.7%	83.3 %

CoSeal® significantly reduced the tenacity of adhesions as compared to controls and Tissucol®.

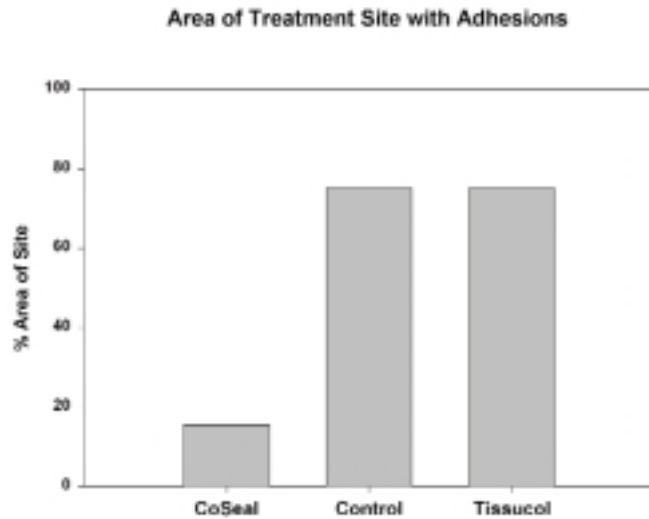


Figure 3. CoSeal® significantly reduced the percentage of the treatment site of the heart with adhesions compared to controls and Tissucol®.

cardium) has been plagued by infection, late calcification, dense adherence to the overlying bone, and, most significantly in some cases, a severe epicardial reaction obscuring the coronary anatomy [Mathisen 1986, Mitchell 1994b, Pacholewicz 1994]. Reconstruction using a permanent synthetic material, notably polytetrafluoroethylene (PTFE) reportedly has been successful at reducing adhesions to the sternum [Zehr 1993, Mitchell 1994b]. However, it has also been found to induce severe obliterative epicardial reactions complicating re-operation. A Poly-2-hydroxyethyl methacrylate (pHEMA) hydrogel reinforced with a polyethylene terephthalate (PET) mesh has been tried, but a thick fibrous layer formed on the heart [Walker 1992]. Duncan reported that the use of

polyvinylpyrrolidone (PVP) and methylcellulose (MCD) prevented postoperative pericardial adhesions in a canine model [Duncan 1988]. There has been no report of the successful use of these materials in humans.

More recently, resorbable materials for inhibiting cardiac adhesions have been tested. A hyaluronic acid, bioabsorbable membrane was shown to significantly reduce the formation of adhesions in dogs [Mitchell 1994a, Mitchell 1994b]. Resorbable films of polyethylene glycol (PEG) and polylactic acid placed between epicardium and sternum and sutured to the edge of pericardium, significantly reduced retrosternal adhesion formation in a rabbit model [Okuyama 1998]. Solutions or gels have also been shown to inhibit adhesion formation in animal models, including Dextran 70 in rabbits [Robison 1984].

The ideal adhesion barrier would be a material, agent, or substance that would prevent drying, necrosis, and inflammation of the tissue. The material should be easily placed on the tissue site without requiring sutures. The material should not increase risk of infection. It should prevent tissue surfaces from contacting, prevent bleeding from tissue surfaces, and prevent blood clot formation from sticking tissue planes together. In addition, the material would not provide a matrix for fibroblasts to infiltrate which could bridge the gap between internal tissue surfaces.

As an adhesion barrier, CoSeal® appears to have these characteristics. CoSeal® is comprised of two PEGs. An inherent property of polyethylene glycol (PEG) is that cells do not attach to the material surface [Drumheller 1995]. This property has been exploited in its use as a coating for vascular catheters, preventing cellular attachment and blood clotting [Merrill 1992]. Therefore, CoSeal® is a temporary hydrogel barrier that does not act as a matrix for cellular infiltration. CoSeal® has previously been reported to inhibit bleeding using a canine iliac PTFE graft model [Hill 2000]. In this

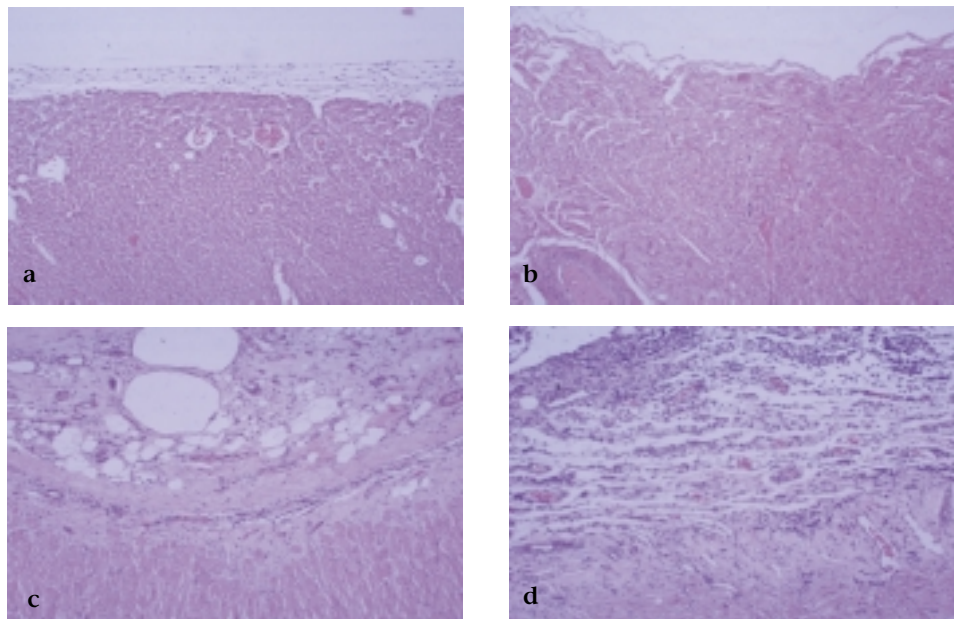


Figure 4. CoSeal® (a) treated hearts showed only a moderate inflammatory response at the site of material application, which was indistinguishable from un-operated (b) and surgical controls (c). Tissucol® (d) treated hearts showed marked inflammation around sites of residual material. (H&E, $\times 100$).

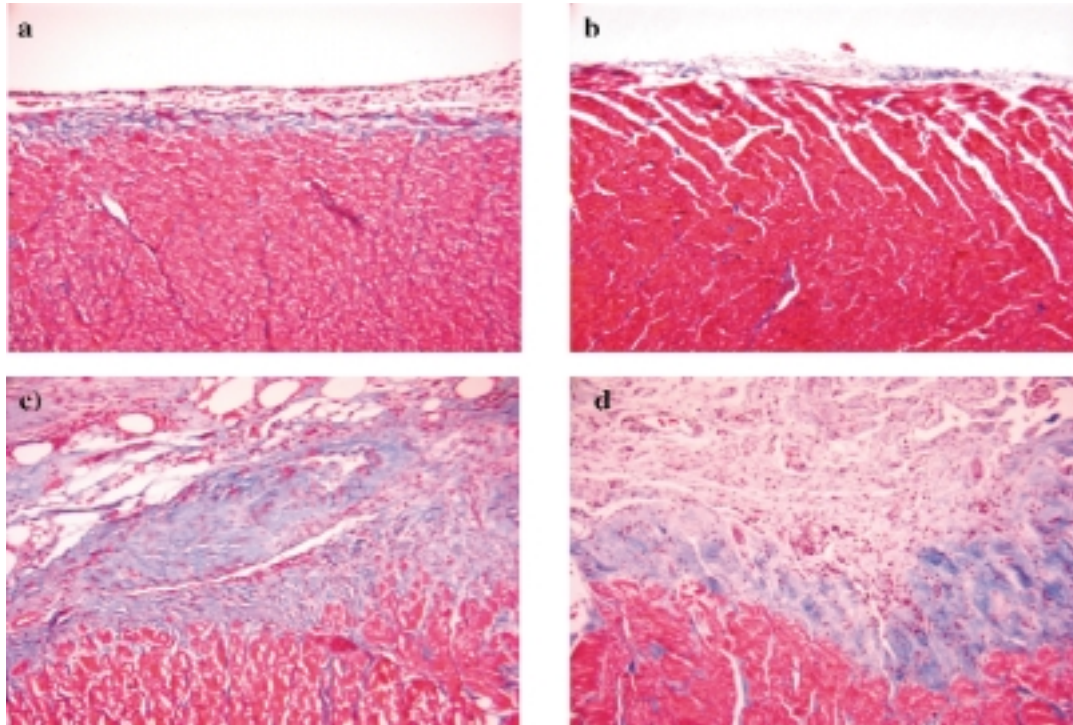


Figure 5. CoSeal[®] (a) treated hearts showed re-establishment of the mesothelial layer with mild fibrosis and small amounts of new collagen deposition at the abraded site. The resulting overall tissue morphology was similar to an un-operated heart (b). Control (c) and Tissucol[®] (d) treated hearts showed marked fibrosis at the treated site with increased collagen deposition. (Trichrome, x100).

model, CoSeal[®] significantly reduced the time to hemostasis (average of 5 minutes vs greater than 15 minutes, $p < 0.05$) and blood loss (19 g vs 284 g, $p < 0.05$) versus control (tamponade). CoSeal[®] was resorbed by 30 days and the healing response was similar to tamponade controls. CoSeal[®] did not produce marked necrosis or inflammation or increased rates of infection in animals in this study or in a previous study [Hill 2000]. CoSeal[®] was easily placed on the heart without sutures. It was easily applied onto the tissue surface and gelled within seconds of application. The material is a flexible, degradable hydrogel that adheres to tissue, thereby preventing additional blood loss and acts as a barrier inhibiting the formation of adhesions.

Our data on Tissucol[®] show that the commercial fibrin glue did not reduce adhesions in cardiac procedures. Fibrin from blood is known to be a factor in adhesion formation, therefore depositing fibrin to the site as with Tissucol[®], and is anticipated to produce adhesions [Holmdahl 1999]. In this study, Tissucol[®] did not inhibit the formation of adhesions. Researchers have presented mixed data on the subject of the ability of fibrin sealants to act as adhesion barriers [Joyce 1991, de Virgilio 2000].

In the clinic, CoSeal[®] is an effective vascular sealant [Mersman 2000]. In addition, the authors report on four cardiac surgery patients who were treated with CoSeal[®] in two types of procedures. All procedures employed cardiopulmonary bypass and cold cardioplegia. Three of the patients had double-bypasses in which the left internal mammary artery (LIMA) was grafted to the left anterior descending

artery and the saphenous vein was grafted to the posterior descending artery. CoSeal[®] was applied along the length of the LIMA graft after the patient was removed from by-pass and just prior to closing. (See Movie 4, human case showing application of CoSeal[®] along the LIMA). The fourth patient had CoSeal[®] applied to the suture line on the aorta after the aortic valve was replaced. The patient was still on by-pass at the time of CoSeal[®] application. CoSeal[®] was allowed to set for 60 seconds at which time the standard operation proceeded. CoSeal[®] was quickly prepared for application within 2 - 3 minutes. It was easily applied onto the tissue surface and gelled within seconds of application. After application of CoSeal[®] to the LIMAs, there was no loss of blood or blood oozing. The aortotomy to which CoSeal[®] was applied did not leak when circulation was restored.

Surgeons use Tissucol[®] (fibrin glue) to inhibit or control the oozing that occurs at suture lines. CoSeal[®] appears to work as well as Tissucol[®] in these applications. CoSeal[®] was quicker to prepare and easier to use than Tissucol[®]. In addition, the preclinical data reported here supports the use of CoSeal[®] to significantly reduce the formation of adhesions, the tenacity of the adhesions, and the percent of site with adhesions as compared to Tissucol[®]. All Tissucol[®] treated animals formed adhesions and showed no significant difference from the surgical control in any parameter of adhesion formation. Tissucol[®] was still present in 50% of the treated animals at sacrifice, while no CoSeal[®] was visible grossly or histologically. CoSeal[®] is an effective surgical sealant, as demonstrated in our clinical experience and as previously

reported [Mersman 2000], and it has the added benefit of inhibiting the formation of adhesions.

In conclusion, CoSeal® significantly inhibited the formation, tenacity and area of adhesion formation as compared to surgical control and Tissucol® treated animals. CoSeal® treated animals showed re-establishment of the mesothelial layer and tissue morphology similar to a normal un-operated heart. CoSeal® warrants further evaluation to determine if the results in the aforementioned animal studies can be replicated in the clinic.

Acknowledgment

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Disclosure

Dr. Hendrikx and Dr. Mees have no financial interest in CoSeal® or Cohesion Technologies. Dr. Hill is a clinical advisor of Cohesion Technologies. Drs. Estridge, Coker, and Egbert are currently employees of Cohesion Technologies. The animal study was funded by Cohesion Technologies.

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REVIEW AND COMMENTARY

1. Editorial Board Member KE221 writes:

- a) Who manufactures the product?
- b) How much does it cost?
- c) What stage is it in regarding FDA approval?

Authors' Response by Marc Hendrikx, MD:

- a) Cohesion Technologies
- b) Costs for the different sizes of CoSeal® have not yet been established in the US.
- c) A PMA for CoSeal® was filed April 17th. A copy of the news release can be found at URL <http://www.prnewswire.com/micro/CSO>.

2. Editorial Board Member EK34 writes:

This is well written and designed. The authors need to say why rabbits were used versus other animal models. What's the next step?

Authors' Response by Marc Hendrikx, MD:

Rabbits were used in the study based on previous work showing similar methods produced cardiac adhesion in the rabbit model. Literature information on larger animals was limited at study initiation.

3. Editorial Board Member SC389 writes:

Please explain if this is an appropriate time postop to assess the adhesions.

Authors' Response by Marc Hendrikx, MD:

The authors believe that 14-21 days postop is appropriate as adhesions begin to form postop and are recognizable at 7 days.

4. Editorial Board Member GX21 writes:

- a) The bar graph figures (1 and 3) are data-sparse, just 3 values plotted; not enough to justify a figure. They could be augmented with numbers or ranges or individual value.
- b) 22 rabbits were assigned to 3 groups. How many rabbits in each group, and why not the same number in each group? Was the assignment done randomly?

Authors' Response by Marc Hendrikx:

- a) The authors have explored various methods of presenting the data and have chosen the method that they believe

accurately and quickly allow the reader to see the results.

- b) The 22 rabbits reported in the study, include only animals that finished the study. Twenty-five animals were obtained for the study (8 for each group and 1 spare). However, due to surgical complications in 2 animals (one each, Tissucol® and Control) and an infection in 1 control animal full randomization of animals was not achieved in the study. Of the 22 rabbits, 8 animals were in the control and CoSeal® groups and 6 in the Tissucol® group.

5. Editorial Board Member DB515 writes:

It would be interesting to see some sort of (semi) quantitative evaluation of the amount of bleeding with the different sealants' since bleeding I believe is a very important reason for applying the sealants. It would also be interesting to see a longer term effect on adhesions since that definitely is the main interest. The authors sacrificed the animals at 14-21 days. That could introduce quite a bias if the different groups were not sacrificed at the same time point. This point should be clarified.

I agree with the authors that a sealant that could reduce adhesions with few or no side effects would be a very important tool in cardiac surgery. This study on early adhesions is certainly promising.

Authors' Response by Marc Hendrikx, MD:

There was no significant difference in the adhesion data between the 14 and 21 timepoints in the treatment groups. The authors reference in the manuscript a previous study using CoSeal® for sealing PTFE grafts that would address the reviewers question.

6. Editorial Board Member NE55 writes:

Could the authors comment on the situation with the pericardium closed (probably this is a more realistic and superior assessment technique). Could they comment about what happened at the pericardial rim, and speculate about prevention of pleural adhesions?

Authors' Response by Marc Hendrikx, MD:

Closing of the pericardial rim was not done in the rabbit model nor was specific assessment done of that site. The authors have not done testing of CoSeal® in pleural adhesions. However, they have recently presented data on CoSeal®'s ability to prevent adhesions in abdominal rabbit models.