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ANESTHESIOLOGY AND RESPIRATORY THERAPY DEVICES PANEL

Gaithersburg, Maryland

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NeoMend Inc. ProGEL™ Surgical Sealant

PMA P010047

SPONSOR'S SUMMARY OF PRECLINICAL DATA

PRECLINICAL OVERVIEW

All biocompatibility, toxicity, and animal effectiveness studies were performed in compliance with current Good Laboratory Practices, 21 CFR Part 58, and the human safety study (Human Repeat Insult Patch Test-HRIPT) was conducted in compliance with Good Clinical Practices, 21 CFR Part 50.

Biocompatibility Studies

Cytotoxicity studies conducted on extracts, *in situ* polymerization, and individual components of the sealant demonstrated materials to be non-cytotoxic.

Topical application of Sealant was non-irritating to the skin of rabbits. Intracutaneous injection of Sealant, allowed to polymerize *in situ*, was moderately to severely irritating to rabbits. When saline and polyethylene glycol (PEG) 400 extracts of polymerized Sealant were injected intracutaneously in rabbits, the extracts were non-irritating.

Saline extracts of the Sealant were non-hemolytic in human whole blood and were not pyrogenic in rabbits.

The initial Guinea Pig Maximization Test with saline extracts of Sealant caused a hypersensitization response. Additional animal studies attributed this immune response to cross-species interaction between the animal model and the human albumin component of the device. To address the concern of human dermal sensitization, an HRIPT study was conducted, which shows the Sealant not to be a sensitizer.

Saline extracts of polymerized Sealant were administered as a single intravenous injection to mice. Sesame oil extracts of the cured Sealant were administered via intraperitoneal injection to mice. Neither extract of Sealant exhibited acute systemic toxicity in mice.

No systemic effects were noted. Acute segmental hemorrhagic enteropathy was noted at the implantation contact sites at day 8 but no anatomical pathological findings were present at day 29. A 7 day follow-up study demonstrated that the enteropathy was mitigated by the instillation of saline into the peritoneal cavity post implantation. It was concluded that the enteropathy was caused by the hygroscopic nature of the Sealant.

The Sealant was not mutagenic nor clastogenic.

Mass balance studies in rats indicated the Sealant degrades readily (within 14 days) and is rapidly excreted primarily in the urine within 72 hours.

Animal Efficacy And Tissue Healing Studies

In 7-Day efficacy and 28-Day tissue healing pig studies, *in situ* polymerized Sealant, when applied to air leaks in the lungs of pigs, was successful in sealing severe air leaks >1,000 cc/min. No immune response or adverse tissue effects were observed. Tissue healing progressed normally in the presence of the Sealant.

Summaries of the preclinical studies are presented in the table below.

Preclinical Testing for the Sealant

Study	Test Article(s) Preparation	Findings
Histopathology - Pig 7 Day Efficacy	*In situ* polymerization[1]	No evidence of an immune response.
Tissue Healing - Pig 28 Day Study	*In situ* polymerization[2]	No evidence of an immune response. Wound healing progressed normally.
Efficacy Study- Pig	*In situ* polymerization[1]	Thoracotomy procedure in 6 pigs. Sealant applied to ALs >1000 cc/min. No leaks at day 7, original test sites remained closed.

[1]NS-lH: Sealant containing human albumin component, gamma sterilized.
[2]NS-1H(e): Sealant containing human albumin component, e-beamed.

Preclinical Studies

INTRODUCTION

Air leaks (ALs) are one of the most common complications of pulmonary surgery. They can develop from suture/staple lines and other types of surgical manipulation, or simply be due to the fragile state of the diseased lung tissue. Without prompt and effective treatment, ALs can lead to increased morbidity and extended hospitalization. Traditionally, suture techniques and stapling devices have been used to seal parenchymal defects. Both can exacerbate rather than remedy the AL. Consequently, there has been a recognized clinical need for a device that effectively seals intraoperative air leaks during pulmonary surgery.

The product developed to address this need, and which is the subject of this PMA, was originally developed by 3M Corporation and was called the 3M Polymeric Patch. In 2007 NeoMend, Inc. acquired all of the assets of the 3M Polymeric Patch business unit and renamed the product the NeoMend ProGEL™ Surgical Sealant ("ProGEL"). ProGEL is identical in formulation, materials, and chemistry to the 3M Polymeric Patch, and all preclinical tests of the 3M Polymeric Patch described in the following are equally applicable to ProGEL.

For the purposes of this PMA the term "Sponsor" applies to both 3M and to NeoMend and should be considered synonymous throughout this document. Similarly, the terms "3M Polymeric Patch" and "ProGEL" should be considered synonymous throughout this document, and are referred to as the "Sealant."

Since the mid 1990's Sponsor has made a substantial investment in the development and testing of the Sealant to ensure its safety and effectiveness for sealing lung air leaks. Among the studies performed in support of IDE G980283) were animal tests to evaluate tissue wound healing and biodegradation (mass balance) as well as small and large animal tests of the Sealant for pulmonary ALs. All biocompatibility/toxicity studies and additional animal safety/efficacy studies were performed in compliance with current Good Laboratory Practice, 21 CFR Part 58.

The results of these preclinical studies have indicated that the Sealant is suitable for its intended use.

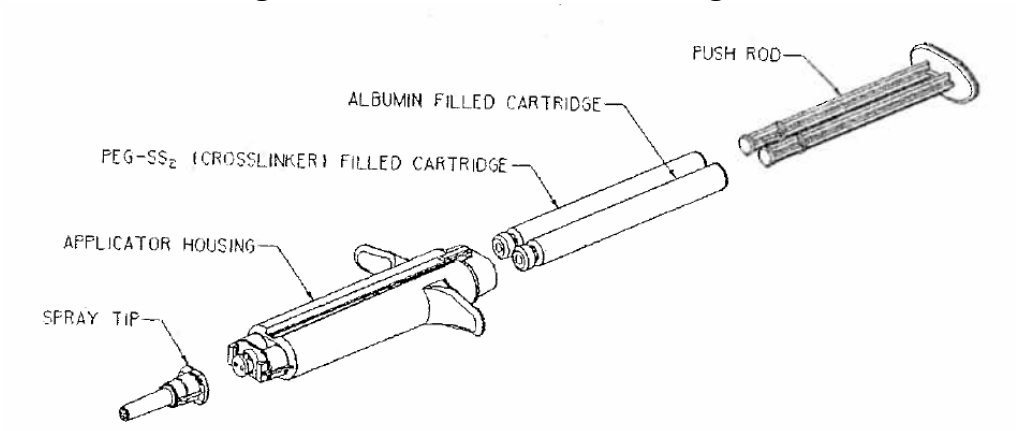
1.0 DEVICE DESCRIPTION

The Sealant consists of a synthetic cross-linking component, polyethylene-glycol (PEG), and a component derived from human serum albumin USP [redacted]. The PEG based cross-linker component is functionalized with succinate groups, and reacts with the HSA component to form a clear, pliant hydrogel. The PEG component is provided to the end user as a powder, which is reconstituted with sterile water. Following reconstitution of the cross-linker, the two liquid components in glass cartridges are placed in an applicator that mixes them within a spray tip, initiating polymerization upon application to the lung tissue. Polymerization is essentially completed in less than 30 seconds, without the need for additional equipment or energy sources, and does not generate any heat. The gel strength is sufficient to withstand 30 mmHg air pressure in two minutes and 90 mmHg in less than ten minutes. After application, the material forms a flexible seal over the

surface of the tissue around the AL thereby providing a seal. The clear hydrogel remains soft and compliant and does not harden or turn brittle. The Sealant degrades and is completely resorbed within two to three weeks.

The Sealant (Figure) is packaged as a single use, sterile chemistry component kit (polyethylene-glycol based cross-linker, functionalized with succinate groups (PEG(SS) 2) , and Human Serum Albumin - USP) and a single use, sterile applicator kit (push rod, tip assembly, applicator housing, a vial of sterile water for injection - USP, and a syringe). Both the cross-linker and albumin components are individually contained within hermetically sealed cartridges.

Figure: Neomend Inc. ProGEL Surgical Sealant



The applicator is designed to mix the two solutions and deliver the Sealant as a spray to the target site. Once mixed, the Sealant polymerizes to form a cross-linked, clear, flexible hydrogel matrix that adheres to the lung tissue. The polymerization process does not require peripheral equipment, such as light sources or heating elements to allow for proper device function.

When the Sealant contacts lung tissue, it conforms to the tissue by adhering to the microstructure of the lungs. The Sealant stays in place and allows for the expansion and relaxation of the lung tissue until it biodegrades (less than 30 days). Based on information from animal experiments, the hydrogel first swells, loses mechanical strength and then undergoes breakdown primarily by hydrolysis. As the Sealant biodegrades it is cleared primarily through the kidneys or locally metabolized.

2.0 SEALANT CHARACTERIZATION

To evaluate the ability of the Sealant to conform to the company's predetermined design specifications regarding sealant characteristics, the following tests and results were evaluated. Table 2.0 presents an overall summary of the information provided in sections 2.1 through 2.3.

Table 2.0 Sealant Verification Summary (Vol. 1, Table 5.3, p60)

Test	Acceptance Criteria	Sealant
Burst Strength	Average> 90 mm Hg	114.3 mm Hg
Gel time	8 ≤ sec ≤ 40	Average = 13.7 sec
Device Pyrogenicity*	≤ 20 EU/device	<5 EU device

*See section 1.3 Device Pyrogenicity, 1.3.2 Test Standards — PMA Device

2.1 Burst Strength

Testing was performed to verify that the air pressure needed to rupture Sealant covering a simulated air leak meets the design specification. In addition, this test is also an indirect measurement of the adhesiveness of the Sealant to a simulated tissue.

Table 2.1.2 Burst Strength Results (Vol. 1, Table 5.3.1.3, p61)

Acceptance Criteria	Sealant	Pass/Fail
At 20 minutes hydrolysis time: Average> 90 mm Hg	**# Samples Tested** 20 Cartridges **Results** Average = 114.3 mm Hg SD = 12.01	Pass

In the clinical setting, peak inspiration pressure for ventilation of the lung after surgery is typically set at a maximum of approximately 30 mm Hg. For the Sealant's design specification Sponsor targeted a burst strength of at least 3 times the pressure of a severe air leak in the clinical environment, or >90 mm Hg. Based on these results, Sponsor concluded the Sealant has sufficient burst strength to seal an air leak.

2.2 Gel Time

Measurement of the polymerization rate of the Sealant components ("gel time") was performed to verify that the time required for a mixture of crosslinker and albumin to form a hydrogel meets the design specification.

Table 2.2.2 Gel Time Results (Vol. 1, Table 5.3.2.3, p62)

Acceptance Criteria	Sealant	Pass/Fail
8 ≤ Seconds ≤ 40	**# Samples Tested** 7 **Results** Average = 13.7 sec S.D. = 1.25 sec	Pass

Sponsor conducted experimental studies and discussions with clinical experts to determine optimal gel time performance for the Sealant. Sponsor determined that gel time should be at least the practical lower limit for working time during Sealant application, and no longer than the time for the Sealant to cure prior to testing the lung for remaining air leak in a timely manner. Based on these results, Sponsor concludes the Sealant has an acceptable gel time.

2.3 Device Pyrogenicity

The objective of this testing was to verify that the endotoxin level of the Sealant is less than or equal to 20 endotoxin units per device.

Table 2.3.2 Device Pyrogenicity Results (Vol. 1, Table 5.3.3.3, p 63)

Acceptance Criteria	Sealant	Pass/Fail
< 20EU/Device	**# Samples Tested** 10 Devices **Results** < 5 EU/Device	Pass

Based on these results, Sponsor concluded that the Sealant has an acceptably low level of endotoxin units so that any pyrogenic reaction to its presence on lung tissue will not represent an adverse effect in its application.

3.0 BIOCOMPATIBILITY TESTING

Biocompatibility tests selected for this device were determined based on FDA's blue book memorandum #G95-1, "Use of International Standard ISO-10993, Biological Evaluation of Medical Devices Part 1: Evaluation and Testing" dated May 1, 1995.

The following Table 3.0 provides a summary of all biocompatibility testing performed on the Sealant and results. This is followed by more detailed supporting text.

Table 3.0 Biocompatibility Testing for the Sealant (Vol. 1, Table 6.0, p73-75)

Study	Test Article Preparation	Findings
Cytotoxicity	Extraction, Neat[1]	Non-cytotoxic
Cytotoxicity	*In situ* polymerization, Neat[6]	Non-cytotoxic
Irritation, Primary Dermal — Rabbit	*In situ* polymerization[1]	Non-irritant
Irritation, Ocular — Rabbit	*In situ* polymerization[1]	Mild irritant
Irritation (IC) — Rabbit	Extraction[1]	Non-irritant
Irritation (IC) — Rabbit	*In situ* polymerization[1]	Moderate—Severe irritant
Hemolysis	Extraction[1]	Non-hemolytic
Pyrogenicity — Rabbit	Extraction[1]	Non-pyrogenic
Sensitization — Guinea Pig	Extraction[1]	Sensitizer
Sensitization — Guinea Pig	Neat[2]	Sensitizer
Sensitization — Guinea Pig	*In situ* polymerization[3]	Non-sensitizer
Human Repeat Insult Patch Test	*In situ* Polymerization[4]	Non-irritating/non-sensitizer, when applied topically to 10 subjects
Acute Systemic Toxicity — Mice	Extraction[4]	No systemic toxicity
Subchronic Toxicity — Rat 7/14 Day Study	*In situ* polymerization[1,5]	No systemic effects noted. Enteropathy noted at implantation contact sites.
Subchronic Toxicity — Rat 28 Day Study	*In situ* polymcrization[1]	No systemic effects noted. Enteropathy noted at implantation contact sites at day 8 but no anatomical findings at day 29.
Subchronic Toxicity — Rat 7 Day Follow-up Study	*In situ* Polymerization[1,4]	No systemic effects noted. Enteropathy noted at implantation contact sites. The enteropathy was mitigated by the instillation of saline into the peritoneal cavity post implantation.
Ames Mutagenicity	Extraction[1]	Non-mutagenic
Ames Mutagenicity	Extraction[4]	Non-mutagenic
Ames Mutagenicity	Neat[6]	Non-mutagenic
Mouse Lymphoma	Extraction[4]	Non-mutagenic
Chromosome Aberration	Extraction[1]	Non-clastogenic
Micronucleus — Rat	*In situ* Polymerization[4]	Non-genotoxic
Pilot Mass Balance — Rat	*In situ* Polymerization[7]	No gender difference, urine was primary route of excretion. Virtually all of the Sealant was eliminated 14 days past application.
Full-Scale Mass Balance — Rat	*In situ* Polymerization[8]	No gender difference. Virtually all of the Sealant was eliminated 14 days past application.
Efficacy Study — Pig 7 Day Efficacy Study	*In situ* Polymerization[1]	Thoracotomy procedure in 6 pigs. Sealant applied to air leaks> 1000 cc/mm. No leaks at day 7, original test sites remained closed.
Histopathology — Pig 7 Day Efficacy Study	*In situ* Polymerization[1]	No evidence of an immune response
Tissue Healing — Pig 28 Day Study	*In situ* Polymerization[4]	No evidence of an immune response. Wound healing progressed normally.

[1] NS-lH: Sealant containing human albumin component, gamma sterilized.
[2] GP: Commercially available Guinea Pig serum albumin, processed, e-beamed.
[3] NS-1G(e): Sealant containing cross-linked low endotoxin 3M prepared Guinea Pig albumin component, e-beamed.
[4] Ns-1H(e): Sealant containing human albumin component, e-beamed.
[5] NS-1R: Sealant containing rat albumin component gamma sterilized.
[6] NS-1H(e) Component 1000: PEG-(SS)2 crosslinker, e-beamed.
[7] C14-NS-1H: C14 Sealant
[8] C14-NS-1H(e): C14 Sealant, e-beamed.

3.1 Cytotoxicity

None of the test article and test article extract concentrations evaluated were cytotoxic to cultured BALB/c-3T3 cells. The RCEs ranged from 95% to 113% for the unpolymerized liquid test article and from 101% to 134% for culture media test article polymer extracts. The positive control reference materials and the negative solvent control produced the expected responses, confirming that the assay was valid. A median inhibitory concentration could not be determined for the test article and its extracts because none of the concentrations evaluated were cytotoxic.

3.2 Guinea Pig Sensitization Studies

The series of experiments reviewed below were performed in a systematic approach to determine which of the components of the Sealant, if any, were responsible for eliciting any observed hypersensitivity.

A first Guinea Pig Maximization study was conducted to determine if the Sealant (NS-1H) would be a potential allergen. A hypersensitivity response to NS-1H saline extracts was elicited, suggesting that it may be an allergen. The human serum albumin component was found to cause hypersensitivity in the guinea pig as well.

The second Guinea Pig Maximization study was performed to see if commercially available Guinea Pig serum albumin could be used in lieu of the human serum albumin. The plan was to use this Guinea Pig albumin to make the Sealant and to test its immunogenic potential in the Guinea Pig. This would avoid the confounding aspect of "cross-species proteins" exposure in the Guinea Pig sensitization model. Unfortunately, this Guinea Pig albumin was contaminated with a high level of endotoxin, which elicited a hypersensitivity response in the Guinea Pig.

A small quantity of "endotoxin-free" Guinea Pig albumin was produced using an "e-beam" sterilization process and a third Guinea Pig Maximization study was performed using this Guinea Pig albumin. The results indicated that in the Guinea Pig animal model, the "endotoxin-free" Guinea Pig albumin has a low risk to elicit either a Type I or Type IV sensitization response.. Thus, the hypersensitivity response that was observed in the first Guinea Pig Maximization study was determined to be human albumin component related.

The fourth Guinea Pig Maximization study using the Sealant containing crosslinked "endotoxin free" Guinea Pig albumi also resulted in a low risk potential to elicit either a Type I or Type IV sensitization response. In this study, the Sealant was applied to "breached skin" at first induction and second induction, as well as at challenge, allowing for the evaluation of sensitization potential of Sealant components or polymerization by-products that would not pass through intact skin. This crosslinked Guinea Pig albumin study was conducted to supplement the information from the Human Safety Study described in section 7.0, below.

3.3 Irritation

The experiments reviewed below were performed in a systematic approach to determine which of the components of the Sealant, if any, were responsible for eliciting irritation reactions.

The first irritation study examined dermal irritation by examining the irritant and/or corrosive effects of the Sealant on the skin of rabbits. The exposure produced no edema or erythema during the test period. Based on this data, the Sealant is considered to be non-irritating to the intact skin of the rabbit.

The second irritation study examined eye irritation studies in rabbits. Exposure caused iritis in 1/6 eyes with resolution within the 24-hour scoring interval. Conjunctivitis was noted in 5/6 eyes and resolved completely by day 14 of the study. Based on this data, the Sealant is considered to be a mild irritant to the ocular tissue of the rabbit.

The third irritation study examined intracutaneous reactivity to Sealant extracts in rabbits. The study observed no apparent skin reaction in the rabbits following administration of either the PEG400 or saline components of the Sealant. Based on this data, the extracts of the Sealant are not considered to be irritants for rabbits when injected intracutaneously.

The fourth irritation study examined intracutaneous reactivity in rabbits following injection with test article and in situ polymerization. The injection and subsequent polymerization caused moderate to severe irritation as determined by PIT scores. A number of factors may explain this reaction including osmotic shift and localized pressure necrosis. When used as indicated during pulmonary surgery to seal air leaks, with no localized pressure necrosis, the sealant is not likely to pose a risk of irritation to patients.

3.4 Systemic Toxicity (Acute)

The objective of this test was to evaluate extracts of the test article for its potential to cause systemic toxicity by standard observational measurements.

There were no observed signs of systemic toxicity. No mortality occurred during the study. None of the mice in any of the groups exhibited clinical signs after injection. A slight body weight loss was noted for several animals during the test period, and was not considered test article extract related. It was concluded that saline and sesame seed oil extracts of Sealant e-beamed are not associated with systemic toxicity.

3.5 Subchronic Toxicity

3.5.1 Pilot Study - 7 and 14 Day Pilot Rat Implantation

Title: Pilot Toxicity Study of Implanted Biodegradable Polymeric Patches NS1H and NS-1R in Rats

> Test Article: NS-1H, Sealant containing human albumin; NS-1R, Sealant containing rat albumin

3.5.1.1 Objective

The purpose of this pilot study was to evaluate and compare the toxicity of two biodegradable Sealants at 1 and 2 weeks following surgical implantation to the serosal surface of the peritoneal cavity of male Sprague-Dawley Crl:CD®Br rats. Results from this study were used to select the most appropriate Sealant formula for a subsequent definitive rat implant study. Two formulations of Sealant were assessed. One was prepared by combining cross-linker with human albumin (NS-1H) and the other by combining cross-linker with rat albumin (NS-1R).

3.5.1.2 Findings

No systemic toxicity effects noted. Both NS-1H and NS-1R caused minimal irritation at implant contact sites.

The study demonstrated essentially no test article-related systemic toxicity, minimal surgical response at the implant site and no apparent difference in response to the two patches' formulation. All rats survived until scheduled termination, except for one Group 1 rat. This rat died after blood collection, but prior to euthanasia, on Day 15. The death was not considered test article related. One rat in each group had a sore/scab at the incision site, and one rat in each group had swelling at the surgical site on multiple days. The clinical signs were considered related to the surgical procedure and unrelated to the test article. Body weight gain and food consumption was similar for both groups and was not indicative of a decrement in health. The hematology and coagulation results were generally unremarkable and comparable between the groups at both intervals.

At week 1 post implant, tissue reaction at the implant site was similar between the 2 groups. Slight to moderate inflammation (consisting of thickening of the peritoneum due to increased infiltration of fibroblasts, macrophages, as well as a few lymphocytes, mast cells, and eosinophils) was observed. Collagen was slightly increased. Inflammation was limited to the peritoneum, and did not involve the underlying skeletal muscle. At 2 weeks post implant, the tissue reaction was reduced in both groups.

There were no apparent test article related histopathologic changes noted in the spleen. One rat had a slight increase in extramedullary hematopoiesis, and this was most likely associated with a skin abscess at the incision site of this animal. The subcutis of all 12 animals had mild to severe inflammation, which is consistent with the trauma commonly associated with a surgical incision.

3.5.1.3 Conclusions

In summary, there were no significant toxicological findings in this study, and response to the two test articles was minimal and comparable. Results from this study demonstrated essentially no test article-related systemic toxicity, minimal surgical response at the implant site and no apparent difference in response to the two patches' formulation. Based on this data, a decision was made to use cross- linked Human Albumin in the Main (28-day) rat implant study.

3.5.2 28-Day Rat Implantation

Title: 28-Day Toxicity Study and Peripheral Blood Micronucleus Assay of Implanted Biodegradable NS-1H Polymeric Patch in Rats

Test Article: NS-1H, Sealant

3.5.2.1 Objective

The purpose of this study was to evaluate the toxicity and mutagenicity of the Sealant after surgical implantation to the serosal surface of the peritoneal musculature of rats for 7 and 28 days.

3.5.2.2 Results: Micronucleus Assay

No systemic effects noted. Enteropathy noted at implantation contact sites at day 8 but no anatomical pathological findings at day 29.

The test article induced a statistically significant increase in micronuclei in peripheral erythrocytes (Group 4,0.448 ml); however the response is not considered biologically significant nor indicative of a positive response. Although the Group 4 response was minimal (0.05 + 0.01%, sexes combined), the vehicle control (Group 1) was even lower, at or near zero. The historical negative control value for the laboratory is 0.14 + 0.008% (sexes combined), a value much greater than the statistically significant mean value obtained for Group 4. For perspective, the historical mean positive control value (Cyclophosphamide, 60 mg/kg) is 2.25 + 0.13% for the combined sexes, some 45 times greater than the value obtained for Group 4.

3.5.2.3 Conclusion: Micronucleus Assay

Implanted Sealant was considered negative in the rat peripheral blood micronucleus test under the conditions of this study.

3.5.2.4 Results: Toxicity Study

There were no test article-related effects on survival, clinical observations, body weight, food consumption, ophthalmic findings, clinical pathology parameters (hematology, coagulation, clinical chemistry, urinalysis, and urine chemistry) and absolute or relative organ weights.

At the interim sacrifice (Day 8), discrete darkened segments or bands were observed on the small intestines (duodenum, jejunum, and/or ileum) of several mid- (Group 3) and high- (Group 4) dose animals. This gross observation corresponded to a microscopic finding of acute hemorrhagic enteropathy with or without minimal inflammation and/or tissue necrosis. This finding was not observed either grossly or microscopically and appeared totally reversed by the terminal sacrifice on Day 29.

Gross examination of the serosal surface of the ventral peritoneal musculature at Day 8 revealed an increased incidence of dark and/or raised areas in both sexes of Group 4 compared with the other groups. The dark/raised areas were located at the test (implant) site. This gross observation correlated with a microscopic finding of increased neovascularization and hemorrhage and possibly a small amount of test article (identified as foreign material) at test sites of Group 4.

Microscopic evaluation of the peritoneal musculature at Day 8 revealed subchronic inflammation, neovascularization, hemorrhage and foreign material at both treated and sham sites of all groups. There were no apparent gender differences. The incidence of inflammation was similar when treated sites were compared across the groups and when treated and sham sites were compared within each group. Severity of inflammation was slightly greater for treated sites versus sham sites within each group, but was similar when treated sites were compared across groups. This would suggest a treatment, and not a test article- related effect.

The incidence of neovascularization was greater for Groups 2-4 treated sites (peritoneal musculature) versus Group 1 treated sites, although a similar finding occurred for Group

2-4 sham versus Group 1 sham sites. Severity of neovascularization was similar for treated and sham sites in Groups 1-4 (minimal- slight) for treated and sham sites. Hemorrhage was observed more frequently in Groups 2-4 versus Group 1 treated sites and for treated versus sham sites within each group. Severity of hemorrhage (minimal-slight) was similar when treated sites were compared across groups and when treated and sham sites were compared within groups. Increased hemorrhage and neovascularization at the implant site appear test article-related at Day 8, although the severity is very low. Foreign material (minimal-slight) was observed more frequently in treated versus sham sites within each group, and was increased at the high dose when treated sites were compared across groups. This could indicate the presence of a small amount of residual test article at the treated site at Day 8.

At Day 29, a decreased incidence of inflammation, hemorrhage, neovascularization and foreign material were observed, and all appeared treatment, but not test article, related. There was no evidence of residual test article at any of the implant sites.

3.5.2.5. Conclusions: Toxicity Study

The only test article-related effect observed in this study was acute segmental hemorrhagic enteropathy in the small intestines. This lesion was seen only at Day 8. This finding was not observed either grossly or microscopically and appeared totally reversed by the terminal sacrifice on Day 29. The lesion most likely occurred in segments of the intestine in close apposition to the implant site on the abdominal musculature. Such an effect was not seen at the implant/abdominal musculature interface.

The hemorrhage appeared to have been induced by some stress at the time of euthanasia or necropsy. It is hypothesized that the Sealant (a polymer known to absorb its own weight in fluid) could have caused increased fragility of the small vessels on the intestine. This increased susceptibility of the vessels may not have become apparent until some stress occurred at the time euthanasia (e.g., intraperitoneal injection of barbiturate) and/or necropsy (e.g. manipulation of the intestine). Hemorrhage has not been seen in pig lungs administered the Sealant either within 24 hours of application or up to 7 days post application.

The intestinal lesion observed in the rat abdomen appeared to be specific to the model, and most likely is not relevant to the clinically indicated use of the product for sealing pulmonary air leaks in the thoracic cavity.

3.5.3 Follow-up Study: 7 Day Rat Implantation

Title: 7-Day Toxicity Study of Implanted Biodegradable Polymeric Patches NS-1H and NS-1H(e) in Rats

Test Article: NS-1H, Sealant and NS-1H(e), Sealant “e-beam” sterilized

3.5.3.1 Background

To test for the existence of pulmonary air leaks, the thoracic cavity of pigs is filled with sterile saline and the lungs are submerged. Air leaks are visualized as bubbles rising from the lung tissue through the saline. Therefore, in the simulated clinical situations with pigs, the Sealant is applied to lung tissue, polymerizes in about 20 seconds, and the

thoracic cavity is then filled with saline to determine whether or not the leaks have been sealed. Most of the saline is then aspirated from the thoracic cavity prior to closure, although much fluid remains and much is generated from the wounds after closure. A thoracic tube is routinely placed to drain this excess fluid. In the clinical situation, the Sealant is exposed to large amounts of fluid that could satisfy the hygroscopicity of the Surgical Sealant. In the rat implant model, however, no saline was placed in the abdominal cavity post-implantation. There may not have been enough fluid available to prevent a local osmotic shift. The local effect on small blood vessels and intestinal hemorrhage that occurred under stressful conditions (euthanasia) 7 days later may have been blocked if saline had been added to the abdominal cavity to meet the fluid needs of the Sealant. This would have more closely mimicked the clinical situation.

The acute small intestinal hemorrhage was the only test article, NS-1H, related effect observed in study T-6922.3 (see 3.5.2 in the preceding), and only at the mid-(20X) and high dose (50X) levels. Note that the test article, NS-1H, consisted of e-beam sterilized cross-linker combined with human albumin that was not e-beam sterilized. E-beam sterilized human albumin is preferred for inclusion in the marketed finished device. This method of sterilization does not affect the efficacy or functionality of the device, and is unlikely to alter its local or systemic toxicity. The local and systemic toxicity of the test article containing cross-linker and human albumin that have both been e-beam sterilized NS-1H(e) will be evaluated and compared to the findings of 3M study T-6922.3 where NS-1H was evaluated (cross-linker only e-beam sterilized).

In the previous 28-day rat abdominal implant study (3M Study T-6922.3, Covance 6329-232), the local and systemic toxicity of test article NS-1H polymerized *in situ* in the rat abdominal cavity was evaluated. The test article (NS-1H) consisted of e-beam sterilized cross-linker combined with human serum albumin (HSA) that was not e-beam sterilized. The implant site was located on the ventral serosal surface of the peritoneal musculature. Dose dependent, gender dependent (4 mid and 5 high dose females, 2 mid and high dose males) acute segmental hemorrhage of the small intestines was observed. Those intestinal segments in close proximity to the implant site appeared to be affected. No significant inflammation or necrosis of the affected intestinal tissues accompanied the hemorrhage. This finding was observed at 8 but not 29 days post-implantation and appeared to occur at or within a few hours of necropsy of the animals. Some red blood cells were observed in the local draining lymph nodes of some of the affected animals.

It is possible that small blood vessels that were in close proximity to the test article at or near the time of *in situ* polymerization may have been adversely affected by the hygroscopic nature of the Surgical Sealant. The Surgical Sealant is a hydrogel that takes up at least its own weight in fluid during the first 1-5 hours after polymerization. The abdominal cavity, and especially the intestinal tissues lying near the implant, may have experienced an osmotic shift around the time of test article polymerization as the polymer drew fluid toward it. The increased fragility of the small vessels did not become apparent until challenged by some stressful event (e.g., intraperitoneal injection of the barbiturate used to euthanize the animals or handling of the intestines during necropsy). The lesion does not appear to have occurred at the time of implantation, and it was not apparent at the Day 29 sacrifice.

3.5.3.2 Objectives

(1) To determine if the acute segmental intestinal hemorrhage associated with NS-1H and NS-1H(e) can be reproduced at necropsy 7 days post-implant. (2) To evaluate the incidence of small intestinal hemorrhage in female rats associated with high dose abdominal implantation of NS-1H and NS-1H(e) (0.448 ml; 50X clinical dose) with and without instillation of sterile saline into the abdominal cavity during implantation. (3) To evaluate the local and systemic toxicity associated with abdominal implantation of a high dose (0.448 ml; 50 X clinical dose) of NS-1H and NS-1H(e) in male and female rats.

3.5.3.3 Findings

No systemic effects noted. Enteropathy noted at implantation contact sites. The enteropathy was mitigated by the instillation of saline into the peritoneal cavity post implantation.

3.5.3.4 Results

There was no NS-lHe and/or NS-1H related effects on survival, clinical observations, body weight, food consumption, ophthalmic findings, clinical pathology, or absolute or relative organ weights (as appropriate). At necropsy on Day 8, dark, raised, or pale areas were observed grossly at the NS-1H, NS-lHe, and sham application sites, which correlated with microscopic findings of subacute inflammation, neovascularization, and hemorrhage at these sites. The incidences of these findings were greater for NS-1H and NS-lHe treated animals than for controls and equal or greater for the females than for the males. The high incidences of findings at the sham application sites are primarily due to the procedure used to apply the test material (i.e., surgical disturbance associated with holding the washer against the musculature). Discrete darkened regions were observed on the small intestines (duodenum and jejunum) of the NS-1H and NSlHe treated animals, which correlated with a microscopic finding of acute hemorrhagic enteropathy. The incidence and severity of this enteropathy were greater for the females when compared to the males. Instillation of sterile saline into the peritoneal cavity post-implant decreased the incidence and severity of the enteropathy. No biologically important differences between NS-1H and NS-lHe were noted locally or systemically.

3.5.3.5 Conclusion

The findings in this study are compatible with the findings in 3M study T-6922.3; Covance Study No. 6329-232. However, the instillation of saline in the abdominal cavity prevented the tissue sealant related hemorrhage of "contact" intestinal surfaces previously observed.

3.6 Genotoxicity

3.6.1 Ames Mutagenicity (NS-1H)

The Ames Mutagenicity (NS-1H) study was conducted to evaluate the test article's potential to cause mutations at the histidine operon of the *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and at the tryptophan operon of *Escherichia coli* strain WP2uvrA.. The study demonstrated that under the experimental conditions the Sealant was not mutagenic.

3.6.2 Ames Mutagenicity (NS-1H(e))

The Ames Mutagenicity (NS-1H) study was conducted to evaluate the polymerized test article's potential to cause mutations at the histidine operon of the *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and at the tryptophan operon of *Escherichia coli* strain WP2uvrA. The study demonstrated that under the experimental conditions the Sealant "e-beamed" was not mutagenic.

3.6.3 Mouse Lymphoma Assay

The Mouse Lymphoma Assay was designed to evaluate saline and DMSO extracts of polymerized NS-1H(e) for the potential to cause mutations at the thymidine kinase locus ofL5l78Y TK mouse lymphoma cells. The Sealant extracts were evaluated both with and without exogenous metabolic activation. The study demonstrated that under the experimental conditions the extracts were non-mutagenic.

3.6.4 Chromosome Aberration

The Chromosome Abberation study evaluated extracts of the polymerized Sealant for their potential to induce chromosome aberrations in cultured human peripheral blood lymphocytes with and without exogenous metabolic activation. The extracts did not induce a statistically significant increase in the percentage of cells with aberrations at any of the concentrations tested when compared to solvent controls or historical data. The study demonstrated that under the experimental conditions the polymerized Sealant was not clastogenic.

3.6.5 Micronucleus Assay after Implantation in Rats for 7 Days

The Micronucleus Assay was designed to evaluate the toxicity and mutagenicity of the Sealant containing e-beamed albumin component after implantation to the serosal surface of the peritoneal musculature of rats for 7 days. The percentage of micronucleated PCEs was not statistically significantly different between the treatment and control group. The study demonstrated that under the experimental conditions the Surgical Sealant "e-beamed" was not genotoxic.

3.7 Hemolysis

The Hemolysis study evaluated saline extracts of the Sealant for hemolytic potential based on cell lysis and hemoglobin release from human whole blood. None of the saline test article extract samples measured in the study had a Hemolytic Index of greater than 2.0. The study demonstrated that under the experimental conditions the Sealant was not hemolytic.

3.8 Pyrogenicity (Rabbits)

The Pyrogencitiy study evaluated extracts of the Sealant for pyrogenicity in female rabbits through intravenous administration. The summed value of the first test group indicated equivocal evidence for mild pyrogenicity, however, when combined with a subsequent trial the results were not indicative of pyrogenicity. The study demonstrated that under the experimental conditions the Sealant was not pyrogenic.

3.9 Toxicology Biocompatibility of PEG-(SS)2 Component

3.9.1 Ames Mutagenicity Test

The study evaluated article NS-1H(e), Component NS1000 (PEG-(SS)2 cross-linker component, e-beam irradiated at 10 kGy) for the potential to cause mutation at the histidine operon of *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and at the tryptophan operon of *Escherichia coli* strain WP2uvrA. The article was evaluated through two assays, one using the plate incorporation method and the second using the pre-incubation method. The study demonstrated that under the experimental conditions the PEG-(SS)2 cross-linker "e-beamed" was not mutagenic.

3.9.2 Hemolysis

The study evaluated article NS-1H(e), Component NS1000 (PEG-(SS)2 cross-linker component, e-beam irradiated at 10 kGy) for the potential to cause hemolytic activity based on cell lysis and hemoglobin release in human whole blood. None of the PEG-(SS)2 cross-linker "e-beamed" concentration had a Hemolytic Index of greater than 2.0. The study demonstrated that under the experimental conditions the PEG-(SS)2 cross-linker "e-beamed" was not hemolytic.

3.10 Local Lymph Node Assay (LLNA) and Micronucleus Assay

This experimental study was undertaken to develop a potential new animal test method for evaluating sensitization and genotoxicity potential of experimental biopolymer materials (JC-1 (NS-1H(e) dissolved in 50% N-methyl pyrollidinone + H_{20} (Solvent A) and JC-2 (NS-1H(e) dissolved in 25% N-methyl pyrollidinone + H_{20} (Solvent B)). The Local Lymph Node Assay sensitization component of the study demonstrated that, based on the stimulation index (CSI, test/control ratio), the two biopolymers induced a hypersensitivity response. The overall study results for the Micronucleus Assay demonstrated some random fluctuation, but concluded that the test components were not clastogenic. The irritant component of the test demonstrated that the test articles were generally minimal to mild irritants. However, the MMC, Solvent A and SLS components produced greater irritation than the other test articles.

4.0 ANIMAL STUDIES

4.1 Safety and Efficacy Study (7-day pig)

Title: Safety and Efficacy Evaluation of a Polymeric Patch in the Pulmonary Pig Model

Test Article: NS-1H, Sealant

4.1.1 Objective

The objective of this study was to evaluate the ability of the Sealant to seal air leaks in the pig pulmonary air leak model.

4.1.2 Findings

Air leaks in excess of 1,000 cc/min (a clinically severe air leak is about 200 cc/min), could be sealed with the Sealant as measured at the end of surgery. At 7 days, the original test sites continued to remain sealed.

4.1.3 Methods and Materials

Six female domestic Yorkshire pigs weighing between 26.5 kg and 39.0 kg were used for the study. The six animals underwent lobe resections of the caudal portion of the cranial lobe of the left lung. An intentional imperfect staple line was placed approximately 4 cm from the tip of the lung appendage. The resections resulted in substantial air leakage of> 1,000 cc/min measured by a flow meter and pressure gauge system placed in line via the endotracheal tube. The air leak was measured for 10 minutes to determine the mean baseline air leak rate. After the 10 minute reading, the lobe was exteriorized through the thoracotomy.

Sealant was applied across the staple line and stump of the resected lobe. The Sealant formed a gelatinous film after 20 seconds. The resected lobe was observed for air leakage by pouring warm sterile saline over the test sites with the restoration of normal respirator assistance. Observations of air leaks were recorded. Up to three applications were allowed prior to considering the Sealant a failure. Immediately post-operatively and at 7 days, each animal had a lateral and ventral dorsal radiograph taken to note chest tube positioning and to assess the absence or presence of a pneumothorax. Chest tube manipulations were performed periodically in an effort to maintain tube patency.

On the seventh day, the animals were anesthetized and an air leak assessment was performed using the endotracheal tube placed in circuit with the air leak apparatus described in the protocol. Following the air leak test, the animals were sacrificed. All tissues were placed in labeled containers containing 10% neutral buffered formalin. The harvested tissues were evaluated histologically. Histology results are presented in section 4.1.6 following.

4.1.4 Results

Acute efficacy was defined as the sealing of air leaks at the end of the surgical procedure. Four of six resection sites were sealed with a single application of the Sealant. One of six animals required two applications, because a small air leak was missed during the initial Sealant application. Finally, one of six animals required three applications for complete closure. In this particular animal, a larger than expected air leak was created, and thus three applications were used to ensure complete closure of the air leak. All six animals had 100% closure of the resected lung sites. The following table summarizes the acute air leak data.

Table 4.1.4 Animal Efficacy Air Leak Results (Vol. 1, Table 7.1.4, p121)

Pig Number	**Air leak rate after resection (cc/min) (correction for leaks from system)**	**Status After Patch**
8S1	1060	No leak*
8S2	2520	No leak
8S3	3160	No leak
8S4	1230	No leak
8S5	4340	No leak**
8S6	Excluded due to pre-existing pathology	NA
8S7	3600	No leak
Mean	2652± 1310 cc/min	No leaks

* Two applications of the Sealant.
** Three applications of the Sealant.

4.1.5 Conclusions

Results indicated that substantial air leaks in excess of 1,000 cc/min, well beyond typical clinical situations in terms of severity (a clinically severe air leak is about 200 cc/min), could be sealed with the Sealant as measured at the end of surgery. At 7 days, the original test sites continued to remain sealed.

4.1.6 Histopathology of 7-Day Pig Efficacy Study

Title: Safety and Efficacy Evaluation of a Polymeric Patch in the Pulmonary Pig Model

Test Article: NS-1H, Sealant

4.1.6.1 Objective

The objective of this study was to evaluate the histologic response to the Sealant after its use to seal air leaks in the pig pulmonary air leak model.

4.1.6.2 Findings

Wound healing progresses normally in the presence of the Surgical Sealant and there was no evidence histologically of an immune response.

4.1.6.3 Methods and Materials

The application of the Sealant is described in Section 4.1.3. At 7 days, the explants were processed for histopathologic evaluation. Sections were processed using standard methods of anatomic pathology. All samples were fixed in buffered formalin, and stained using hematoxylin and eosin and trichrome.

4.1.6.4 Results

The response in all animals to the Sealant was identical. It was difficult to identify the Sealant. Since the Sealant is based on albumin, the staining of the Sealant was very similar to the staining of nature albumin and other proteins. Subtle staining differences were identified, and these differences were reinforced, and at times magnified, by the use of trichrome staining in addition to hematoxylin and eosin. In one animal, the histology was suggestive of, but not definitive for, a focal multinucleated giant cell reaction involving foreign body type giant cells. However, the response area was adjacent to degenerating skeletal muscle related to surgical injury and no definitive statement can be made regarding this reaction. All other sections revealed small amounts of material remaining. The Sealant material was surrounded by granulation tissue. The granulation tissue extended into the Sealant material. There was no giant cell reaction, and no macrophage reaction at the surface.

4.1.6.5 Conclusions

There was no evidence of an immune response involving the Sealant in any section in any animal. Wound healing progressed normally in the presence of the Sealant and/or its degradation products.

4.2 Pulmonary Tissue Healing Study (28-day pig)

Title: Wound Healing Evaluation of a 3M Polymeric Patch in the Pulmonary Pig Model
Test Article: NS-1H(e), e-beam sterilized Sealant

4.2.1 Objective

To examine the healing of the lung tissue over time in the presence of the Sealant.

4.2.2 Findings

Wound healing progressed normally in the presence of the Sealant. There was no evidence of an immune response involving the Sealant in any section. The Sealant was effectively absent by 7 days post implantation and definitely absent by 14 days post implantation.

4.2.3 Methods and Materials

Experimental samples were taken at 24 hours (1 day), 4, 7, 14, and 28 days from each of two pigs for each experimental time frame. Seven wounds were inflicted at the time of surgery. In one wound, no Sealant material was applied. In another wound, the Sealant was applied to the inside surfaces of the wound prior to closure with staples and application of the Sealant to the pleural surface of the wound. In the remaining five wounds, the Sealant was applied to the pleural surface after stapling. The study was performed in a partially blinded fashion. Although the time in which *in situ* was known by all individuals involved at the time of necropsy, only the surgeons knew which wounds received specific therapy. The microscopic sections were examined in a blinded fashion.

4.2.4 Results

The response in all animals to the Sealant material was identical. It was difficult to identify the Sealant material. Since the Sealant is based on albumin, the staining of the Sealant was very similar to the staining of nature albumin and other proteins. Subtle staining differences were identified, and these differences were reinforced, and at times magnified, by the use of trichrome staining in addition to standard hematoxylin and eosin. The response was determined by the time *in situ.* At one day, only hemorrhage was present. By 4 days, granulation tissue had moved into the pleura and the Sealant was largely absent. By 7 days, only isolated fragments of the Sealant were apparent. By 14 days, the Sealant was no longer observed. There was no giant cell reaction and no macrophage reaction at the surface of the tissue.

4.2.5 Conclusions

Wound healing progressed normally in the presence of the Sealant and/or its degradation products. There was no evidence of an immune response involving the Sealant in any section. The Sealant was effectively absent by 7 days post implantation and definitely absent by 14 days post implantation. No giant cells were observed at any time frame in any sample, implying that the major, and perhaps only, method of degradation was by hydrolysis.

4.3 Pharmacokinetics Studies

4.3.1 Pilot Mass Balance in Rats

Title: Pilot Excretion Study of Implanted Biodegradable^{14} C-Polymeric Patch NS-1H in Rats

Test Article: ^{14}C NS-1H, ^{14}C-Surgical Sealant

4.3.1.1 Objective

To assess the absorption, distribution, and excretion of radioactivity in a pilot study in rats following implantation of the ^{14}C NS-1H to the serosal surface of the peritoneal musculature.

4.3.1.2 Findings

No gender differences were noted and urine was the primary route of excretion.

4.3.1.3 Methods and Materials

Four Sprague Dawley Crl:CD'BR rats (2M12F) received about 0.5 ml (285 mm^2, 1.6 mm thick) of ^{14}C NS-1H implanted to the serosal surface of the ventral peritoneal musculature. The ^{14}C NS-1H was formulated by combining a cross- linker labeled with ^{14}C at the PEG moiety with an unlabeled human albumin component. This study was designed to determine the temporal characteristics of ^{14}C NS-1H degradation over 14 days based on ^{14}C elimination via the urine, feces, and expired air. The ^{14}C was also determined at the implant site and in the digested whole carcass at the termination of the study. Results from this study were used to design a subsequent definitive 14-day study to evaluate the absorption, distribution and excretion of ^{14}C NS-1H in rats.

4.3.1.4 Results

Twenty-four hours after implantation of ^{14}C NS-1H, an average of 47.3% and 3.26% of the administered radioactivity was recovered in the urine and feces, respectively. The administered radioactivity was then cleared at a slower rate through 336 hours (14 days) post-dose. There were no apparent gender differences in the excretion of radioactivity. The mean overall recovery of total radioactivity was 93.0% at 336 hours post-dose. Recovery in individual matrices (overall percent of administered dose) is shown in the following summary table.

Table 4.3.1.4 Recovery in Individual Matrices (Vol. 1, Table 8.2.5, p125)

Matrix	**Percent of Dose**
Urine	70.2
Feces	12.4
Expired Air/Volatiles	0.69
Recovery Cage Wipe	3.64
Cage Wash	0.80
Cage Wipe	0.21
Implant Site (336 hours)	0.49
Residual Carcass (33 hours)	4.50
TOTAL	93.0

4.3.1.5 Conclusions

This study revealed the following: there were no gender differences, urine is the primary route of excretion, respiratory excretion is negligible and the majority of excretion occurred early (1-3 days) post-implant. This information was used to design the definitive pharmacokinetic study for the Sealant (section 4.3.2 following).

4.3.2 Full-Scale Mass Balance in Rats: 14C Absorption, Distribution and Excretion after Implantation in Rats

Title: Absorption, Distribution, and Excretion of Polymeric Biodegradable Patch ^{14}C NS-1H (e) Following Implantation in Rats

Test Article: ^{14}C NS-1H(e), ^{14}C-Surgical Sealant "E-beamed"

4.3.2.1 Objective

To assess the absorption, distribution, and excretion of radioactivity in rats following implantation of the ^{14}C NS-1H(e) to the serosal surface of the peritoneal musculature. The test article consisted of e-beam sterilized human albumin and cross-linker labeled with ^{14}C at the PEG moiety.

4.3.2.2 Findings

The ^{14}C NS-1H(e) was widely distributed in the rats post application. Over 50% of the ^{14}C NS-1H(e) was eliminated in 1 day and virtually all of it was eliminated by day 14.

4.3.2.3 Methods and Materials

Groups of animals (3/sex) were sacrificed at 1, 3, 7 and 14 days post- implantation and specified tissues (including the implant site) were evaluated for radioactivity. In addition, animals scheduled for the day-14 sacrifice had serum, urine, feces and expired air monitored daily for radioactivity throughout the study. Whole body distribution of radioactivity was evaluated in one male per time point (1, 3, 7 and 14 days post-implant) by whole body autoradiography.

4.3.2.4 Results

The maximum mean concentrations of radioactivity in plasma occurred 72 hours after implantation with maximum concentrations for males and females of 409 and 437 pg equivalents ^{14}C NS-1H(e)/g, respectively. The tissues with the highest overall maximum mean concentrations in both males and females were the application site, liver and pancreas.

Autoradiographic data indicated that the lymphatic system was involved in the transport of ^{14}C NS-1H(e)-derived radioactivity. Radioactivity was primarily eliminated in the urine. By 24 hours post-dose, over 61% of the administered radioactivity had been recovered with the urine and feces, and thereafter, the administered radioactivity then cleared steadily but relatively slowly through 336 hours post-dose.

The mean recovery of total radioactivity in urine and feces at 336 hours post-dose was 77.2% and 14.6% respectively in males and 98.4% and 22.4%, respectively in females. The overall recovery for all matrices, including carcass was 96.4% in males and 126% in females.

4.3.2.5 Conclusion

The study revealed the following: following implantation of the ^{14}C NS-1H(e) Sealant in male rats, radioactivity slowly migrated from the dose site into the visceral spaces of the peritoneal cavity. ^{14}C NS-1H(e) was widely distributed in the tissues but mainly distributed to the plasma at the early time points. There was no gender differences in the distribution or excretion of ^{14}C NS-1H(e)-derived radioactivity and urine is the primary route of excretion. Over 50% of the ^{14}C NS1H(e) was eliminated in 1 day and virtually all of the ^{14}C was eliminated 14 days past application.

5.0 PERSPECTIVE ON IRRITATION AND HYGROSCOPIC NATURE OF SEALANT

A collective review of the Biocompatibility and Animal Efficacy/Tissue Healing studies data and the literature was conducted to address any lingering issues with regard to potential Sealant-induced Irritation and the clinical implications of the perceived hydroscopic nature of the material.

5.1 Sealant Induced Irritation

Any Sealant-induced irritation appears to be related to the amount of fluid surrounding the Sealant, rather than the N-hydroxysuccinimide (NHS) by-product of the polymerization reaction**,** which is a known irritant. Of all the routes tested, injection of the Sealant by the subcutaneous route presents the polymer with the least volume of fluid after polymerization. The moderate to severe irritation that was observed in rabbits after intracutaneous injection of the liquid Sealant was probably reflective of a local osmotic shift due to polymer hydration. All other preclinical data as reviewed in the following indicates that irritation is absent or minimal when fluid is available for polymer hydration. Therefore, application of the Sealant in a fluid rich environment, such as that in the lung surgery setting, should not induce irritation as in the rabbit studies as supported by the following.

• Pig Efficacy Study. *In situ* polymerization of the Sealant on the pig lung (high fluid environment) was not associated with any irritation to the lung tissue.

• 28-Day Rat Implantation Study and 7-Day Follow-up Rat Implantation Study. *In situ* polymerization of the Sealant on the serosal surface of the peritoneal musculature of rats (without filling the peritoneal cavity with saline) was associated with acute segmental hemorrhagic enteropathy 7 days after the Sealant was implanted. There was little/no inflammation at the abdominal muscle/implant interface. This resulted in a situation where the Sealant interfaced intimately with the abdominal muscle, and also (although less intimately) with the opposing intestinal tissue. Fluid content of the environment was low (compared to a thoracic cavity filled with saline), although not as low as in the subcutaneous location. Filling the peritoneal cavity with saline significantly blocked the incidence and severity of this response, further supporting that a high fluid environment present when the Sealant polymerizes and hydrates will minimize any risk of local irritation.

• Primary Dermal Irritation in Rabbits. When the Sealant was polymerized *in situ* on intact, saline moistened, rabbit skin and occluded with a saline saturated Hilltop chamber (high fluid environment) no irritation occurred at the Sealant/skin interface.

• Ocular Irritation in Rabbits. When the Sealant was polymerized *in situ* in the conjunctival sac of the eye mild irritation was noted at the 1-hour scoring interval and resolved completely by the end of the study.

The Sealant is indicated for use in reducing and eliminating air leaks associated with pulmonary surgery. This indication offers an environment rich in fluids. Little or no irritation would be expected in this type of situation, and this is confirmed by the pig efficacy data and the human clinical testing data. To address any specific concerns regarding the irritation potential of NHS, no additional pre-clinical tests exist. The calculated maximum concentration of NHS formed within the Sealant is 0.4%. A review of the scientific literature regarding the irritation potential of NHS showed no specific irritation testing that has been performed on this chemical, nor has irritation been reported as an adverse event when the chemical has been used in biological applications. The MSDS for NHS does claim that 98%+ concentrations of NHS may be a skin and eye irritant, but provides no testing data.

Conclusion: The risk of irritation to the lung tissue and/or nearby thoracic tissues after application of the Sealant to the lung during lung surgery is considered low.

5.2 Hygroscopic Nature of Sealant

Some of the preclinical study suggests that some reversible gross anatomic changes observed in the peritoneal organs can occur because the product is very hygroscopic. This hygroscopic nature of the polymeric Sealant is not believed to impact its clinical use. The body fluids in the region of surgery at the time of application of Sealant and/or saline to the surface of the lung can each be sufficient to avoid the hygroscopic effect of the Sealant, especially when one considers that the clinical use of the Sealant does not in any way approach the surface/volume ratio or volume/body mass ratios used in the preclinical studies.

This is supported by the following:

• A segmental acute hemorrhagic enteropathy that was originally observed in the 28-Day Rat Implant Study was reproduced in the subsequent 7-day study in terms of incidence, severity and gender difference (females affected more than males) at the 50X dose (normalized for body weight).

• Saline instillation into the peritoneal cavity significantly blocked the incidence and severity of the intestinal lesion.

• There was no difference between NS-1H (Sealant with e-beam irradiated crosslinker only) and NS-1H(e) (Sealant with e-beam irradiated crosslinker and human albumin) in terms of: no test article-related clinical signs, mortality, effects upon body weight, food consumption, minimal tissue reaction at the implant site on the abdominal muscle, and no test article-related clinical pathology.

• NS-1H(e) was negative at 7 days post implant in the peripheral blood micronucleus assay.

Conclusion: The observed hygroscopic nature of the Sealant is not believed to adversely impact its clinical use.

6.0 KINETICS OF SEALANT RESORPTION

The clinical requirement for the Sealant is the need to seal air leaks until such time as normal healing processes occur. Normal healing leads to an influx of proliferative elements (fibroblasts) within 4 days. Collagen deposition begins shortly thereafter. As the collagen displaces the Sealant, the clinical need for the Sealant decreases. It is anticipated that a sealing lifetime of 7 days is a necessary requirement.

The kinetics of resorption from the radiolabeled Sealant experiment conducted in rats, based on review of relevant literature and discussion with the Sponsor's clinical and scientific consultants, are considered by the Sponsor to be applicable to Sealant use in patients. The resorption studies performed in rats necessarily involved high surface to volume ratios when compared with humans. Since the breakdown of the sealant is via hydrolysis, the high surface to volume ratios studied in the rats favor accessibility of the polymer to hydrolysis and would favor faster dissolution rates. In contrast, the human clinical study was expected to use approximately 2.5 units/subject. The preclinical study was designed to use 1 unit of Sealant per animal. The rat studies, therefore, represent a worst case scenario with regard to the potential hydrolysis and resorption of the Sealant. The volumes used and the surface area covered in the clinical and preclinical studies will differ, precluding direct quantitative comparison of Sealant degradation. Recognizing the expected difference in methods, a theoretical comparison between the clinical and preclinical use suggests that more Sealant on one site will seal longer.

The high surface to volume ratios in the rat model, which are unlikely in a clinical setting, demonstrated that the Sealant persists at least until the influx of healing elements. In the planned clinical use of Sealant, where lower surface to volume ratios exist, device resorption is expected to be further attenuated and Sealant will be present at least in the situation where healing is normal.

Conclusion**:** Results of the radiolabeled rat studies support the expectation that Sealant resorption rates in the clinical setting will allow for the necessary 7 day sealing lifetime.

7.0 HUMAN SAFETY STUDY

Title: Human Repeat Insult Patch Test (Jordan-King Modification of the Draize Sensitization Procedure)

Test Article: NS-1H(e), Sealant "e-beamed"

7.1 Objective

A Human Repeat Insult Patch Test (HRIPT) was performed on 10 normal healthy volunteers to determine the potential for the Sealant "e-beamed" to induce Type IV immune response (delayed contact hypersensitivity) after repeat topical application to the intact skin, according to the attached protocol. All ten subjects were Caucasian, with five males and five females, ranging in age from 27 to 70 years old, without allergic history.

7.2 Findings

The Sealant "e-beamed" showed no signs of irritation or sensitization.

7.3 Study Design

The Sealant "e-beamed" was applied and polymerized *in situ* on the skin three times per week over approximately a three week period. The Surgical Sealant "e-beamed" remained in place for approximately 48 to 72 hours (continuous exposure during induction), with patch removal and applications performed at the clinic by authorized personnel.

The rest period between induction and challenge was greater than three weeks for all subjects. For the Challenge dose, the patch samples (and corresponding saline negative controls) were applied for approximately 48 hours to the original test sites and to naïve test sites. Dermal response was scored at approximately 48 and 96 hours post-application for signs of contact sensitization.

7.4 Application/Induction

Test sites were moistened using saline prior to Sealant "e-beamed" application. About 0.2 ml of Sealant "e-beamed" (liquid mixed components) was expressed from the delivery device into a Teflon template placed on the skin over the test site. The Sealant "e-beamed" was allowed to polymerize on the skin inside the template for 2 minutes, after which time the template was removed. The test site was occluded using a Hilltop chamber that had been saturated with 0.3 ml saline. Subjects remained at the test facility for approximately one hour for observation after each patch application.

At the end of each induction application period (i.e., after 48 or 72 hours), test article was removed from the site by gently wiping the site using a saline moistened gauze pad. If the subject was absent for a regularly scheduled application during the induction, they received a make-up application during week 4. Subjects who received a make-up application began their rest period after the final induction exposure to the Sealant "e-beamed".

Ten subjects completed the induction phase with nine Sealant "e-beamed" applications each during the period of February 8 to March 8, 1999.

7.5 Rest Period

Following the Induction period, the subjects did not receive test article for at least three weeks. All ten subjects received their challenge doses on March 29, 1999 (actual rest periods: 3 subjects at 24 days, 6 subjects at 26 days and 1 subject at 28 days).

7.6 Challenge

About 0.2 ml of the Sealant "e-beamed" was applied, as described for Induction, to the original induction site (or last alternate site) and to one naïve site. The Sealant was allowed to polymerize on the skin inside the Teflon template for 2 minutes, after which time the template was removed. The challenge sites were occluded using a Hilltop chamber that had been saturated with 0.3 ml saline. For the saline controls, about 0.2 ml saline was applied to a naïve site, and the site was occluded in a similar manner as the test article site. Subjects remained at the test facility for approximately one hour after patching for observation. The test article and saline patches remained in place for 48 hours. Dermal response was scored at approximately 48 hours (approximately 30

minutes after patch removal) and at approximately 96 hours after Sealant "e-beamed" application.

7.7 Results

Of the original 18 volunteers, 10 completed the study. One of the subjects was dropped from the study due to initiation of decongestant and antibiotic therapy. 7 subjects withdrew due to scheduling conflicts that did not allow them to complete the study as planned. 10 subjects completed all phases of the protocol. Skin responses were graded according to the following scale:

0 = No visible reaction and/or erythema
+ = Slight, confluent, or patchy erythema
1 = Mild reaction - macular erythema (faint, but definite pink)
2 = Moderate reaction - macular erythema (definite redness, similar to a sunburn)
3 = Strong to severe reaction - macular erythema (very intense redness)

The following results were observed: No adverse events, other than tape dermatitis (see below), were observed during the induction phase. Of the ten subjects who completed the study, none received scores within the test site of greater than + (slight, confluent or patchy erythema).

At challenge, no adverse events were observed during the one-hour observation period that followed the challenge dose. The original test article sites of three subjects were scored as + (slight, confluent or patchy erythema) at 48 hours. This response was no greater than responses recorded during the induction phase of these subjects, and the responses all fell to 0 at 96 hours. The remaining seven subjects showed scores of 0 at 48 and 96 hours at the original or alternate test site.

7.8 Adverse Events

Patients were observed for one hour after application of the Sealant "e-beamed" during the Induction and Challenge phases of the study. Irritation and/or allergic reaction to the tape adhesive (i.e., tape dermatitis) that may occur during the induction period were defined as an adverse event within the protocol.

Four (4) subjects experienced tape dermatitis, unrelated to the test article. Two of those subjects had the patch moved to an alternate site during induction, due to this reaction to the tape. No medical treatment was necessary. All four of these subjects completed the study throughout Challenge and scoring.

No other adverse events were reported for any of the 10 subjects at any time point during induction or Challenge.

7.9 Conclusions

All ten subjects showed no signs of the Sealant-related irritation or sensitization during the induction and challenge phases. The Sealant "e-beamed" presents a low risk of delayed contact hypersensitivity reaction when applied topically to intact human skin and allowed to polymerize *in situ*.

8.0 SUMMARY

As described in the preceding, the Sponsor completed a comprehensive series of studies to assure that the Sealant would be safe and effective for use in the treatment of air leaks.

The Sponsor conducted three tests to characterize the Sealant. The company assessed the device's burst strength, polymerization rate and pyrogenicity. The Sealant performed satisfactorily across all three categories.

The Sponsor conducted rigorous testing to evaluate the Sealant's biocompatibility. Biocompatibility was measured through multiple tests of the following aspects of biocompatibility: cytotoxicity; irritation; hemolysis; pyrogenicity; sensitization; toxicity; mutagenicity and efficacy. The Sealant performed satisfactorily for all aspects of biocompatibility.

The Sponsor also conducted additional animal tests to assess the efficacy of the product for closing air leaks. The Sponsor designed its experiments to either mimic clinical conditions, or conditions more severe than clinicians are likely to encounter. The company's robust and extensive testing demonstrated that the product was effective in a variety of in-vivo models.

The Sponsor performed a variety of tests and conducted an extensive literature review to quantify the Sealant's resorption rate. Its data support the expectation that resorption will extend beyond the necessary healing time.

The Sponsor evaluated the Sealant in an initial human safety study in 10 healthy volunteers. The study demonstrated that while minor tape dermatitis was observed, the Sealant did not produce adverse reactions.

The positive supporting data generated from the rigorous in-vitro and in-vivo testing provided the sponsor with the initial assurance of safety and efficacy it needed to justify advancing its product into clinical trials.