

Adherus Dural and Spinal Sealant as Adjuncts to Sutured Dural Repair in a Canine Lumbar Durotomy Repair Model

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SUMMARY: The objective of this GLP study was to evaluate the safety and effectiveness of Adherus Dural Sealant and Adherus Spinal Sealant when used to achieve watertight dural closure in a canine lumbar durotomy repair model. Another important objective of this study was to determine whether Adherus Dural and Adherus Spinal Sealants would inhibit the formation of peridural fibrosis and dural adhesions as normal healing occurred. The formulations were easy to apply, setting in the expected time frame to form an approximate 2 mm hydrogel film over the durotomy site. Application of Adherus Dural Sealant or Adherus Spinal Sealant provided 100% water-tight closure at time of surgery, as well as at four or five days, two months and four months post-operatively. There were no adverse test article-related effects in clinical observations, body weight, food consumption, physical or neurological parameters, clinical pathology parameters, CSF total cell count or chemistry parameters during this study. In addition, there was no histopathological evidence that application of Adherus Dural Sealant or Adherus Spinal Sealant had any adverse effects on the adjacent tissues, including the spinal cord and spinal nerve roots. Furthermore, the formulations did not appear to impede healing of the surgical site. Adherus Dural and Adherus Spinal Sealant, in general, significantly limited dura adhesions at both the two month and four month necropsy intervals. Peridural fibrosis and dura thickening/fibrosis was also reduced in animals that received Adherus Dural or Spinal treatment over that in control animals which did not receive either of the test articles.

INTRODUCTION

Two of the prevalent difficulties associated with spinal procedures are the prevention of cerebrospinal fluid (CSF) leaks and the prevention of adhesions between epidural scar tissue and the dura. CSF leaks are either the result of an incidental dural tear or an intentional durotomy and, if not properly managed, may significantly increase hospitalization times and the cost of patient treatment.¹ Furthermore, as the surgical site heals, peridural scar tissue is produced, often blending almost imperceptibly with the dura in the months following the procedure. The formation of this scar tissue may lead to complications such as radicular and low back pain.

A variety of adjunctive treatments have been used to either augment dural closure or to minimize dural adhesions. These products typically lack the mechanical and adhesive strength to provide confident water-tight closure or lack the residence time and durability to serve as a barrier against the formation of dural adhesions. Additionally, some of the existing products have the tendency to expand in situ, creating the risk of symptoms or physical deficit from compression of neural structures.

Adherus Dural and Spinal Sealants both have been designed specifically to both limit postoperative CSF leaks and inhibit dural adhesions. The products form densely crosslinked

hydrogels that swell minimally and adhere persistently to the dura while forming a robust barrier to both prevent CSF leaks and dural adhesions. Degradable ester linkages allow the product to degrade after sufficient time has lapsed to permit the dura to heal and the initial healing response to subside.

To date, these sealants have undergone extensive *in vitro* biocompatibility and preclinical safety and effectiveness testing². These studies indicate that Adherus Dural and Spinal sealants are safe and perform well as adjuncts to standard methods of dural repair. In a pre-clinical canine cranial durotomy repair model³ there were no adverse clinical effects related to the application of Adherus Dural Sealant and physical and neurological evaluations were normal. During this study, Adherus Dural Sealant was 100% effective in sealing cranial CSF leaks intraoperatively and postoperatively at six months when challenged at CSF pressures up to 44 mm Hg. In addition, Adherus Dural Sealant allowed the dura to heal without complication over the following months. Monthly MRI evaluations demonstrated a consistent degradation profile over approximately three months. Histopathological examination following the two necropsy time points revealed no adverse changes in the brain, calvarium, dura, meninges or non-nervous system organs associated with the test article in any of the animals.

The following study extends the use of Adherus Dural Sealant to spinal procedures and examines the use of a new but similar Adherus Spinal Sealant formulation. Adherus Spinal Sealant contains all of the same components and the same ratio of crosslinking components as Adherus Dural Sealant, but differs in the overall crosslinker content (10 wt% versus 15 wt% in Adherus Dural Sealant) and set time

(approximately 30-35 seconds versus 1-2 seconds for Adherus Dural Sealant)

MATERIALS and METHODS

PEG-Based Surgical Sealants

Adherus Dural Sealant

The Adherus Dural Sealant system (HyperBranch Medical Technology, Inc.) is a synthetic hydrogel sealant designed for use as an adjunct to standard methods of dural repair to provide watertight closure. At time of use, the crosslinking components, an activated polyethylene glycol (PEG) and polyethyleneimine (PEI), are reconstituted with their respective reconstitution buffers and withdrawn into 5 mL syringes. The two syringes are then coupled to a Micromedics Spray Applicator Kit.

To dispense the sealant system, the user depresses the plunger cap which forces equal amounts of each solution down separate cannulas inside the applicator toward the mix tip. As the solutions pass through the mix tip, the solution paths merge immediately prior to exiting the tip. The mixed solution is expressed as a spray and deposited on the surgical site where it will rapidly polymerize to form a compliant, well adhered film. Once the plunger assembly is released, the flow of solution ceases.

Adherus Spinal Sealant

The Adherus Spinal Sealant system (HyperBranch Medical Technology, Inc.) is a synthetic hydrogel sealant designed for use as an adjunct to standard methods of dural repair to provide watertight closure, and as an adhesion barrier for the inhibition of post surgical peridural fibrosis and dural adhesion. The applicator for Adherus Spinal Sealant is ideally designed to deliver the formulation to tight surgical sites and allow for precise application of the mixed formulation into

smaller spaces which can be encountered during spinal surgery.

At time of use, the polyethyleneimine (PEI) crosslinker is first reconstituted by the buffer within the same syringe. Following reconstitution, the syringe is coupled to the vial adapter assembly, the PEG powder in the glass vial is attached to the vial adapter spike and an angled applicator tip is also attached, via luer lock, to the other end of the three-way valve. At this stage, the device is ready for use.

To apply Adherus Spinal Sealant, the solution in the syringe must be pushed through the syringe chambers, through the three-way valve and into the glass vial. This action reconstitutes the PEG powder in the glass vial, allowing for the mixing of the two components and the initiation of a crosslinking reaction. The syringe plunger is then allowed to recoil, pulling the solution out of the vial and back into the syringe assembly. The PEG is fully reconstituted by depressing and withdrawing the syringe plunger one additional time. Following an approximate 10 second reconstitution, the vial adapter and vial are removed, automatically turning the three way valve to allow solution to flow down the applicator tip. The mixed solution is expressed by depressing the syringe plunger to deliver the desired amount of the crosslinking formulation. Once delivered, the formulation will set within approximately thirty seconds.

Adherus Sealants

Once applied, the Adherus Sealants adhere tenaciously to the underlying tissue providing a smooth, lubricous coating which prevents cerebrospinal fluid leaks and minimizes or prevents adhesions to bone and other tissue structures. The hydrogels are resorbed as the underlying tissue heals, establishing a competent CSF barrier and separating the dura from other

tissues to prevent dural adhesions. The degradable ester linkages are designed to allow controlled degradation of the hydrogel over the course of eight to twelve weeks. Due to the structure of the hydrogel, the Adherus Sealant systems swell minimally after implantation, exhibiting at most only a 6% dimensional change in any axis. The degradation byproducts (mainly PEG) are water-soluble and are cleared through the renal and hepatic pathways. No toxic byproducts are created and the sealant does not interfere with tissue healing.

Surgical Procedure

Group	Treatment	Number of Animals / Necropsy Interval	
		2 Month	4 Month
1	Control	3	3
2	Adherus Dural Sealant	3	3
3	Adherus Spinal Sealant	3	3

Table 1 Treatment groups and number of animals at each necropsy interval.

This study was conducted at Northern Biomedical Research, Inc, in accordance with the United States Food and Drug Administration (FDA) Good Laboratory Practice Regulations (GLP) (21CFR Part 58), the Japanese Ministry of Health, Labor, and Welfare (MHLW) Good Laboratory Practice Standards Ordinance 21, and the Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice [C (97) 186/Final]. Eighteen male beagle dogs (Covance Research Products, Inc., Kalamazoo, Michigan) were utilized for this study. The dogs were approximately 9-18 months old and weighed 10.0 to 13.7 kg. The animals

were randomly assigned to three treatment groups as illustrated in Table 1.

All animals were pretreated with atropine sulfate SQ (0.04 mg/kg). Approximately fifteen minutes later, an IV dose of sodium thiopental (16 mg/kg) was provided to induce sedation. If necessary, an anesthetic mask (4% isoflurane, 1 liter/min oxygen) was used to aid in sedation. Animals were intubated and maintained on approximately 1 liter/minute of oxygen and approximately 2.0% isoflurane. Prednisolone sodium succinate IV (30 mg/kg) and flunixin meglumine IM (2 mg/kg) were administered prior to surgery.

In surgery under aseptic conditions, a midline lumbar incision was made and the skin and the musculature reflected from the L₃ spinous process and lamina. A hemilaminectomy was made at L₃ using a cutting burr drill bit. A 0.9 mm OD x 0.5 mm ID polyurethane catheter was inserted into the subarachnoid space for collection of CSF and a baseline CSF pressure reading. For the first control animal, a purse string suture (6-0 nylon) was used to secure the catheter to the dorsal dura. Sutures were not used to secure the catheter in subsequent surgeries due to the technical difficulty of suture placement inside the laminectomy defect. Dental acrylic, lightly applied within the laminectomy defect, was subsequently used to secure the catheter in the remainder of the experimental animals. The catheter was connected to an intracranial pressure (ICP) transducer (Codman Microsensor ICP Transducer) and three way stop cock via a stainless steel blunt-tipped needle. Once the catheter was implanted, a baseline CSF pressure was recorded using the pressure transducer. After obtaining the baseline reading, additional musculature was reflected over the L₂ spinous process and lamina. The dorsal aspect of the L₂ lamina was removed with a burr drill bit and the dura was exposed. The dura and arachnoid were



Figure 1 Intraoperative view of the approximate 1 cm dural incision closed with 4 interrupted 6-0 nylon sutures.



Figure 2 Intraoperative view of the approximate 1 cm dural incision closed with 4 interrupted 6-0 nylon sutures and approximately 2 mm of Adherus Spinal Sealant over the suture line.

incised to a length of approximately 1.0 cm in the parasagittal plane and CSF was allowed to freely egress. The dural incision was then re-approximated with four evenly spaced simple interrupted 6-0 nylon sutures and blotted dry (Figure 1). In group 2 and 3 animals, one of the two test articles was then applied to the sutured dural closure to a thickness of approximately 2 mm over the defect (Figure 2). No test article was applied to the Group 1 control animals. The same procedure was followed for the L₅ vertebral segment. Adequacy of dural closure was evaluated intraoperatively by infusing saline through the previously implanted intradural catheter and monitoring the ICP with the pressure transducer. The maximum intradural pressure reached and maintained for 30 seconds in both control and experimental animals was recorded. The intradural catheter was then tied off in all animals. The paraspinous muscle and connective tissues were closed in layers with absorbable

sutures, and the skin was closed with absorbable sutures and tissue adhesive. The animals were recovered from anesthesia, placed in a protective jacket, administered butorphanol IM (0.05 mg/kg) and placed on a post surgical antibiotic (ceftiofur sodium IM (5 mg/kg) b.i.d., one injection during or immediately prior to surgery followed by three injections post operatively).

Following surgery, clinical observations were performed daily, food consumption was monitored daily after the first week, body weights were monitored weekly, clinical pathology was collected prior to necropsy, CSF samples were collected before necropsy, and neurological and physical examinations were performed at one week and at necropsy.

Immediately prior to necropsy at the specified endpoints the animals were sedated with sodium thiopental IV (16 mg/kg) taken to surgery, maintained on inhalant anesthesia (approximately 1 L/min of oxygen and 2-5% halothane or isoflurane) and the durotomy sites were surgically re-explored. A midline lumbar incision was made and the musculature was reflected to expose the durotomy sites. At both the two month and four month necropsy, nearly all of the surgical sites were found to have significant connective tissue overgrowth. The sites were not further explored and the animals were immediately euthanized and given an IV bolus of heparin sodium, 200 IU/kg. The animals were subsequently perfused via the left cardiac ventricle with 0.001% sodium nitrite in saline followed by 10% neutral buffered formalin fixative. Tissues were then procured from all animals at necropsy and maintained in 10% neutral buffered formalin. The surgical sites were removed en bloc in order that those areas could be scored for fibrosis and adhesions histologically.

Durotomy Site Evaluations by MRI

On study Day 5 or 6, the animals were dosed with atropine SQ, 0.04 mg/kg and thiopental sodium IV, 16 mg/kg (50 mg/mL), intubated and maintained on inhalant anesthesia (isoflurane). A 20 or 22 gauge IV catheter coated with heparin was inserted into the cephalic vein and attached to a slow drip of 0.9% sodium chloride. The animals were subsequently taken for MRI evaluation on a Philips Achieva 3T MRI Scanner. T2 sagittal (DRIVE and FLAIR), pre-contrast sagittal T1-weighted turbo spin echo (TSE), axial turbo field echo and post-contrast sagittal T1-weighted sequences were performed for the evaluation. Gadolinium IV (gadodiamide) was the contrast agent used at a dose of 0.1 mmol/kg (0.2 ml/kg).

Histopathology

Histopathologic evaluations were completed on all designated tissues harvested at necropsy. At the L₂ and L₅ surgical sites, the vertebrae/spinal cord/dura specimen was decalcified. The section was then cut as close to the middle of the surgical site as could be determined in the wet tissue. The cranial side of the site was embedded in one block. The caudal side of the site was embedded in the following block. Dorsal nerve roots were examined along with the spinal cord sections. All sections were embedded in paraffin and stained with hematoxylin and eosin.

At each surgical site, the bone, muscle/soft tissue, peridural tissue/space, dura, spinal cord, catheter track (for the indwelling pressure recording catheter) and spinal nerve roots were examined. Sections of the caudal cervical spinal cord and cauda equina were examined in order to investigate the possibility of distant spinal cord effects. Multiple sections at each surgical site were examined.

Both peridural adhesions and fibrosis were evaluated histologically. For the purposes of this

study, adhesions are defined as the extent of attachments between the dura and other tissues in the epidural space. Fibrosis is defined by the extent of fibrous connective tissue formation in the epidural space. The grading scheme used to evaluate dural adhesions is outlined in Table 2. For the determination of dural adhesions, the pathologist was blinded to treatment groups. The grading scheme used to record peridural fibrosis is outlined in Table 3.

Grade	Characterization	Description*
0	0-25%	Adhesions present on small portion of the dura
1	25-50%	Adhesions present on up to half of the dura
2	50-75%	Adhesions present on a majority of the dura
3	75-100%	Adhesions present on all of the dura

*Percentages based upon the portion of the dura exposed or adjacent to the surgical site. If a complete bone roof had formed over the dura, the scar extent was considered a 0.

Table 2 Grading scheme for dural adhesions.

Grade	Description
0	No tissue affected
1	Severity grade slight; A severity grade of 1 denotes a very slight change, barely discernable in the section, and affecting less than 1% of the potentially affected tissue.
2	Severity grade minimal; A severity grade of 2 denotes a microscopic change slightly more pronounced than a grade 1, but still affecting a very small portion (less than 5%) of the potentially affected tissue.
3	Severity grade mild; A severity grade of 3 denotes a microscopic change slightly more pronounced than a grade 2, still limited in distribution, but readily apparent in the section.
4	Severity grade moderate; A severity grade of 4 denotes a microscopic change slightly more pronounced than a grade 3, affecting a majority of the potentially affected tissue or space, readily apparent in the section.
5	Severity grade severe; A severity grade of 5 denotes the change was among the most pronounced observed in the study.

Table 3 Grading scheme for peridural fibrosis.

Statistical Analysis

Body weights, body weight changes, clinical pathology data (hematology, serum chemistry and coagulation), CSF total cell count, CSF chemistry, heart rate, body temperature, respiration, and food consumption data were analyzed by a one-way analysis of variance and comparison of the control group to the treated groups by Dunnett's test. Analysis was two-tailed for significance levels of 5% and 1%. A probability value <0.5 was considered statistically significant.

For dural adhesion and peridural fibrosis analysis, an F test was performed to determine if the variances in the two test populations were equivalent. In instances where the variances were determined to be equivalent, a t-test for equivalent variances was used to determine statistical significance of the difference in means. Likewise, in cases where the F test showed unequal variances, a t-test for unequal variances was used to determine statistical significance of the difference in means.

RESULTS

Clinical and Neurological Evaluations

All animals recovered from the surgical procedure, and the animals remained neurologically intact throughout the course of the study. The surgical sites healed as expected and showed no signs of infection or delay in healing. Furthermore, the application of Adherus Dural or Adherus Spinal Sealant produced no adverse test article-related effects in clinical observations, body weight, food consumption, clinical pathology parameters or CSF total cell count or chemistry parameters during this study.

Pressure Testing at Surgery

At surgery, baseline CSF pressures (before durotomy) were recorded for each animal in

Groups 1 through 3. The individual durotomy sites were tested for CSF leakage following dural closure and maximum intradural pressure achieved was recorded. Results of this analysis are summarized in Figure 3.

The average baseline (i.e., pre-durotomy) intradural pressure for the Group 1 controls was 10 mm Hg. Following the sutured closure of the approximate 1 cm dural incisions, none of the controls maintained a maximum pressure over 5 mm upon saline infusion and all incisions were observed to leak CSF. The average baseline intradural pressure for the Group 2 Adherus Dural Sealant animals was 11 mm Hg before dural incisions. During saline infusion, all of the hydrogel-treated sites maintained an intradural pressure over 30 mm Hg for at least thirty seconds and the average maximum pressure was 44 mm Hg. Similar to Group 2, the Group 3 Adherus Spinal Sealant treated animals had an average baseline intradural pressure of 12 mm Hg and all of the treated sites maintained an intradural pressure over 30 mm Hg for at least thirty seconds with an average maximum pressure of 43 mm Hg. Visual confirmation of watertight closure was confirmed in all Adherus-treated animals at peak intradural pressure.

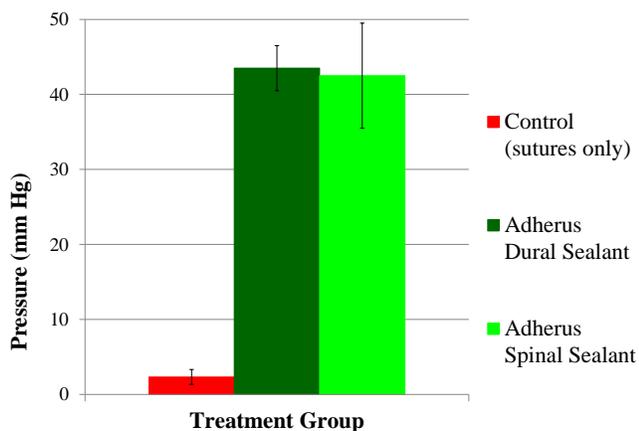


Figure 3 Average maximum achieved intradural pressure in Groups 1-3 during saline infusion.

MRI Evaluations

Animals in Groups 1 through 3 were evaluated by MRI on Study Day 5 or 6 to assess the surgical sites. All animals were observed to have varying degrees of hyper-intense signal in the perivertebral and/or subcutaneous tissues likely related to (expected) perioperative edema and blood products. The hydrogel was consistently visualized as a thin, uniform, high signal “plaque” immediately adjacent to the dura. CSF was visualized as an irregular, hyperdense signal abnormality contiguous with the epidural space and typically trailing into the adjacent soft tissues.

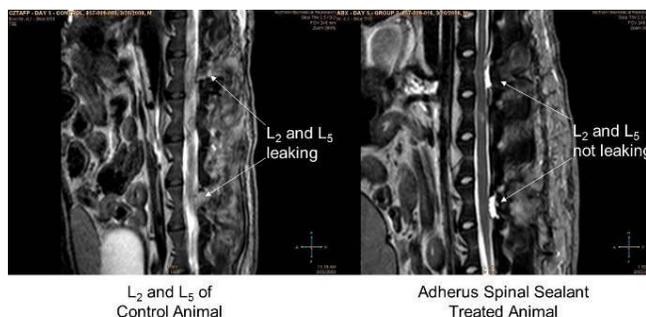


Figure 4 T2 Drive MRI of control animal (left) and Adherus Spinal Sealant treated animal (right) at day 5/6.

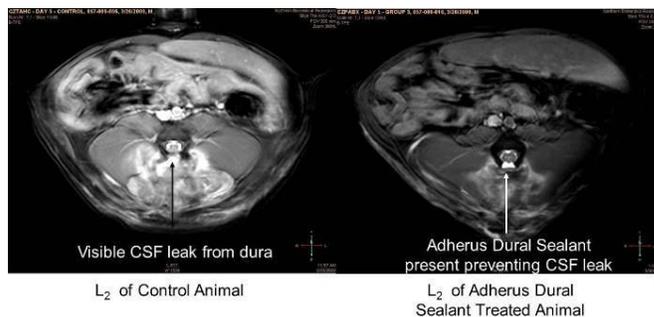


Figure 5 Axial MRI of control animal (left) and Adherus Spinal Sealant treated animal (right) at day 5/6.

Imaging evidence of CSF leak was found in 100% (12/12) of the durotomy sites in the six Group 1 (control) animals (Figures 4 & 5). In Groups 2 and 3, 12.5% (3/24) of surgical sites could not be definitively evaluated secondary to the existence of diffuse hyper-intense signal in the perivertebral tissues (thought to be blood products and/or tissue

edema). In contrast to unequivocal radiographic examples of CSF fistula in the control animals, this imaging feature was not contiguous with the subdural space, presented above the hydrogel and lacked homogenous signal characteristics. In the 21 evaluable experimental surgical sites, there was no radiographic evidence (0%) of CSF leak. (Figures 4 and 5).

Histopathology

Two Month Necropsy

There were no significant histological differences between the control and test article treated groups at the level of the cervical spinal cord or cauda equina. Between the surgical sites (i.e., at L₃/L₄), slight to minimal nerve fiber degeneration was observed and was likely due to local spinal cord damage occurring secondary to the surgical procedure (laminectomy) or (more likely) the placement/presence of the catheter used to monitor cerebrospinal fluid pressure.

The dura was observed to be intact without any visible gaps (healed) in all treatment groups at both surgical sites (L₂ and L₅). Foamy macrophages were noted at the periphery of the site of test article application in the Adherus-treated groups, but were not observed at the control sites. Overall, there was no evidence that application of Adherus Dural Sealant or Adherus Spinal Sealant had any detrimental/adverse effects on the local tissues (including the spinal cord) or impeded healing at the surgical site.

As compared to the control animals in which adhesions in three of six sites were graded as a 3 on the adhesion scale, two sites were graded as a 2, and one site lacked any adhesions, administration of Adherus Dural Sealant to a durotomy site appeared to essentially eliminate dura adhesions based on microscopic examination of the surgical sites two months post-surgery (average scores presented in Figure 8). Although

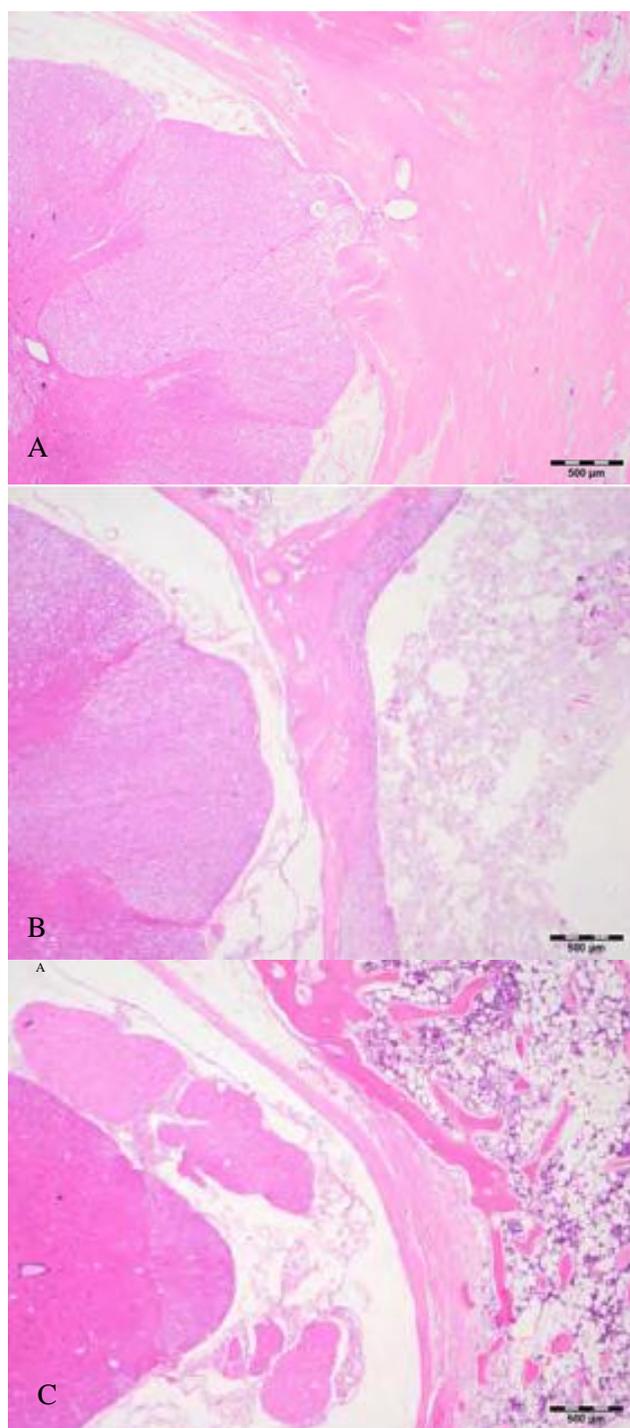


Figure 6 Representative photomicrographs of histology slides from Group 1 (A), Group 2 (B), and Group 3 (C) at 2 month necropsy. Figure 6A shows the spinal cord, dura and peridural tissue are all adhered and prominent peridural fibrosis is evident in the control while Figure 6B and Figure 6C show little peridural fibrosis and no dural adhesions in Adherus-treated animals. In figure 6B, the space to the right of the dura was most likely occupied by the Adherus sealant. In Figure 6C, bone has formed a nearly complete shelf over the dura. Bar = 500 µm.

the hydrogel typically does not survive histological processing, in five of six sites that were treated with Adherus Dural Sealant, an approximate 1.5 mm thick space adjacent to the dura and/or persistent material (thought to be Adherus Dural Sealant) appeared to completely block the formation of dura adhesions. One site, which appeared to lack the same epidural space as previously described, was graded as a 3.

Dura adhesions were also decreased in the Adherus Spinal Sealant treated animals. Three of six sites had no adhesions, one site was graded as an adhesion score 1, and two other sites were graded as a 2 (average score presented in Figure 8). At microscopic examination, the presence or absence of the test material or an epidural space was not as noticeable when compared to the Adherus Dural Sealant-treated sites.

There was, in general, also a decrease in peridural fibrosis in the Adherus Dural Sealant and Adherus Spinal Sealant groups as compared to the controls (average scores presented in Figure 9). The difference was most notable in the Adherus Dural Sealant group in which three of six sites had no peridural fibrosis, one site was graded as a 1 on the fibrosis scale, and two other sites were graded as a 2 as compared to the control animals in which five of six sites were graded as a 4 and one site was graded as a 3. In the Adherus Spinal Sealant group, one of six sites was graded as a 1, two sites were graded as a 2, and three sites were graded as a 3.

Four Month Necropsy

Again, there were no significant histological differences between the control and test article treated groups at the cervical spinal cord or cauda equina. Slight to minimal nerve fiber degeneration was observed at the L 3/4 level secondary to the laminectomy and/or

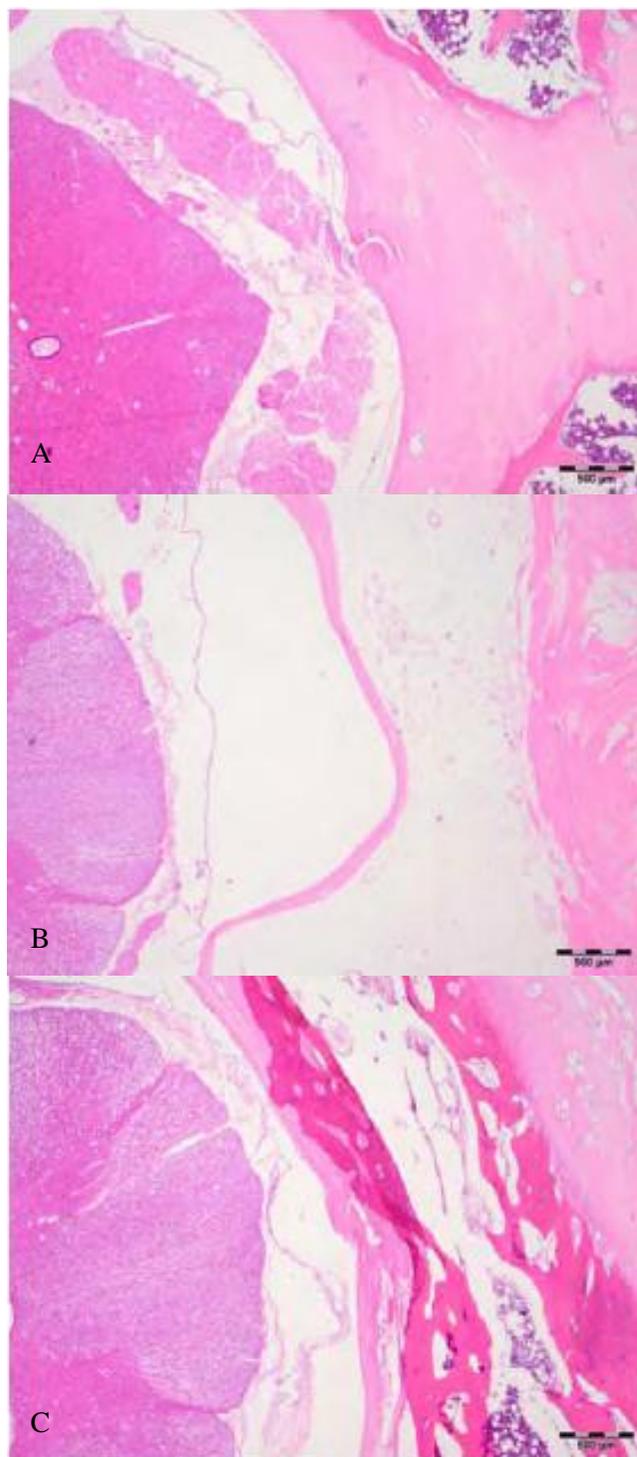


Figure 7 Representative photomicrographs of histology slides from Group 1 (A), Group 2 (B), and Group 3 (C) at 4 month necropsy. Figure 7A shows the dura completely adhered to the overlying connective tissue while Figure 7B and Figure 7C show little peridural fibrosis and no dural adhesions in Adherus-treated animals. In each photomicrograph, the spinal cord is visible on the left hand side. Bar = 500 µm.

placement/presence of the catheter used to monitor intradural cerebrospinal fluid pressure.

The dura was observed to be healed in all treatment groups at both durotomy sites (L₂ and L₅). Foamy macrophages in the peridural tissue, noted at the two month sacrifice in the Adherus-treated animals, were nearly (Adherus Dural Sealant) or totally (Adherus Spinal Sealant) absent in both treatment groups by the four month sacrifice. Furthermore, there was no evidence that application of Adherus Dural Sealant or Adherus Spinal Sealant had any detrimental/adverse effects on the local tissues (including the spinal cord) or impeded healing at the surgical site.

As compared to the control animals (Group 1), dura adhesions were markedly decreased (on average) in the Adherus Dural Sealant and Adherus Spinal Sealant groups. For the control sites, four of six sites were graded as a 3 on the adhesion grading scale and two sites did not have adhesions. Three of six Adherus Dural Sealant treatment sites did not have adhesions, one site was graded as a 1 and both sites on one animal were graded as a 3. Five of the six Adherus Spinal Sealant treatment sites did not have adhesions and one site was graded as a 2 (average scores presented in Figure 8).

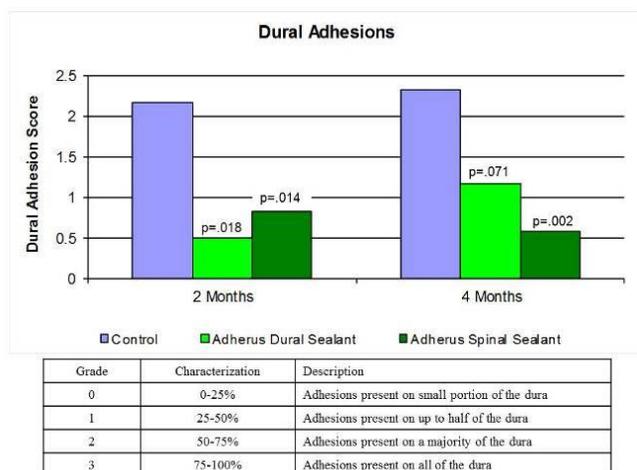
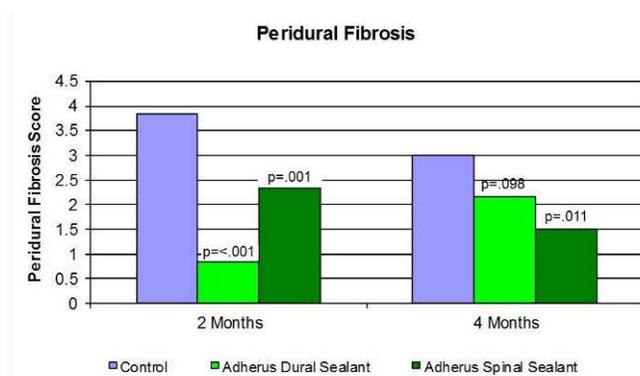


Figure 8 Dural adhesion scoring for all treatment groups at two and four months.

Peridural fibrosis was also somewhat decreased at 4 months in both Adherus treated groups as compared to controls. In contrast to the two month sacrifice group, the distinct space above the dura/peridural tissue at the surgical site in the Adherus Dural Sealant animals was not apparent at four months. This observation likely indicates resorption of the test article during the four month period followed by connective tissue filling the space.

During peridural fibrosis evaluation, two of the six control sites were each graded as either a 2, 3, or 4. Two of the six Adherus Dural Sealant sites were each graded as either a 1 or 2 respectively on the fibrosis scale while one site was graded as a 3 and one site was graded as a 4. The Adherus Spinal Sealant group was observed to have one site free of peridural fibrosis, two sites graded as a 1, two sites graded as a 2 and one site graded as a 3 (average scores presented in Figure 9).



Graded on a sensitive 0 – 5 scale with scores of 1 and 2 representing less than 5% of affected tissue

Figure 9 Peridural fibrosis scoring for all treatment groups at two and four months.

DISCUSSION

Following spine surgery, Adherus Dural and Spinal Sealants successfully and safely established a watertight seal at all sites. As the dura healed, the sealants were able to maintain this watertight seal and served as a physical barrier between the dura and the epidural space, limiting dural adhesions.

Pressure testing was performed at surgery following closure of the durotomy sites. This test was performed to rigorously test the hydrogel's strength intraoperatively and is intended to simulate the pressures produced within the lumbar cistern after patients assume an upright posture in the peri-operative period.⁴ In the Group 1 control animals 0/6 animals were able to maintain the protocol stated pressure of 30 mm of Hg for 30 seconds. Following dural incision and primary repair, all control animals leaked at a pressure at or below 5 mm of Hg. The Group 2 and Group 3 animals, treated with Adherus Dural Sealant and Adherus Spinal Sealant respectively, all were able to withstand CSF pressures over 30 mm Hg for 30 seconds. This suggests that Adherus sealants can significantly augment standard dural closures and provide watertight seals that can withstand supra-physiological pressures for sustained periods.

MRI analysis was utilized to determine if the test articles were able to maintain a watertight seal on Study Day 5 or 6. The control animals were also examined to determine whether the untreated durotomy sites still had evidence of CSF leakage. All control animals (Group 1) were found to have evidence of spontaneous CSF leaks at both surgical sites. Animals treated with Adherus Dural Sealant (Group 2) or the Adherus Spinal Sealant (Group 3) showed no evidence of spontaneous CSF leaks, although three sites could not be definitively evaluated based on extensive hyper-intense signal at or near the surgical sites. As noted previously, varying degrees of hyper-intense signal within the perivertebral or subcutaneous tissues was observed in all animals and is believed to be secondary to post surgical effects (likely tissue edema and/or blood products). Individual variation and slight differences in surgical technique could account for the more prominent soft tissue signal found in three of the animals. Although the presence of a CSF leak cannot be ruled out at these three sites,

the lack of communication between this radiographic finding and the subdural space suggest that a CSF leak was not present in these few animals.

Although precise comparisons cannot be made between intraoperative photographs of the Adherus hydrogels within the confines of the laminectomy site at time zero (which document approximately 2 mm of hydrogel over the incision and approximately 3 mm near the edges of the laminectomy site) and subsequent post operative MRIs, it is of interest that MRI images at day 5-6 suggest no appreciable change in the size of the hydrogel within that timeframe. This dimensional stability appears to be confirmed with MRI images obtained at post-op days five, 14, and 28 during a pilot laminectomy animal study of the same design⁵. Necropsy at the 1 month time interval during this pilot laminectomy study also confirmed that the Adherus hydrogel does not undergo any appreciable dimensional changes⁵. These findings are confirmed by in vitro data which has documented minimal swelling of the Adherus hydrogel over time.⁶ This finding is of great clinical importance as volumetric expansion within the epidural space runs the risk of inducing neurological deficit. This possibility is of more than theoretical concern. Other hydrogel dural sealants possessing less favorable dimensional stability have been observed to create neurological signs and symptoms secondary to volumetric expansion in both spine and cranial surgeries^{7,8}.

Clinically, there were no test article-related changes in hematology, serum chemistry and coagulation parameters throughout the study. There were also no test article-related changes in CSF total cell counts or CSF chemistry.

During histopathological examination, foamy macrophages were present at the periphery of the site of test article application in the Adherus Dural

Sealant and Adherus Spinal Sealant groups. It was presumed that the macrophages were recruited in response to the test articles. The presence of the foamy macrophages indicated phagocytosis of the test article.

Histopathologic examination and grading also indicated that both hydrogel sealants successfully limited dural adhesions and epidural fibrosis while not impeding the rate of dural healing. Administration of Adherus Dural Sealant to a durotomy site essentially eliminated dura adhesions when examined two months post surgery. At four months following the application of Adherus Dural Sealant, dura adhesions were decidedly decreased as compared to control animals that had a durotomy but were not treated with a sealant. Administration of Adherus Spinal Sealant to a durotomy site dramatically reduced dura adhesions when examined two months post surgery. When examined at four months post surgery, Adherus Spinal Sealant essentially eliminated dura adhesions as 5 of 6 Adherus Spinal Sealant treated surgical sites examined were without any visible dura adhesions. Peridural fibrosis and dura thickening/fibrosis in general were reduced at two and four months post surgery following the application of Adherus Dural Sealant and Adherus Spinal Sealant.

Finally, there was no evidence that application of Adherus Dural Sealant or Adherus Spinal Sealant had any adverse effects on the adjacent tissues, including the spinal cord and spinal nerve roots. Also, Adherus Dural Sealant or Adherus Spinal Sealant application did not appear to impede healing of the surgical site.

CONCLUSIONS

Combined results from cranial and spine studies suggest that the Adherus Dural and Spinal Sealants are ideal adjuncts to standard methods of dural repair to provide watertight closure.

Adherus Dural and Adherus Spinal Sealants appear to lack neuro or systemic toxicity, consistently seal the dura to prevent CSF leaks (in both the intraoperative and postoperative time periods) and allow normal healing of the dura while reducing dural adhesions and epidural fibrosis.

DISCLOSURE

Dr. Asher is a compensated consultant to HyperBranch Medical Technology, Inc. and has received compensation in the form of consultant fees, stock and stock options.

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