Adherus Dural Sealant as an Adjunct to Sutured Dural Repair in a Canine Cranial Durotomy Repair Model

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SUMMARY: The objective of this study was to evaluate the safety and effectiveness of Adherus Dural Sealant when used as an adjunct to sutured closure in a canine durotomy repair model. The effectiveness of dural repair was evaluated at time of surgery, one week post surgery and again at approximately six months post surgery by pressure testing. The safety and endurance of the test article was evaluated over approximately six months using magnetic resonance imaging, clinical evaluations, clinical pathology, and histopathology. During pressure testing at all specified time points, Adherus Dural Sealant provided 100% water-tight closure. None of the Adherus Dural Sealant-treated incisions leaked at intracranial pressures up to 50 mmHg. MRI scans obtained at two to four days post surgery and monthly thereafter showed the test material to be clearly visible in all imaging sequences (T2, FLAIR, T1, and T1 with contrast). The test article continued to be visible, although clearly degrading, through the three month scan, was barely discernible in a portion of the scans at four months, and not present in the five and six month scans (T2 images). Adherus Dural Sealant was well-tolerated and none of the treated animals experienced test article related clinical signs after surgery. There were no test article related changes in body weight, food consumption, neurological/physical examination parameters, and clinical pathology parameters. Histopathologic evaluations indicate that there were no unanticipated morphologic changes associated with a single topical administration of Adherus Dural Sealant to a durotomy site in beagle dogs at eight days or six months post surgery.

INTRODUCTION

Despite meticulous sutured closure of the dura following neurosurgical procedures, cerebrospinal fluid (CSF) leaks are frequently observed.[1,2] As such, a variety of onlay grafts, haemostatic agents, and surgical sealants/adhesives are commonly used to augment sutured dural closures. Most of these products either do not provide watertight closure or are hemostats that actively participate in the clotting cascade and are not specifically indicated for use in promoting robust tissue closure/healing in neurosurgical applications.

To be effective, dural sealants require at least three key bio-mechanical characteristics: tenacious tissue adherence to provide a mechanical CSF barrier, minimal swelling to prevent mass effects (and resultant neurological complications) and an extended rate of degradation since dura mater is relatively slow to heal.

Adherus Dural Sealant was specifically designed to provide a strong, watertight barrier to compromised dura and maintain its strength over the entire course of dural healing while remaining dimensionally stable. The architecture and ratio

of the unique crosslinking components within Adherus Dural Sealant impose the formation of a densely crosslinked network. This constricted network and the selection of relatively robust degradable linkages make the sealant strong over the course of multiple weeks and dimensionally stable in the presence of bodily fluids.[3]

Following the completion of a battery of biocompatibility tests which demonstrated that the synthetic hydrogel was non-toxic, non-hemolytic, non-irritant [4] and not neurotoxic,[5] the safety and efficacy of Adherus Dural Sealant was confirmed using an established canine durotomy repair model.[6,7]

MATERIALS and METHODS

Adherus Dural Sealant

The Adherus Dural Sealant system (HyperBranch Medical Technology, Inc.) is a hydrogel sealant designed for use as an adjunct to standard methods of dural repair, such as sutures, during neurosurgical intervention to provide watertight closure. The hydrogel sealant requires the preparation of two precursor solutions that, once mixed within the supplied applicator, rapidly cross-link in situ to form a solid, absorbable, biocompatible PEG-based hydrogel. The resultant hydrogel is primarily composed of water (approximately 85% by weight) and the remaining components are fully synthetic, containing no human or animal derived products. The first precursor contains a modified polyethylene glycol (PEG) polymer with terminal electrophilic ester groups, while a second precursor solution possesses a component containing nucleophilic amine groups. The complementary end groups undergo an electrophilic-nucleophilic reaction, resulting in crosslinking and the formation of a hydrogel. Once implanted, Adherus Dural Sealant minimally swells, exhibiting only an 8% dimensional change in any axis.[3] It slowly degrades over approximately 90 days through the hydrolysis of ester linkages. The hydrolyzed polymer constituents are primarily cleared through the renal and hepatic pathways.

Surgical Procedure

Fifteen male beagle dogs (Covance Research Products, Inc., Kalamazoo, Michigan) were quarantined for at least ten days before the study was initiated. The 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]. The dogs were approximately 13-16 months old and weighed 11.1 to 14.2 kilograms. The animals were randomly assigned to the treatment groups as illustrated in Table 1.

	Number of Animals		
Treatment Group	**1 Week (Histopathology, pressure test)**	**6 Month (MRI, Histopathology, pressure test)**	**6 Month Histopathology Only**
Adherus Dural Sealant	3	4	1
Control	3	3	1

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

The animals were pretreated with atropine sulfate (0.04 mg/kg at 0.54 mg/ml) as a subcutaneous injection. Approximately 15 minutes later, an IV dose of thiobarbiturate (16 mg/kg at 50 mg/ml) was provided to induce sedation. Animals were then intubated and maintained on approximately 1 liter of oxygen and 2.0% isoflurane. The anesthetic gases and mixtures varied as required for each individual animal. Prednisolone sodium

succinate IV (30 mg/kg at 50 mg/ml) and flunixin meglumine IM (2 mg/kg at 50 mg/ml) were administered prior to surgery. Using routine sterile techniques, an approximate 3 cm x 2 cm bone flap was removed from the left frontal parietal region of the skull using a 1.4 mm burr drill bit and the dura and arachnoid were incised to a length of approximately 2 cm in the parasagittal plane for all animals. The incision was then loosely approximated with interrupted 6-0 nylon sutures, leaving a gap of approximately 2 mm. All durotomy sites continued to leak CSF following primary closure with suture. The treated animals then had the durotomy site blotted dry and test article applied to a depth necessary to cover the suture knots (see the intraoperative images in Figures 5 and 8). Once the test article had cured, confirmation of closure was evaluated using a Valsalva maneuver up to 20 cm of H_2O for approximately 5 seconds. If leakage occurred, additional test article was applied. The control animals received no treatment upon the observation of a leaking wound (see the intraoperative images in Figures 6 and 9). The bone flap was replaced and secured with 3-0 nylon sutures and dental acrylic (DuraLay, Reliance Dental Manufacturing Company, Worth, Illinois). The skin and musculature were closed in layers with sutures (3-0 nylon and 2-0 nylon respectively), followed by tissue adhesive. Animals were recovered from anesthesia, provided butorphanol tartrate IM (0.05 mg/kg at 2 mg/ml) and placed on a post surgical antibiotic, ceftiofur sodium IM (5 mg/kg at 50 mg/ml) b.i.d. (one injection during or prior to surgery followed by three injections post surgery).

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

One week following surgery, the six animals in the one week group were sedated, evaluated, pressure tested, perfused with saline and 10% neutral buffered formalin, and tissues were harvested for histopathological analysis.

Monthly scans were conducted on the 6 Month (MRI, Histopathology, Pressure Test) animals to determine the presence of the test article. Six months following surgery the remaining seven animals had MRI scans and were sedated, evaluated, pressure tested (except those in the histopathology only group), perfused with saline and 10% neutral buffered formalin, and tissues were harvested for histopathological analysis.

At the end of their treatment periods, all animals were subjected to a full necropsy. Prior to necropsy, animals were provided with an I.V. bolus of heparin Na, 200 IU/kg. The animals were perfused via the left cardiac ventricle with 0.001% sodium nitrite in saline followed by 10% neutral buffered formalin fixative. Tissues from all animals were harvested and saved in 10% neutral buffered formalin.

Durotomy Site Evaluations by MRI

Two to three days following the surgical procedure, four Adherus Dural Sealant and three control animals in the 6 month group were sedated and taken to the onsite MRI for evaluation. For the MRI, an IV catheter was inserted and the animals were maintained on inhalant anesthesia. The animals were placed in a nylon stereotaxic head holder prior to imaging so that the positioning could be replicated at subsequent time points. The animals were then scanned on a Picker (Phillips) 1T MRI Scanner. The following sequences were utilized for the evaluation: T2-weighted, FLAIR, T1-weighted, and T1 weighted with contrast. Gadodiamide IV

(0.2 ml/kg at 287 mg/ml) was utilized as the contrast agent. The sequence parameters are listed in Table 2. These same animals were reevaluated monthly after the initial surgery until the test article was no longer visible with MRI. The animals were anesthetized in the same manner as previously stated and subsequent MRI evaluations were performed using the same sequences.

	T2 Weighted	T1 Weighted	FLAIR
Echo Time (ms)	96	16	128
Repeat Time (ms)	4000	800	14231
Field of View (cm)	18	18	18
Slice Thickness (mm)	3	3	3
Gap (mm)	0.5	0.5	0.5
Resolution	256 x 256	256 x 256	256 x 256
Flip Angle	90	90	90
Signal Averages	2	2	2

Table 2 Sequence Parameters for MRI evaluation.

Pressure Testing

All animals subject to necropsy at one week and seven animals in the six month sacrifice group underwent pressure testing prior to perfusion. Animals were sedated as stated previously and intubated. Under inhalant anesthesia, the surgical site was exposed and the bone flap was carefully removed.

Once the durotomy site was examined for spontaneous CSF leaks, the cisterna magna was cannulated with a 20 gauge spinal needle with attached three-way stopcock containing the ICP (intracranial pressure) transducer (Codman Microsensor ICP Transducer) and saline. Once the baseline intracranial pressure was measured using the ICP transducer, saline was slowly injected to a maximum ICP of at least 40 mmHg and the maximum intracranial pressure reached was recorded. During the procedure, the durotomy site was closely monitored for the presence of any CSF leakage. If leakage occurred, the pressure at which it began to leak was noted.

Histopathology

Three full coronal (transverse) sections through the brain (including the pia/arachnoid layers of the meninges) underlying the durotomy site and three sections through the calvarium and dura (also through the durotomy site) were trimmed and embedded from each animal. The tissues from each section were placed into oversized blocks. The other tissues, including the bone flap, were trimmed and embedded in regular sized blocks. All tissue sections were embedded in paraffin, sectioned at approximately 5 µm, and stained with hematoxylin and eosin (H&E). The resulting glass slides were examined by a board certified veterinary pathologist with specific expertise in the examination of the nervous system.

Statistical Analysis

Body weights, body weight changes, clinical pathology data (hematology, serum chemistry and coagulation), 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 group by Dunnett's test. Analysis was two-tailed for significance levels of 5% and 1%.

RESULTS

Clinical and Neurological Evaluations

All animals recovered quickly from the surgical procedure, the surgical sites healed as expected and the animals remained neurologically intact throughout the course of the study. Furthermore, there were no test article-related clinical observations, changes in food consumption or body weight, nor were there any changes in clinical pathology parameters for the six animals sacrificed on day eight or for the nine animals sacrificed after six months.

MRI Evaluations

Seven animals, four treated and three controls, in the six month group were subject to MRI throughout the study. Animals were scanned two to three days following surgery and monthly thereafter to assess the degradation profile of the test article as well as any morphologic changes that may have occurred.

A review of the scans showed the test material clearly visible in all images two to three days following surgery and the hydrogel remained evident on MRI images for approximately three months. Test material degradation, indicated by a decrease in size/volume over time, was most clearly observed in the T2 images which initially showed the sealant as a conspicuous hyperintense signal becoming isointense over time due to the inherently large amount of water in the hydrogel (which is slowly reabsorbed) (Figure 1) Partial degradation was noted at the one month scan. This degradation continued through the two and three month scans and the test material was barely discernible in some of the four month T2 images. The test material was not discernible in the five and six month T2 images. Test article degradation can also be followed in the T1 images (in which the sealant initially appears as a hypo intense signal object) but is not as dramatic as what is observed in the T2 images.

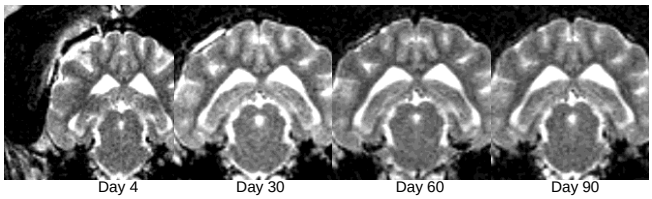

Figure 1 Representative T2 MRI images of one treated animal over the course of the first 90 days.

Following the administration of IV contrast, the test material exhibited a hyper intense signal visible in the T1 images suggesting that it was contrast positive. The signal was not visible in the test material at the Day 3/4 scan, suggesting there was no uptake of contrast by the test material. Then, at one month, contrast was clearly visible in the test material and the pattern of contrast enhancement followed a similar imaging pattern to what is seen in the T2 images, presumably reflecting the degradation of the test material. Contrast uptake was clearly visible through the four month scan on all animals then dissipates in the five and six month scans.

A hyper intense signal can be seen near the bone flap and in the surrounding musculature in the T2 and T1 with contrast images (somewhat in the T1 images) on all seven animals, two to three days following surgery and was suggestive of edema due to retraction at the surgical site. This edema was attributed to the large amount of musculature that had to be reflected to expose a large enough area to accommodate removal and subsequent replacement of an approximate 2 cm x 3 cm bone flap. This hyperintensity, however, substantially subsided by the one month scan in both the control and treated animals.

Minor displacement of the cerebral cortex was noted in the MRI images adjacent to the site of the craniotomy in all animals. The displacement was typically more pronounced when the bone flap was placed over the test material, displacing the brain within a closed vault. The flattening can be seen in the treated animals at one month, but resolved as the test material degraded and was no longer present at six months, an observation confirmed at necropsy and during histopathological examination.

Pressure Testing

Prior to sacrifice, the six day eight necropsy animals had pressure testing conducted to challenge the durotomy repair methods. The results are presented in Table 3 and the average maximum ICP reached at the day eight time interval is presented in Figure 2. All three of the

animals in the control group were leaking at baseline pressures, but there were no leaks at baseline pressures in the test group. Furthermore, all of the Adherus Dural Sealant-treated repair sites remained leak free at maximum ICP between 40 and 50 mmHg.

	Initial ICP (mm Hg)	Maximum ICP (mm Hg)	CSF Leakage[a]
Adherus Dural Sealant Group	12	50	No leaks
	12	40	No leaks
	13	45	No leaks
Control Group	10	NA	Baseline
	9	NA	Baseline
	9	NA	Baseline

Table 3 Results of pressure testing prior to necropsy on day eight. [a]Baseline indicates that CSF was leaking when the bone flap was removed. NA=Not applicable.

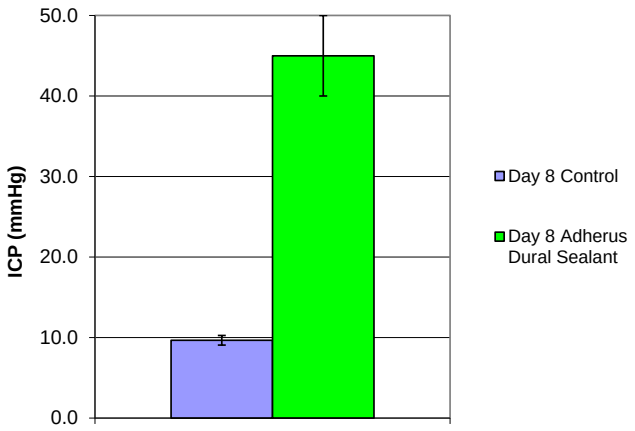

Figure 2 Average terminal CSF leakage pressure for control and Adherus Dural Sealant-treated groups at day eight. Note: None of the Adherus Dural Sealant treated sites leaked at a terminal pressure between 40 and 50 mmHg.

Results of the pressure testing procedures conducted prior to the six month necropsy are presented in Table 4. Two of the three animals in the control group were leaking at baseline pressures, although one of the leaks was most likely caused by a tear in the dura during bone flap removal. No leaks were observed at baseline pressures in the test group. Furthermore, all of the Adherus Dural Sealant-treated repair sites remained leak free at maximum ICP pressures between 46 and 49 mmHg. Only one of the control animals was leak free at maximum ICP pressure of 47 mmHg.

	Initial ICP (mm Hg)	Maximum ICP (mm Hg)	CSF Leakage
Adherus Dural Sealant Group	8	46	No leaks
	4	49	No leaks
	7	47	No leaks
	8	46	No leaks
Control Group	2	NA[a]	NA[a]
	3	47	No leaks
	NA[b]	NA[b]	NA[b]

Table 4 Results of pressure testing prior to necropsy at six months. [a]Dura was leaking when bone flap was removed. [b]Unable to obtain data due to tear in dura. NA=Not applicable.

Histopathology

Day Eight Necrospy

During tissue trimming (dissection), there were no adhesions noted between the dura and the pia mater. The cerebral cortex underlying the durotomy site was flattened in most of the test and control animals at the day eight timepoint. Since this observation was made for animals with and without the sealant, the process of replacing the bone plate was identified as a likely contributing factor. Because the flattening of the cerebral cortex did not correlate to any microscopic changes and was resolved by the six month necropsy interval, this change was not interpreted to be an adverse effect.

During microscopic examination, there were no adverse morphologic changes in the brain or the overlying pia/arachnoid meninges. The cerebral hemisphere underlying the site of the durotomy was flattened, but this displacement of cerebral cortical tissue did not cause any microscopic changes.

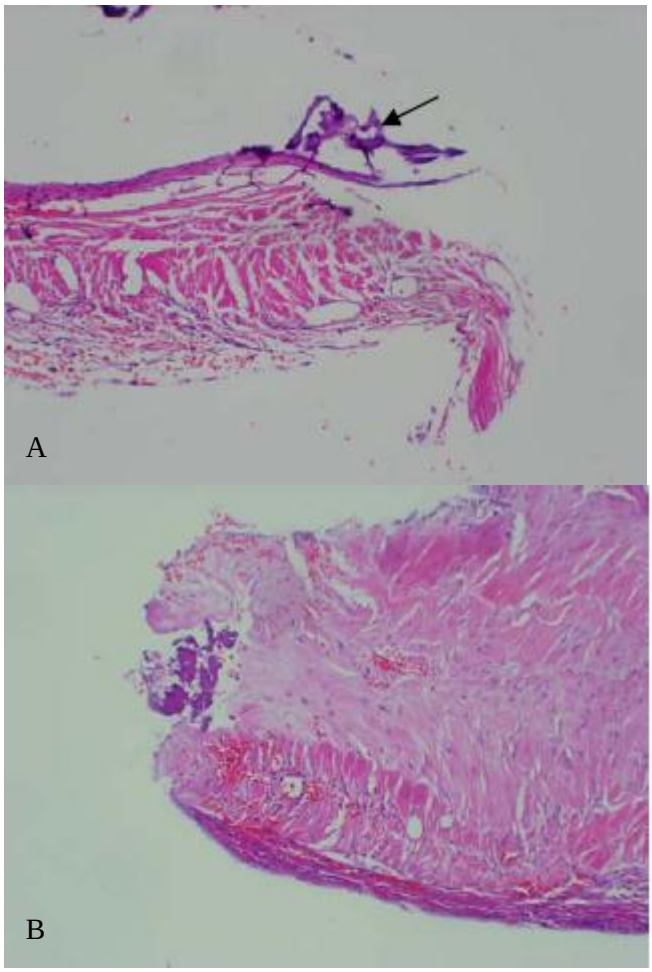

Figure 3 Representative photomicrographs of histology slides from Adherus Dural Sealant (A) and Control (B) at day eight necropsy showing incised dura. In Figure 3A, Adherus Dural Sealant is present on the periosteal surface of the medial dural flap (indicated with arrow). Figure 3B shows the medial dural flap of a control animal in which granulation tissue is evident.

Minimal infiltrates of mononuclear cells (macrophages and plasma cells/lymphocytes) and neutrophils into the pia mater layer of the meninges were present in control and treated animals, an expected observation in any procedure that results in dural penetration. Minimal focal vacuolation, noted in a single treated and control animal, was only superficial and immediately adjacent to an area of thickened meninges (thickened by an influx of inflammatory cells and possibly hypertrophy or slight hyperplasia of meningeal cells). Focal necrosis in the brain of a single control animal was due to trauma related to the initial creation of the bone flap. This iatrogenic lesion was of no biologic significance. All histological changes in the brain and meninges were thought to be fully consistent with the surgery and are commonly observed in the region of durotomies/craniotomies.

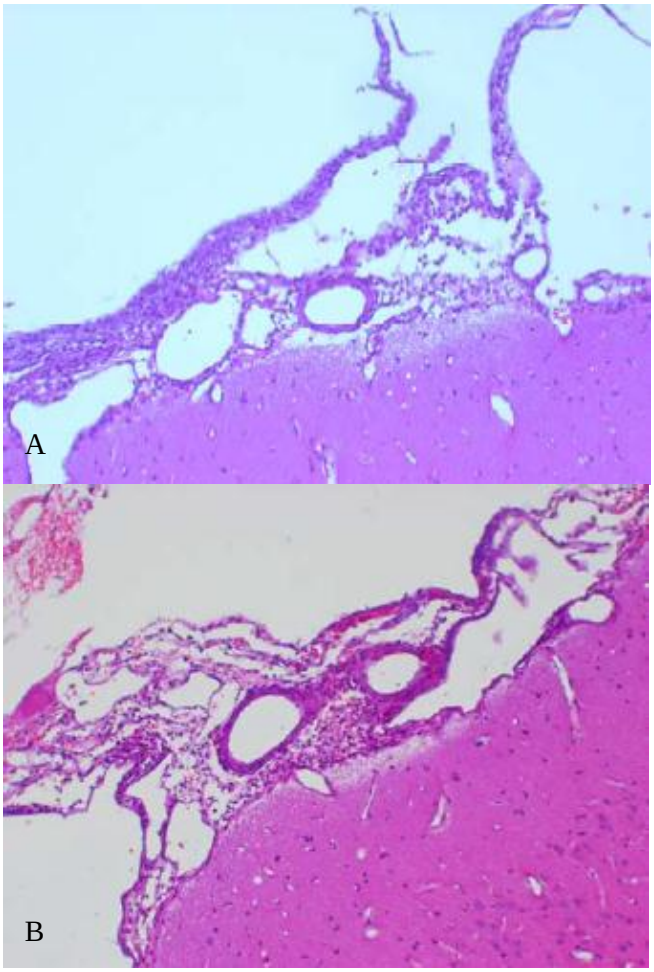

Figure 4 Representative photomicrographs of histology slides from Adherus Dural Sealant (A) and Control (B) at day eight necropsy showing the pia (meninges) with minimal infiltrates (original magnification x 10).

The dural incision was identified histopathologically in all animals. Adherus Dural Sealant was present on the periosteal surface of the dura in all three treated animals, indicating resorption of the sealant was not complete at the one week necrospy (Figure 3A). As would be expected one week post surgery, the 2 mm gap in the dura was not fully healed in either the test or control animals. Although there consistently was

a gap between the medial and lateral dura flaps, it is likely that the process of trimming, processing, paraffin embedding and microtomy caused some interruption to the surgical site, making differences in the determination of early healing impractical.

Overall, there was no histological indication that the presence of the sealant had any adverse effect on the dura, the surrounding tissues (including the brain) or the rate of dural closure.

Six Month Necrospy

During trimming, the brain, arachnoid, and dura for each animal sacrificed at six months were observed to be grossly normal. Dural attachments to the pia mater were minimal in both groups. One pin-point adhesion between a suture and the underlying pia was noted in one of the Adherus Dural Sealant-treated animals and pinpoint adhesions were noted in one control animal. The flattening of the cerebral cortex, noted in the animals at the Day eight sacrifice was not present.

There were no test article related changes in the animals sacrificed six months after surgery and there were no microscopic changes in the brain of any animal. Cellular infiltrates noted in the animals sacrificed at day eight had completely resolved.

Dura bridged the previous durotomy site in all control and test article treated animals. The dura was thickened in all animals, but the thickening was consistent with normal dura tissue (essentially a specialized fibrous connective tissue). Adherus Dural Sealant was no longer visible on the dura of any of the treated animals.

In all animals, the bone flap was partially vascularized and partially populated by osteoblasts.

The calvarium was normal in all but one control and one treated animal. In one control animal, foreign body giant cells were present around remaining suture material in soft tissue adjacent to the bone of the calvarium. In one Adherus Dural Sealant-treated animal, a portion of bone at the medial side of the previous craniotomy site was necrotic and associated with granulation tissue, mononuclear cell infiltrates, osteoblast proliferation and focal necrosis at the periosteal surface of the dural side. These changes were consistent with the resorption of a necrotic piece of bone. The most likely cause of these changes was trauma to the bone at the time of craniotomy, resulting in death of the resident osteoblasts and subsequent resorption reactions. There was no evidence that these changes were in any way related to the test article, especially given the lack of histological changes in the other animals.

DISCUSSION

Once applied to the target tissue, Adherus Dural Sealant sets in approximately one second, limiting potential device “run-off”. The sealant appeared tenaciously adherent to the dura and compliant with the pulsations caused by the dynamic changes in intracranial pressure.

Following application of Adherus Dural Sealant to a canine durotomy repair, there were no adverse clinical effects related to the application of the test article. There were no test article related clinical observations made in the six month period following application. Body weights and food consumption data remained consistent with that of the control animals over the same period. Physical and neurological evaluations performed over the six month period revealed no significant adverse findings that could be attributed to the test article such as mass effect, epidural or subdural hematomas, infection, or unusual inflammatory reactions. Clinical pathology parameters taken prestudy, one week,

two months and six months following application showed no significant differences between treated and control groups. At necropsy, there were no significant differences in organ weights between control and treated animals.

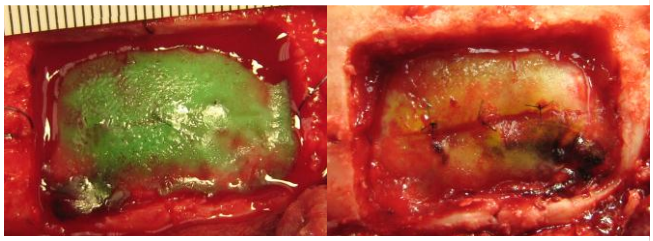

Figure 5 Photographs comparing an Adherus Dural Sealant-treated durotomy site at day one (intraoperative), on the left, with the same site at day eight, on the right.

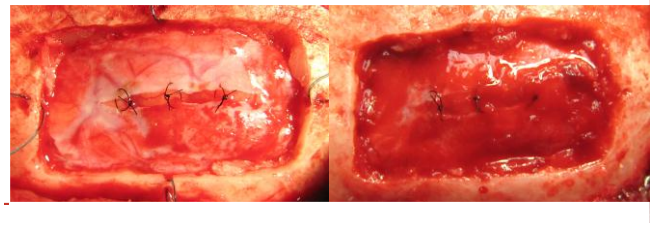

Figure 6 Photographs comparing a control durotomy site at day one (intraoperative), on the left, with the same site at day eight, on the right.

As expected, based on in vitro data[3], MRI evaluations showed a consistent degradation profile over approximately three months with accompanying tissue ingrowth (evidenced by thickening of the periosteum) at the durotomy site continuing for the next three months. The hydrogel was best visualized with T2-weighted images. When compared to control animals, the hydrogel was consistently visualized as a uniform, high signal band immediately adjacent to the dura. CSF collections and/or edema were typically visualized as irregular, hyperdense signal abnormalities.

During the first month after surgery, there were minimal changes in the size of the hydrogel. These findings were also noted with more frequent MRI imaging obtained at post-op days one, 14, and 21 during a pilot cranial durotomy repair study of the same design[8]. Furthermore, necropsy at the 14 and 21 day time intervals during this pilot animal study visually confirmed that the Adherus hydrogel did not undergo any appreciable dimensional changes. Volumetric expansion was likely constrained by the dense crosslinked network and the hindered degradation rate of the Adherus Dural Sealant. In comparison, other hydrogel sealants may undergo up to five times more volumetric expansion, especially during the first few weeks after implantation. This characterisctic has been observed to create signs and symptoms secondary to compression of neurological tissue in both spine and cranial surgeries[9,10,11].

The test article continued to be visible, although degrading (as indicated by a decrease in volume and signal intensity of the hydrogel), through the three month MRI scans. The hydrogel was barely discernible in a portion of the scans at four months and not present in the five and six month scans (T2 images). Bone growth was not impeded by the sealant and can be observed between the bone flap and skull in the T1 images at the four, five and six month scans. Bone healing was confirmed macroscopically at necropsy.

Edema was noted near the bone flap and in the surrounding musculature in the T2-weighted and T1 with contrast MRI images for all animals. In the Adherus-treated animals, this imaging feature was not contiguous with the subdural space, presented above the hydrogel and lacked homogenous signal characteristics (characteristics of CSF). These observations provided no radiographic evidence of a CSF leak in any of the six month animals receiving Adherus Dural Sealant.

The flattening of the underlying cortex that was noted histopathologically in the one week necropsy animals was also visible in the MRI images. As previously noted, this flattening is

likely due to displacement caused when the bone flap was replaced over the space occupying test material. The beagle brain takes up almost all the space within the skull magnifying the space occupying effect of any additional material. In the treated animals, the cortical depressions were apparent at one month, but resolved as the test material degraded and were no longer present at 6 months (confirmed at necropsy and histopathologically). This flattening did not result in any microscopic changes to the cortical tissue.

Intracranial pressure (ICP) testing at the 1 week necropsy (P = 0.003) and the 6 month necropsy (P= 0.043) both showed statistically significant increased baseline pressures over that of the control. The differences noted were small and all baseline pressures were in the physiological range. In the one week necropsy animals, this finding almost certainly reflected the difference between a "closed system" in the treatment group and the uniformly leaking dural closures in control animals. The clinical significance of the baseline pressure differentials at 6 months is less certain. The relative changes were on the threshold of statistical significance, and may not reflect any meaningful clinical difference between the groups.

More importantly, after establishing a baseline pressure, all (n=7) Adherus Dural Sealant-treated animals (100%) were able to reach a maximum ICP of at least 40 mmHg during pressure testing. Only one of the control animals pressure tested at 6 months reached this level and the remaining animals (n=5) were leaking at baseline. Although the ICP pressures were limited in this study to prevent inadvertent trauma to neurological tissues such as brain stem herniation, higher pressures were reached during the pilot cranial durotomy repair study. In this study, pressures of 81 and 77 mmHg were reached at 14 and 21 days respectively without any leaks.[8] The ability to sustain elevated ICP pressures for several weeks is unique to Adherus Dural Sealant (note that most other tissue adhesives/sealants have significantly or completely degraded within two to three weeks) and corroborates previous in vitro testing[3].

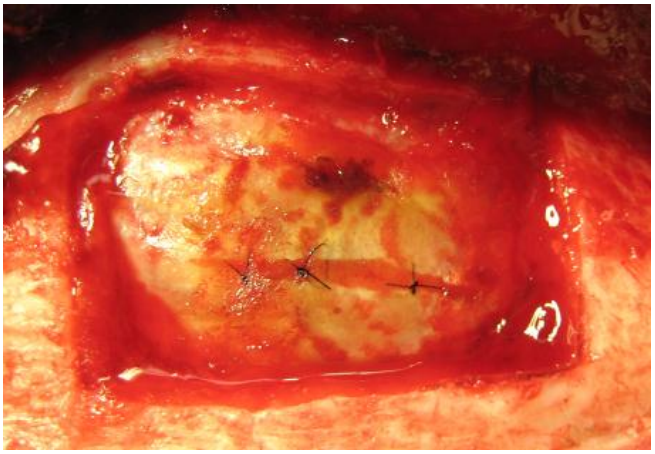

Figure 7 Adherus Dural Sealant-treated durotomy site at one week necropsy with CSF pressure at 45 mmHg. Adherus Dural Sealant remained elastic and tissue compliant under high pressures which caused dura to bulge and gape.

Pressure testing at up to three weeks highlights the strength and durability of Adherus Dural Sealant, especially when comparing the one week necropsy interval to other dural sealants. Under similar test conditions with the same dural defect model, DuraSeal Sealant System (Confluent Surgical, Waltham, MA) was leak free at pressures greater than approximately 40 mmHg in only 38% of the adequately sealed animals within the first week of testing. Furthermore all three animals tested at the one week time point reportedly experienced a CSF leak at intracranial pressures between approximately 27 and 40 mmHg.[7] These tests indicate DuraSeal begins to lose a significant amount of its functionality before the dura has had time to completely heal.[12]

Over the course of the study, the dura and surrounding tissues healed as expected. Adherus Dural Sealant did not seem to cause adhesions between the dura and underlying pia mater even

though it was directly applied over the gaping hole in the dura, crosslinking on and directly contacting the cerebral cortex. Finally, there were no unexpected histopathological changes in the brain, calvarium, meninges, or non-nervous system organs associated with the test article in any of the treated animals.

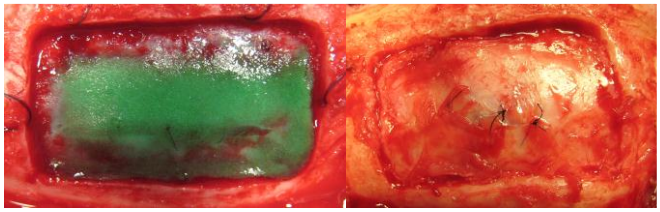

Figure 8 Photographs comparing an Adherus Dural Sealant-treated durotomy site at day one (intraoperative), on the left, with the same site at six months, on the right.

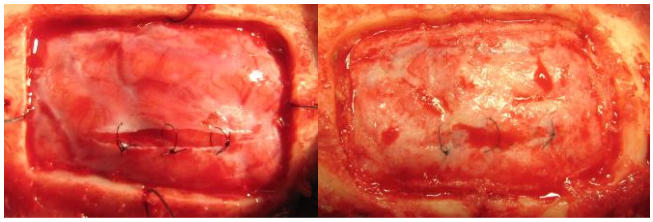

Figure 9 Photographs comparing a control durotomy site at day one (intraoperative), on the left, with the same site at six months, on the right.

CONCLUSIONS

Following surgical procedures involving compromised dura, Adherus Dural Sealant provides a safe fluid barrier with exceptional strength and durability, minimizing postoperative complications associated with CSF leaks and pseudomeningoceles. Once applied, the sealant practically maintains its implantable dimensions and acts as a part of the native dura while it is gradually replaced by new tissue over approximately three months.

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.

REFERENCES

[1] Hoffman RA: Cerebrospinal Fluid Leak Following Acoustic Neuroma Removal. **Laryngoscope 104**: 40-58, 1994.

[2] Selesnick SH, Liu JC, Jen A, Newman J: The Incidence of Cerebrospinal Fluid Leak after Vestibular Schwannoma Surgery. **Otol Neurotol 25**: 387-393, 2004.

[3] Data on file at HyperBranch Medical Technology, Inc. and summarized in 2010-TR-04867-04867 R1

[4] Data on file at HyperBranch Medical Technology, Inc.

[5] Data on file at HyperBranch Medical Technology, Inc. and summarized in 2011-TR-05583-05583 R1

[6] Alleyne CH, Cawley CM, Barrow DL, Poff BC, Powell MD, Sawhney AS, Dillehay DL: Efficacy and biocompatibility of a photopolymerized, synthetic absorbable hydrogel as a dural sealant in a canine model. **J Neurosurg 88**: 308-313, 1998.

[7] Preul MC, Bichard WD, Muench TR, Spetzler RF: Use of a Novel Hydrogel Sealant in a Canine Durotomy Repair Model. **Neurosurgery 53**: 1189-1198; discussion: 1198-1199, 2003.

[8] Data on file at HyperBranch Medical Technology, Inc.

[9] Mulder M, Crosier J, Dunn R: Cauda Equina compression by hydrogel dural sealant after a laminotomy and discectomy. **Spine 34**: E144-E148, 2009.

[10] Blackburn SL, Smyth MD: Hydrogel-induced cervicomedullary compression after posterior fossa decompression for Chiari malformation**. J Neurosurg (4 Suppl Pediatrics) 106**: 302-304, 2007.

[11] Thavarajah D, De Lacy P, Hussain R, Redfern R: Postoperative Cervical Cord Compression Induced by Hydrogel (DuraSeal). **Spine 35**: E25-E26, 2009.

[12] Cain Jr. JE, Lauerman WC, Rosenthal HG, Broom MJ Jacobs RR: The Histomorphologic Sequence of Dural Repair, Observations in the Canine Model. **Spine 16**: S319-S323, 1991.