

In-Vitro Properties of Adherus Dural Sealant

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SUMMARY: The physical and mechanical properties of Adherus Dural Sealant were evaluated through in vitro testing which included set time, water uptake, Young's modulus and burst strength. Using product up to two years old, Adherus Dural Sealant repeatedly set in one second, remained dimensionally stable, swelling only 8% in any one axis when exposed to an excess of solution, and was able to provide a firm barrier to fluid leaks for three to four weeks when exposed to pressures corresponding to spinal CSF pressures and five to six weeks when exposed to cranial physiological pressures.

INTRODUCTION

The potential for a cerebrospinal fluid (CSF) leak following neurosurgical intervention has led to the use of a variety of adjunctive techniques to bolster suture lines and duraplasty onlays. Whether approved for use in neurosurgery or not, surgeons often augment dural closure with biological adhesives, surgical sealants, hemostatic agents and other preparations. These products are used despite disadvantages associated with their use which include a lack of biomechanical strength, poor durability and significant swelling after polymerization.

In order to meet the needs of neurosurgeons, Adherus Dural Sealant was designed with an emphasis on limiting run-off, minimizing swelling, and maximizing adhesion while forming a watertight barrier over dural gaps until the compromised dura has had sufficient time to heal.

The following report summarizes the most important in vitro attributes of Adherus Dural Sealant which include a rapid rate of polymerization, minimal dimensional change, and a clinically advantageous burst strength that is maintained over an extended period of time.

MATERIALS and METHODS

At least three lots of the Adherus Dural Sealant (HyperBranch Medical Technology, Inc., Durham, NC) were available for the in vitro tests. Once reconstituted, the Adherus Dural Sealant system is composed of two solutions, a polyethylene glycol (PEG) ester solution and a polyethyleneimine (PEI) solution. The two solutions are mixed while expressed from the supplied applicator to form the hydrogel sealant. During each of the tests described herein, the product was prepared and tested under ambient conditions.

Set Time

The set time of Adherus Dural Sealant was determined by adding 100 μ L of the reconstituted PEG solution to an inverted microcentrifuge tube with the conical portion of the tube removed, containing a magnetic micro stir bar (6.35 x 3 mm, VWR,) rotating at approximately 1000 rpm. Subsequently, 100 μ L of the reconstituted PEI solution was added to the vial and a calibrated stopwatch was used to record the time between the addition of the PEI solution and a stop in stir bar rotation. The reported set time for each lot is

an average of at least 7 separate tests. The average set time was measured using Adherus Dural Sealant units produced 25 months prior to use and reconstituted 2 hours prior to use.

Water Uptake

To measure water uptake, hydrogel samples were created by expressing the reconstituted solutions from a Micromedics applicator (NUS001-2) into a Teflon mold (9.5x7.8x2.5 mm). The sealant plugs were weighed immediately after creation, and after soaking for 1, 5, 12, and 19 days and at least weekly thereafter in 37 °C PBS pH 7.4. The percent gravimetric swelling at various time intervals was calculated using the following formula:

$$\%Swelling_t = [(Mass - Initial Mass)/Initial Mass] \times 100\%$$

where Mass = Mass at any time t.

Although direct measurements of the change in dimension were not made, the following formula was used to relate the percent swelling to the percent change in dimension by assuming uniform dimensional change in each axis and a gel density approximating 1.0 g/cc:

$$\{[\%Swelling_t/100\% + 1]^{1/3} - 1\} 100\% = \% \Delta S_t$$

where $\%Swelling_t$ = percent swelling as a function of time t
and $\% \Delta S_t$ = percent change in dimension (S) as a function of time t.

The reported percent swelling or percent dimensional change for each lot is an average of at least 7 separate tests. The average swelling was measured using Adherus Dural Sealant units produced 25 months prior to use and reconstituted at least 2 hours prior to use.

Young's Modulus

To obtain defect free samples for this test, once reconstitution of the components was complete, a

small amount of phosphoric acid solution was added to the reconstituted PEI solutions and mixed thoroughly to obtain a set time of approximately 30-45 seconds. Approximately 500 μ L of the PEI and PEG solutions were subsequently mixed, quickly place into a 1 cc syringe and centrifuged prior to hydrogel polymerization to eliminate any air bubbles. The resulting hydrogel samples were allowed to fully cure over 18 to 24 hours in the sealed syringe before they were removed from the 1 ml polypropylene syringe with compressed air to produce uniform cylindrical samples with a diameter of approximately 4.7 mm. The hydrogel samples were cut to a length of approximately 25 mm. The hydrogel samples were glued to a test fixture using Gorilla glue. Once inside the fixture a Shimadzu AGS-J (Shimadzu Corporation, Kyoto, Japan) test machine with 20 N load cell was used to stress each specimen at a crosshead speed of 10 mm/minute with a chord modulus taken at 0.1N and 0.2N points on the stress strain curve. The reported Young's modulus is an average of at least five separate tests for each lot. All data was digitally collected and analyzed.

Burst Strength

Burst pressure testing was performed to examine both the adhesive strength of the interfacial bonding between collagenous materials and the sealant and the cohesive strength of the hydrogel itself (ASTM F2392-04). Collagen casing (#320, Nippi, Inc., Tokyo, Japan) with a uniform 3.0 mm diameter hole, created using a skin biopsy punch, was used as the tissue substrate. Adherus Dural Sealant was applied to the washed collagen casing, bridging the 3 mm defect with an approximate 1 mm thick coating of hydrogel. Sealant thickness was measured with calipers and the sample were either tested immediately, or placed in 37°C PBS pH 7.4. At designated test intervals, specimens were placed into a fixture

and water was pressurized behind the collagen substrate in order to determine the pressure needed to rupture the repair. The water pressure at which sealant failure occurred was recorded as the burst pressure. The method of failure, adhesive or cohesive, was also noted. The reported burst pressure for each lot is an average of at least 5 separate tests. The initial burst strength was measured using Adherus Dural Sealant units produced 25 months prior to use and reconstituted 2 hours prior to use. Burst strength over time was performed on one representative lot of material.

RESULTS

Set Time

A near instantaneous set time is necessary to assure that the mixed solutions contact the dura and form a hydrogel sealant at the desired location without run-off, irrespective of the angle of the dura. To test the reaction rate, two equal volumes of the reconstituted crosslinking components were mixed and the set time was determined based on the length of time necessary to stop a rotating stir bar. During the study, the sealant reaction rate in all instances occurred within one second after mixing (Table 1). This nearly instantaneous reaction rate remained constant throughout the shelf-life of the product even following reconstitution of the product out to 8 hours¹.

Lot	Average Set Time (Seconds)					
	4 Months	6 Months	9 Months	12 Months	18 Months	25 Months
1008116	1	1	1	1	1	1
1008117	1	1	1	1	1	1
1008118	1	1	1	1	1	1

Table 1 Average set time of Adherus Dural Sealant over a 24 month shelf life study.

Water Uptake

Once the surgical sealant has been applied to the dura, the hydrogel must swell minimally and degrade predictably to minimize mass effects and

other neurological complications. Over the first 16-24 hours, hydrogels, when placed in solution, typically absorb a certain amount of solution until the crosslinked hydrogel network is fully hydrated. The amount of solution the hydrogels absorb is usually measured by a change in the hydrogel's mass. The Adherus Dural Sealant hydrogel plugs were analyzed at 24 hours and absorbed a minimum amount of water, an average of 27% by weight, when compared to the gel's initial mass (Table 2). This gravimetric change was converted to a more clinically relevant 8% average change in dimension along any one axis (Table 3).

Lot	Average Gravimetric Swelling after 24 hours in 37 °C PBS pH 7.4 (%)					
	4 Months	6 Months	9 Months	12 Months	18 Months	24 Months
1008116	26	21	29	28	33	29
1008117	26	30	26	24	33	29
1008118	27	24	25	24	32	27

Table 2 Average gravimetric swelling of Adherus Dural Sealant after 24 hours in 37 °C PBS pH 7.4 over its 24 month shelf life.

Lot	Average Dimensional Change after 24 hours in 37 °C PBS pH 7.4 (%)					
	4 Months	6 Months	9 Months	12 Months	18 Months	24 Months
1008116	8	7	9	9	10	9
1008117	8	9	8	7	10	9
1008118	8	7	8	7	10	8

Table 3 Average change in any one dimension of Adherus Dural Sealant after 24 hours in 37 °C PBS pH 7.4 over its 24 month shelf life.

Following this initial uptake of water, ester linkages embedded within the crosslinked structure are hydrolyzed. This decrease in crosslink density of the hydrogel formulation creates a more relaxed polymer network and allows the hydrogel to uptake more water. Therefore, the hydrolytic degradation of the hydrogel is also measured through increased swelling. As the gels were further monitored, gravimetric analysis and conversion to dimensional change over the course of nearly three weeks, demonstrated that Adherus Dural

Sealant undergoes minimal dimensional changes over the first 24 hours and then remains nearly dimensionally stable over the first 19 days (Figure 1). Eventually, enough crosslinks are hydrolyzed to allow complete dissolution of the remaining polymeric materials over the course of 90 to 120 days.

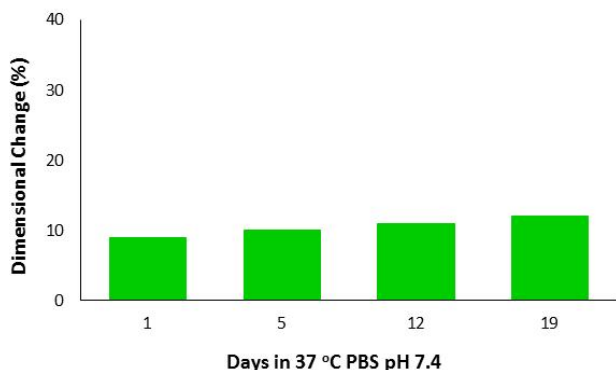


Figure 1 Average change in any one dimension of Adherus Dural Sealant over time in 37 °C PBS pH 7.4.

Young’s Modulus

Young’s Modulus is a measure of the stiffness of the gel. It is important that any repair made to the dura be flexible enough to ensure that stress concentrations do not occur in the native tissue being repaired (i.e. compliance matching) which could result in premature failure of the repaired dura. The Young’s Modulus (or elastic modulus) for the three lots of material tested ranged between 121 and 147 kPa (Table 4). The lots of material used in Young’s Modulus testing were different from those used in other testing since the older lots of material were not available for this test.

Lot	Young’s Modulus (kPa)
0609238	147
0809263	121
0809269	131

Table 4 Average Young’s modulus (elastic modulus) of Adherus Dural Sealant.

Burst Strength

Since the primary purpose of Adherus Dural Sealant is to provide a watertight seal, it is important to confirm that the device is capable of sealing the dura throughout the healing process while withstanding physiological pressures. In general, cranial CSF pressures average approximately 15 mm Hg² and spinal CSF pressures average approximately 30 mm Hg, with pressure spikes that may reach at least 45 mm Hg³. Following the general procedure outlined in the ASTM test, an approximate 1 mm thick layer of Adherus Dural Sealant was able to withstand an average burst pressure well over three times the hyperphysiological levels that may be generated in the spine, with an average burst pressure of 183 mm Hg (Table 5). The slight changes in burst pressure over the shelf life of the product were most likely a result of sample preparation and/or mounting the samples on the test fixture and not a variation in the hydrogel material itself. All failures throughout the study were cohesive, in that the sealant material itself failed, demonstrating very strong tissue adherence.

Lot	Average Initial Burst Strength (mm Hg)					
	4 Months	6 Months	9 Months	12 Months	18 Months	24 Months
1008116	157	209	152	170	254	154
1008117	179	145	147	173	227	163
1008118	227	146	187	151	227	180

Table 5 Average initial burst strength of Adherus Dural Sealant over a 24 month shelf life study.

Although initial burst pressure measurements do indicate the intraoperative strength of a sealant, a hydrogel must maintain a high level of performance post-operatively until the neodura can sufficiently contain CSF on its own. To determine the ability of Adherus Dural Sealant to maintain a high level of performance once implanted, the approximately 1 mm thick hydrogel sealant spanning the 3 mm diameter hole

in the collagen casing was incubated in 37 °C PBS pH 7.4 for up to 7 weeks before it was mounted on the test fixture. One day after implantation, Adherus Dural Sealant maintained most of its burst pressure performance, providing a watertight seal until an average pressure of 135 mm Hg. Over the course of three to four weeks, the sealant continued to maintain pressures above physiological levels that may be experienced in the spine, and was able to withstand representative spikes in CSF pressure up to 45 mm Hg for approximately three weeks. Furthermore, Adherus Dural Sealant continued to provide a watertight barrier for pressures that are typically experienced in the cranium for up to 6 weeks (Figure 2).

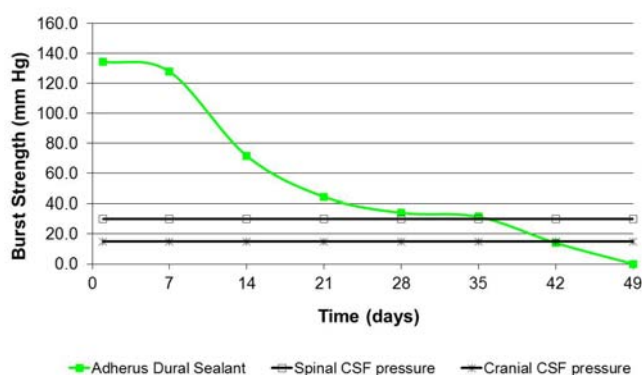


Figure 2 Average burst pressure of Adherus Dural Sealant over time in 37 °C PBS pH 7.4 compared with typical spinal and cranial CSF pressures.

DISCUSSION

In vitro testing confirmed that Adherus Dural Sealant combines mechanical strength and tissue adherence in a low swelling, quick setting device.

During set time testing, the hydrogel sealant consistently set rapidly. Not only did all samples during this testing set in one second, but almost all lots of material are released with a set time of one second. A rapid set time is a critical factor in limiting run-off from the target site, but will not eliminate run-off in all cases. A large amount of solution deposited in one location at a rapid rate

will most likely run before setting up. A thin, light application of Adherus Dural Sealant typically does not run and such an application is recommended.

Recording water uptake revealed that Adherus Dural Sealant changed minimally in any dimension upon implant, and will undergo little change over the most critical post-operative weeks as the dura heals. Adherus Dural Sealant is able to remain nearly the same size after implantation through a dense crosslinked network that firmly locks the size of the hydrogel in place. Furthermore the hydrogel is able to retain this dense crosslinked structure for at least three to four weeks by integrating ester linkages into the network that are less hydrolytically labile than ester linkages used in other commercially available sealants and adhesives. These ester linkages are eventually hydrolyzed and the sealant is typically no longer visible after approximately 120 days in vitro. This hydrolytic degradation rate correlates well with in vivo studies suggesting an approximate 90 day residence time.^{4,5}

While Adherus Dural Sealant typically degrades and is cleared from the body in approximately 90 days in vivo, it is important to draw a distinction between the mere presence of gel, which is often referred to in brochures and literature for other sealants, and a hydrogel that is able to function as a sealant. During burst strength measurements carried out over the course of seven weeks, Adherus Dural sealant provided a functional barrier to CSF flow for as long as six weeks in the cranium where dural edges often are difficult to approximate and may take longer to heal. Afterwards, the hydrogel was still present but the burst strength had eroded. Based on clearance times for other surgical sealants and adhesives, Adherus Dural Sealant is able to maintain functionality when many other products have already been completely cleared from the body.

Initial burst strength and Young's modulus further support the concept of Adherus Dural Sealant forming a strong, well adhered barrier. During the first week following implant, Adherus Dural Sealant provides a barrier that can contain CSF even at pressures up to three times greater than the pressure spikes that may be generated during coughs or Valsalva maneuvers. While the hydrogel is strong, it is also highly mechanically compatible with dural tissue. To minimize stresses between tissues and sealants, it is generally accepted that it is best to use materials with a similar elastic modulus. Although the Adherus hydrogel sealant is not as stiff as native dura⁶, it best matches the elastic modulus of the dura when compared to other commercially available sealants.

CONCLUSIONS

Obtaining a watertight closure of the dura is an important step in preventing post-operative CSF leaks. Adherus Dural Sealant is the optimal choice for obtaining watertight closure because it rapidly sets up once applied, forms a durable bond between the target tissue and the sealant and provides a robust barrier against CSF leaks that is able to function for up to five or six weeks post-operatively. Adherus Dural Sealant also minimizes the risk of neurological complications by remaining dimensionally stable over this same time period. The results of this in vitro testing provide important evidence supporting the performance of Adherus Dural Sealant when used as an adjunct to standard dural repair methods.

REFERENCES

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