Efficacy and biocompatibility of a photopolymerized, synthetic, absorbable hydrogel as a dural sealant in a canine craniotomy model


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Object. A canine craniotomy model was used to evaluate the dural sealing efficacy and biocompatibility of a novel, synthetic, biodegradable hydrogel.

Methods. Bilateral craniotomies were performed in 24 dogs assigned to six survival periods. In each animal a parasagittal durotomy was created and then repaired. At the treatment sites the hydrogel sealant was applied over the dural repair and photopolymerized. The repair was tested for leaks to 20 cm H₂O by using a Valsalva maneuver. At the control sites the incisions were sutured and tested for leaks only. After uneventful survival periods, the leak test was repeated in three of the four animals in each group. Bone–dura adhesion was evaluated, after which the dura and underlying brain were removed, fixed, and examined histologically. En bloc histological investigation was performed on a specimen obtained from the fourth animal in each group.

Over a 56-day period, 18 treated sites were tested for leaks. A leak was detected at a site remote from that of the repair in one animal; this was excluded from analysis. Thus 17 of 17 treated sites remained free of leaks. On the control side of one animal, there was a leak from a new dural tear at the cranial end of the durotomy, which occurred when the bone flap was removed. This site was also excluded from analysis. Eleven of 17 leak-tested control sites remained free of leaks over the study period. Bone–dura adhesions occurred in 15 of 19 control sites and had a mean adhesion score of 1.37 (range 0–4), whereas adhesions occurred in 10 of 19 treated sites with a mean adhesion score of 0.84 (range 0–3). No cortical reaction was noted.

Conclusions. This novel hydrogel sealant is efficacious in sealing dural repair sites measuring up to 2 mm. Healing of the underlying dura is not compromised and exposed cortical tissue is not altered histologically.

KEY WORDS • cerebrospinal fluid leak • dural defect repair • dural sealant • dural tear • hydrogel • meninges • dog

There are several disadvantages in using the aforementioned dural substitutes. Cadaveric human dura mater is often expensive, difficult to obtain, and has been associated with the transmission of Creutzfeldt–Jakob disease. In addition, an immune-type response has been reported, albeit rarely, with the use of cadaveric dura and bovine pericardium. Although the availability of single donor sources of concentrated fibrinogen has reduced the risk of blood-borne disease transmission, a real risk still attends the use of fibrin glue. The use of autologous fascia lata necessitates an additional skin incision and, perhaps, additional operating time. Other complications of synthetic materials include hemorrhage, excessive local tissue reaction, inflammation of underlying nervous tissue, and graft encapsulation. Newer biodegradable substances, including an elastin–fibrin material and hydroxyethylmethacrylate hydrogels, have been used in animals.
A novel synthetic, bioabsorbable hydrogel has been recently used to seal pleural leaks.\textsuperscript{25} We investigated the use of this hydrogel (FocalSeal; Focal Inc., Lexington, MA) as a dural sealant in a canine craniotomy model.

**Materials and Methods**

**Animal Preparation and Operative Procedure**

Twenty-four adult dogs were assigned to six survival periods, with four animals in each group. The survival periods were 1, 4, 7, 14, 28, and 56 days after treatment, and the corresponding animal groups were labeled A, B, C, D, E, and F, respectively. After the dogs had been premedicated with morphine and glycopyrrolate, anesthesia was induced with ketamine and orotracheal intubation was performed. Each animal’s scalp was prepared and draped in sterile fashion, after which a biparietal incision was made. Bilateral paramedian curvilinear incisions were made in the temporalis muscle and fascia 1 cm lateral to their insertion onto bone (which is 1–2 cm from the midline in the dog) and the temporalis muscle was reflected laterally. Curved Weitlander retractors were placed to facilitate exposure and a cranial perforator was used to create a burr hole in the anterior parietal region. Bilateral bone flaps were then raised by means of a craniotome. Treatment and control sides of the skull were determined randomly and a parasagittal durotomy measuring 2 cm was made centrally on the treatment side. The arachnoid was also opened because it is extremely thin and generally is in close contact with the dura in the dog. Three simple, interrupted No. 6-0 Prolene sutures were placed at equal intervals to approximate the dural edges. Gaps ranging from 1 or 2 mm were present in the dural repair with the underlying cortex exposed.

**Description and Application of Sealant**

The FocalSeal surgical sealant consists of a novel, bioresorbable hydrogel delivered in two parts (primer and sealant) and photopolymerized with visible light. The primer consists of an aqueous solution of a copolymer of poly(ethylene glycol)-co-lactide (Mr 3350) with acrylated end groups. The sealant solution consists of a viscous aqueous solution of a copolymer of poly(ethylene glycol)-cotrimethylene carbonate-co-lactide (M, 20,000) with acrylated end groups (Fig. 1). The sealant solution was used as a...
10% (w/w) solution in triethanolamine (90 mM)–buffered saline with eosin Y (20 ppm) added as a photoinitiator. The design and formulation of surgical sealant hydrogels have been described previously.26,27 The formulation tested in this study was designed to be resorbed over a 3- to 4-week time period. The FocalSeal “macromer” (large-molecular-weight polymer) was sterilized in 0.5-g aliquots with ethylene oxide. The sterilized macromer was reconstituted with 4.5 ml of buffer that had been filtered in a sterile fashion through a 0.22-μm filter. The sealant macromer was tested for pyrogens and for microbial growth at an outside testing laboratory to validate its sterility.

The liquid primer was applied to the exposed dura with a small brush. The sealant solution was mixed with the primer in situ to provide a final thickness of approximately 2 to 3 mm. The sealant was photopolymerized by using visible blue–green (450–550 nm) light delivered at 100 mW/cm² from a xenon arc lamp. The liquid sealant stops flowing in 1 or 2 seconds and is completely polymerized in 40 to 60 seconds, forming a clear, flexible, solid sheet (Fig. 2). The sealed durotomy was tested for leaks by maintaining pulmonary inflation at 20 cm H₂O and laterally compressing the thorax. On the contralateral control side, a 2-cm parasagittal durotomy was made and repaired with three interrupted No. 6-0 Prolene sutures, but no hydrogel was applied. The leak test was repeated. The bone flaps were replaced and secured with No. 2-0 Vicryl sutures. The temporal fascia was closed with a running No. 2-0 Vicryl suture, and the skin was closed in layers. The dog was then awakened from anesthesia.

No abnormal findings were noted in any animal in any of the study groups.

Postoperative Study

At the end of each survival period, three of the four animals in the respective groups underwent induction of anesthesia, after which the bone flaps were removed and the leak test was repeated. Using a subjective 5-point scale from 0 (no adhesions) to 4 (tenacious adhesions), healing of the bone flap (bone–bone adhesions) was evaluated in 17 animals (17 treated sites and 16 control sites) and adhesion formation between the bone and the dura in 19 animals. After heparin had been administered, the animal was killed by an overdose of pentobarbital. A thoracotomy was performed and the ascending aorta was cannulated and perfused with 4 L of normal saline followed by 4 L of 10% neutral buffered formalin. The dura and underlying brain were harvested and postfixed in formalin for at least 48 hours. In the fourth animal from each group, a block of tissue consisting of bone, repaired dura, and brain was removed en bloc and placed in a commercial decalcifying agent for several days. Specimens were sectioned, placed in cassettes, and embedded in paraffin. Sections were made and histological studies were performed using hematoxylin and eosin and trichrome stains.

Results

Adhesion Formation

Bone healing proceeded by the formation of a fibrinous bridge that gradually became organized into dense fibrous tissue in both the control and treated sites. Bone–bone adhesion occurred in 16 of 17 treated sites with a mean score of 1.82 (range 0–4), and in 14 of 16 control sites with a mean score of 2.12 (range 0–4). Bone–dura adhesion occurred in 10 of 19 treated sites with a mean score of 0.84 (range 0–3), and in 15 of 19 control sites with a mean score of 1.37 (range 0–4). Scores in both bone–bone adhesions and bone–dura adhesions increased with survival.
Hydrogel as a dural sealant

<table>
<thead>
<tr>
<th>Adhesion Formation Status</th>
<th>No. of Sites (%)</th>
<th>Mean Score (range)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>bone–bone</td>
<td>16 of 17 (94.1)</td>
<td>1.82 (0–4)</td>
</tr>
<tr>
<td>bone–dura</td>
<td>10 of 19 (52.6)</td>
<td>0.84 (0–3)</td>
</tr>
<tr>
<td>leak-free</td>
<td>17 of 17 (100)</td>
<td>—</td>
</tr>
<tr>
<td>bone–bone</td>
<td>14 of 16 (87.5)</td>
<td>2.12 (0–4)</td>
</tr>
<tr>
<td>bone–dura</td>
<td>15 of 19 (78.9)</td>
<td>1.37 (0–4)</td>
</tr>
<tr>
<td>leak-free</td>
<td>11 of 17 (64.7)‡</td>
<td>—</td>
</tr>
</tbody>
</table>

* — = not applicable.
† Scoring system: 0 = no adhesions present; 1 = filmy, transparent adhesions that are easily broken down with blunt dissection (avascular); 2 = adhesions that can be broken down with blunt dissection (vascular); 3 = adhesions that require sharp dissection (small vessels are present); 4 = tenacious adhesions that require sharp dissection (large vessels are present).
‡ Of those sites that leaked, three were in Group A, one in Group B, and two in Group C.

Leak Testing

Initial leak testing revealed intraoperative sealing of all cerebrospinal fluid leaks at treated sites and leakage at all control sites. All treated sites were tested before creating the control durotomy. At the end of each survival period three animals in each group were again tested for leaks. One of the 18 treated sites (in Group B [4-day survival]) leaked at a location under the anterior edge of the craniotomy site, but no leak was noted at the durotomy. This site was excluded from this portion of the analysis. Thus 17 of 17 treated sites remained free of leaks over the 56-day period. One of the 18 control sites (Group F [56-day survival]) leaked from a new dural tear at the cranial end of the durotomy. Because this tear occurred when the bone flap was removed, this site was excluded from analysis. Eleven of 17 leak-tested control sites remained free of leaks over the study period. Therefore, on the control sides there was a leakage incidence of 35.3%. The leaks occurred in three sites in Group A, one in Group B, and two sites in Group C (Table 1). No leaks were noted on the control side in Groups D, E, and F.

Pathological Examination

Gross Examination. Following photopolymerization, the hydrogel formed a transparent or translucent layer that was smooth to the touch. However, by Day 14 the hydrogel became soft and pasty and by Day 21 had been largely resorbed.

Histological Examination. At early time points, the interface between the dura and the overlying hydrogel showed a mild macrophage-mediated cellular response devoid of giant cells. The hydrogel was resorbed by bulk hydrolysis and was not dependent on cell-mediated mechanisms; thus a quiescent macrophage response occurred, as was expected. Cut dural edges were shown to have healed by the formation of a bridging fibrous scar. Trichrome stain-
ing showed a dense band of collagen deposition joining the cut dural edges. Importantly, the dura was shown to have healed “in plane,” which means that the cut edges were prevented from evertting and healing to overlying bone while they were being supported by the hydrogel; thus, the incidence of bone–dura adhesions was markedly reduced. The presence of the hydrogel did not appear to alter the normal time course of wound healing. The fibrous bridge was not attached to the underlying brain and no cortical reaction was noted, despite application of the hydrogel directly on the brain in the gaps between the interrupted sutures. The biological response to this hydrogel sealant was consistent with other bioresorbable materials and resulted in fewer and less tenacious adhesions and no adverse reaction in the underlying cortex (Fig. 3).

**Discussion**

The search for the ideal dural substitute continues despite the use of diverse substances, both natural and synthetic. The hydrogel used in this study sealed 100% of the treated sites over the 56-day study period. This compares with a 35.3% leak rate in the control sites. Both the bone–bone and bone–dura mean adhesion scores were decreased in the treated sites (1.82 and 0.84, respectively) as compared with the control sites (2.12 and 1.37, respectively). This hydrogel offers many advantages over other dural substitutes. It can be applied with ease and with relative speed. No infectious complications were associated with its use in this study. It is bioresorbable and allows in-plane neodura formation through progressive healing of the dural defect. There appears to be no cortical reaction despite the juxtaposition of the hydrogel to the cortex. There is decreased adhesion formation between the dura and bone and no adhesion formation between the dura and underlying cortex.

Although the hydrogel was used in this study to augment primary dural closures with gaps of 2 mm, it is conceivable that it might also be effective without the use of sutures. Further study is needed to corroborate this hypothesis. Although no neurotoxicity was noted in this study, more extensive toxicity studies are warranted.

In summary, FocalSeal is a bioresorbable, synthetic, photopolymerized hydrogel that is efficacious in sealing dural repair sites measuring up to 2 mm. Healing of underlying dura is not compromised and exposed cortical tissue is not altered histologically.

**Acknowledgments**

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C. H. Alleyne Jr., et al.
Hydrogel as a dural sealant


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