In Vivo Tissue Reaction, Resorption, Safety, and Efficacy of a Collagen Dural Substitute in an Animal Model

Bob Sommerich, Anita Barnick, Mark Barakat M.D., and Michael Ward D.V.M.

Codman & Shurtleff, Inc.
Raynham, MA
USA

January 2005
Abstract

The in vivo safety and efficacy of DURAFORM™ Dural Graft Implant, a collagen dural substitute, was evaluated with regard to tissue reaction and long-term resorption after implantation in albino New Zealand rabbits. In addition, the effectiveness of DURAFORM™ Dural Graft Implant as a cerebrospinal fluid (CSF) leak-resistant dural graft implant was evaluated in a comparative analysis with DuraGen® Dural Graft Matrix (DuraGen), with sham-operated animals serving as surgical controls. Post-mortem evaluations of subjects following one, three, six, nine, and twelve months of DURAFORM™ Dural Graft Implant implantation, did not reveal CSF leakage, hydrocephalus or hemorrhaging. In addition, there was no clinical evidence of infection or systemic toxicity. Both DURAFORM™ Dural Graft Implant and DuraGen® Dural Graft Matrix were similar with respect to observed adhesion formation to the brain surface and calvarium and both showed less adhesion formation than the control group. In all but one animal, the DURAFORM™ Dural Graft Implant material was not visible after three months, suggesting a complete degradation of the implanted material. After six months, none of the implanted DURAFORM™ Dural Graft Implant or DuraGen® Dural Graft Matrix material was visible at surgical sites. The results from this study support a conclusion that DURAFORM™ Dural Graft Implant is substantially equivalent to existing DuraGen® Dural Graft Matrix.

Background

The tissues of the central nervous system (brain and spinal cord) are covered by three connective tissue layers collectively called the meninges. Consisting of the pia mater (closest to the CNS structures), the arachnoid and the dura mater (farthest from the CNS), the meninges also support blood vessels and contain cerebrospinal fluid. While the dura mater is a tough fibrous membrane capable of protecting the underlying tissues, head and spinal injuries and/or complex invasive neurosurgical procedures may result in the dura mater being torn, opened, or removed to gain access to the delicate tissues contained within. Effective dural closure helps minimize cerebrospinal fluid leakage and facilitates proper wound healing. Dural defects may be repaired by several techniques, including the use of commercially available dural graft substitutes.

Dural Substitutes

The Food & Drug Administration describes a dura mater substitute as a sheet or material used to repair the dura mater. A number of characteristics are considered when assessing the effectiveness of collagen-based dural substitutes (Table 1).

Table 1: Proposed Characteristics of an Ideal Dural Substitute

- Prevents cerebrospinal fluid leaks
- Easy to handle – pliable, flexible, resistant to tearing, stable
- Biocompatible
- Conforms anatomically to all regions of the brain & spine
- Fully resorbable over time
- Barrier to soft tissue adhesions to brain
- Sutureable
- Provides protection for damaged brain tissue
There are several types of dural substitutes. Cadaveric dura mater substitutes are no longer marketed after a nongovernmental surveillance group for Creutzfeldt-Jakob disease (CJD) in Japan reported its analysis of a 1996 mail questionnaire survey of healthcare institutions throughout Japan. This analysis identified 82 patients with CJD from January 1979 to May 1996, including a large number (43 patients) who had received a cadaveric dura mater graft during a neurosurgical (42) or orthopedic (1) procedure. While the manufacturer of cadaveric dura mater graft (LYODURA, B. Braun Melsungen, AG) did modify its grafting process, healthcare agencies remained concerned, since recipients of contaminated grafts remain at risk for CJD at least sixteen years following receipt of grafts. As of March 2003, the number of cases in Japan of CJD associated with cadaveric dura mater grafts totaled ninety-seven, with one hundred and seventeen cases worldwide.

While collagen dural substitutes have been in development since 1967, it was not until the early 1990s that they became available. Synthetic dural graft material has been made with resorbable collagen matrices. Resorption is facilitated by a cellular infiltration of connective tissue. Grafts from the patient’s own fascia lata (connective tissue from the lateral aspect of the thigh) are a viable alternative, although these require a second incision and a modest increase in operative time. Biotissue from another patient or an animal can be utilized, with positive reports for both ovine and bovine pericardium. A collagen sponge produced from bovine tendons has also been used. Among the twelve currently available dural substitutes, four are synthetic, four are manufactured from bovine pericardium, one from porcine intestine, one derived from fetal bovine skin and two are derived from bovine achilles tendon. In the U.S., dural substitutes created from bovine pericardium and bovine Achilles tendons are harvested in countries claiming to be free from Bovine Spongiform Encephalopathy (BSE). These are manufactured under the United States Food and Drug Administration's Guidance document and European Standards for animal tissue sourcing, handling, and inactivation of BSE. CJD and BSE are very similar in both disease development and symptomology. CJD, or a very similar syndrome, is thought to be associated with exposure to BSE-contaminated bovine tissues.

**DURAFORM™ Dural Graft Implant**

DURAFORM™ Dural Graft Implant is a resorbable porous collagen matrix designed for the repair of dura mater. DURAFORM™ Dural Graft Implant is a soft, white, pliable, non-friable material with reliable handling characteristics. DURAFORM™ Dural Graft Implant is manufactured from Type I collagen obtained from bovine Achilles tendon. This collagen is obtained from a geographical BSE risk level 1 source, the lowest risk category available. The Type I collagen is fashioned into strong, sterile, pliable sheets that provide a rich environment for cell attachment and growth. This tissue has been found to have physical properties required for repairing and replacing dura mater. Over time, DURAFORM™ Dural Graft Implant takes on the characteristics of surrounding dural tissue while it serves as a framework, within which human tissue grows. As the biomaterial is replaced by intrinsically generated tissue, the collagen matrix is resorbed by the body and is no longer needed.

**In Vivo Evaluation in an Animal Model**

The purpose of this in vivo study was to evaluate the safety and efficacy of collagen dural substitutes with regard to tissue reaction and long-term resorption rates after functional implantation in albino New Zealand rabbits.

**Study Objectives**

DURAFORM™ Dural Graft Implant or DuraGen® Dural Graft Matrix implants were compared in rabbits for the following parameters:

1. Ease of implantation and overall conformity to complex neurological surfaces.
2. Overall safety and efficacy.
3. Resorption rates.
5. Incidence of cerebrospinal fluid leakage with sutureless closure.
6. Presence of graft encapsulation or foreign body reaction.
7. Comparative infection rates.
A total of sixty (60) animal subjects were divided among three study groups that included sham-operated animal subjects (controls) and those receiving DURAFORM™ Dural Graft Implant or DuraGen® Dural Graft Matrix. The study design included twelve subjects per time period and four subjects per group and per time period using a random distribution. The materials were implanted for one, three, six, nine and twelve months. During the observation periods, all animals were evaluated for neurobehavioral changes, food consumption, body weight, and incidence of infection and cerebrospinal fluid leakage. A histological analysis of all surgical sites was undertaken at the end of each animal subject's term of study.

Animal Preparation and Anesthesia

All animal subjects were pre-medicated with atropine and anesthetized with tiletamine-zolazepam (25 mg/kg) and xylazine (5 mg/kg). The skin of the dorsal section of the cranium was clipped free of fur and scrubbed with germicidal soap. The surgical site was disinfected with povidine iodine. Heart rate and blood pressure were continuously monitored during surgery.

Surgery

When complete anesthesia had been achieved, an incision of 4 cm in length was made medio-sagitally in the scalp followed by insertion of a self-retaining retractor. Once the parietal bones of the scalp were exposed, osteoplastic trepanation (8 mm in diameter) was performed on each side. A defect of the dura mater was created bilaterally (~4-5 mm in diameter) in all animals. Either one of the two dural substitutes was implanted into the dural defect on each side of the calvarium (8 mm diameter when measured dry); or, in the control group, the dural defect was left without any implant. The dural substitutes were moistened in situ with a saline solution after being positioned directly over the defect created in the dura mater. Once these implants were appropriately positioned, macro-photographs of the implant sites were prepared. The bone flap was then repositioned on the pericranium. The periosteum and subcutaneous tissues were sutured with polyglactin sutures and the skin incision was closed using metallic staples.

Recovery

Upon recovery, animals were individually housed in stainless steel cages for clinical observation. Any animal that died less than nine days post-surgery was considered to have had a poor recovery from either surgery and/or anesthesia, and was replaced. A staff veterinarian examined animals immediately following and at twenty-four-hours post-implantation. Animals were observed once daily thereafter and at study termination for clinical signs and symptoms. Additional observations and tests included:

<table>
<thead>
<tr>
<th>TEST/OBSERVATION</th>
<th>FREQUENCY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Examination of incision sites</td>
<td>Once/day for first 7 days post-surgery</td>
</tr>
<tr>
<td>CBC/differential</td>
<td>Pre-surgery; 7-days post-surgery; at sacrifice</td>
</tr>
<tr>
<td>Neurobehavioral observations</td>
<td>Pre-surgery; weekly thereafter</td>
</tr>
<tr>
<td>Detailed neurobehavioral assessment (staff veterinarian): skin sensitivity, hind paw reflex, toe pinch, ocular response, and auditory startle</td>
<td>Weeks 4, 13, 26, and 52</td>
</tr>
<tr>
<td>Body temperature</td>
<td>Pre-implant; 3-days post-surgery; weekly thereafter</td>
</tr>
<tr>
<td>Body position, locomotor activity, general behavior, tremors, convulsions, piloerection, salivation, lacrimation, and signs/symptoms associated with urination, defecation, or breathing</td>
<td>Daily</td>
</tr>
<tr>
<td>Food consumption</td>
<td>Weekly</td>
</tr>
<tr>
<td>CSF samples for total protein and cells*</td>
<td>Weeks 1, 3, 9, and 12 &amp; at sacrifice; additional, if clinical signs indicated</td>
</tr>
<tr>
<td>Body weight</td>
<td>Day of surgery; weekly</td>
</tr>
</tbody>
</table>

Table 2: Frequency of Tests and Observations During the Study

* CSF analyses by Laboratoire d’Analyses de la Sauvegarde, Lyon, France
Sacrifice and Sampling

Animal subjects were anesthetized with tiletamine-zolazepam (25 mg/kg) and a CSF sample was drawn for analysis. Animals were then sacrificed by lethal injection of pentobarbital sodium (135 mg/kg). The skull was removed by sectioning at the atlanto-occipital joint and submitted to histology. The surgical sites (in block with calvarium, dura mater and brain) were identified and fixed in 10% buffered formalin solution for histopathology. The remainder of the carcass was discarded. One week after the initial formalin fixation and to assure complete fixation of all harvested tissues, the calvarium was separated transversely between each side of the trepanation area using a diamond saw and additional formalin fixation was continued.

Histopathological Assessment

To assess relative biological response, a pathologist reviewed fixed preparations of explanted tissues from animal implant sites. Peri-implant or surgical (sham) responses were graded as absent, slight, moderate, marked or severe. Specific assessments were limited to the following parameters: fibrin deposition; cellular necrosis; degenerative changes; inflammatory cells surrounding the implant; fibrosis; neovascularization; degradation of the implant material; cellular colonization of the dural substitute; neofibroblastic adhesions to calvaria; the brain and/or gap formation; alteration of the brain cortex (i.e. cellular infiltration, scar formation and hematoma); brain compression; and, brain scarring. The meninges and choroid plexus were also examined. Dural adhesion was considered present when there was fibrous adhesion (fibrous bridges) between the calvarium and the brain tissue.

Study Results

There was no observed clinical evidence of infection, as determined by observation of the incision site during the seven days following surgery, or signs of systemic toxicity of the implanted products. Body temperature, blood cell counts and differentials, and CSF total protein and leucocytes were normal during the entire period of the study. During the clinical follow up and at the gross evaluation, there was no clinical evidence of CSF leakage, hydrocephalus, or hemorrhage. While calvarial and brain adhesions were observed in the majority of sites in both implanted materials (DURAFORM™ Dural Graft Implant, DuraGen® Dural Graft Matrix) they were less severe than those observed in the control (sham operated) group. The implant sites for DURAFORM™ Dural Graft Implant and DuraGen® Dural Graft Matrix were similar with respect to adhesion formation at the brain surface and calvarium. The inflammatory reaction to either implanted material was minimal and limited to the presence of a few macrophages; further, degree of inflammatory response decreased in all sites between one and twelve months post-implant. Almost no brain compression was observed and superficial brain tissue degeneration and alteration were attributable to the surgical procedure as they were evident and comparable among all groups at all time-points. Further, the surgical trauma to the brain did not have a significant impact on the tissue reaction to the implanted materials and their resorption rates. By six-months post implant all implanted materials were resorbed.

Conclusions

The results of this study support a conclusion that DURAFORM™ Dural Graft Implant and DuraGen® Dural Graft Matrix are substantially equivalent with respect to:

1. Overall performance
2. Preventing leakage of CSF
3. Creating a non-infectious, non-inflammatory, and non-toxic environment
4. Minimizing adhesion formation
References

1. DuraGen, Dural Graft Matrix. Integra LifeSciences Corporation; 1999


© 2005 Codman & Shurtleff, Inc. All rights reserved