IN VIVO EVALUATION OF DURA SUBSTITUTES

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Abstract

OBJECTIVE: The use of dura substitutes is frequently unavoidable when tension-free dura closure cannot be achieved following neurosurgical procedures or trauma. Biodegradable collagen matrices serve as scaffolds for the regrowth of natural tissue. The aim of this study was to assess the safety and efficacy of 2 dura substitutes (A and B) as sutured grafts in a rabbit model.

METHODS: In this pilot study, 8 adult New Zealand White rabbits were sedated and implanted with dura substitute A or B. At 4 weeks, the rabbits were sacrificed and CSF samples were taken to assess white cell counts. The 8 rabbits were divided into 2 groups; each group consisted of 2 rabbits implanted with implant A and 2 rabbits implanted with implant B. One group (4 rabbits) was used for grossly assessing adhesion to overlying bone and underlying cortex and one group (4 rabbits) was used for histological assessment. For the adhesion group, gross examination of the rabbit dura substitutes for adhesion and strength was performed on a scoring basis of 0-4. For the histology assessment group, the samples underwent both gross evaluation and microscopic evaluation. The Veterinary Pathologist first examined the prepared rabbit heads grossly for evidence of hemorrhage or infection and made coronal cuts through the brain to assess ventricles for signs of hydrocephalus. Then the subsequently prepared brain tissue samples were assessed histologically in a semi-quantitative fashion for several categories: adhesion formation to underlying structures, anchorage to dura, resorption/replacement by host tissue, reformation of dura, vascularization, hemorrhage, infection, and cellular response to the dura substitute.

CONCLUSION: Dura substitution implants A and B appear ill suited in their present forms for dura substitution. Their use resulted in maximal adhesion of the implants to the overlying bone and some degree of adhesion to the underlying brain cortex. At 1 month, there was some integration with the native dura and the implants had weakened from scores of 5 for the virgin (unimplanted) implants to 1-2 as they were easily pulled apart. The chronic inflammation seen in the histology strongly suggests chronic rejection of the implants with no signs of infection.

Introduction

Inadequate closure of the dura exposes the patient to such possible post-operative complications as CSF fistula, infection, hypertensive pneumocephalus, and pseudomeningocele. Consequently, a careful dural closure is an essential step after intradural neurosurgical procedures. When primary suture closure of dural defects in the cranium and spine is not feasible, materials must be used as dura substitutes to repair the defects. Although many experimental and clinical studies have been performed to identify a suitable material to repair defects of the dura mater, no ideal dura substitute is currently available. Many materials have been evaluated in animal models and in humans for repairing dura defects. These materials can be broadly categorized into two groups, namely synthetic materials and natural materials. Each type of material can further be divided into subgroups of resorbable
and non-resorbable materials. In the synthetic resorbable subgroup, lactic acid-caprolactone
copolymer, polyglycolic acid$^1$ and polylactic acid-polyglycolic acid copolymer$^{2,3}$ have been
evaluated, whereas in the non-resorbable synthetic subgroup, polytetrafluoroethylene
(PTFE)$^4$, silicone$^{5,6}$, and hydroxymethylmethacrylate hydrogel$^3$ have been investigated. In the
natural resorbable subgroup, autografts$^7$, allografts$^8,9,10,11,12,13,14,15$, heterografts$^{16,17,18,19}$, and
engineered type I collagen-based implants$^{20,21,22,23}$ are the materials of choice. In the natural
non-resorbable subgroup, glutaraldehyde processed bovine pericardium has been the material
most extensively used clinically$^{24}$.

Thus far, materials used clinically have a wide range of material characteristics as well as
physical, chemical and biological properties. What makes such diverse materials acceptable
clinically for dura repair is that these materials are stable and biocompatible. However,
despite the many materials used clinically, the search for an ideal dura substitute continues,
primarily due to the fact that none of the currently available commercial materials can satisfy
all the requirements for dura repair. Complications associated with the use of many synthetic,
natural, and allogenic dura substitutes include meningoencephalitic scars and seizures,
localized inflammation and necrosis, transmission of infectious disease such as
Creutzfeldt-Jakob disease, systemic immune disorders, myelopathy, and acute intracranial
hemorrhage$^{2,5,25,26,27,28}$.

Using rabbits as the animal model, we report here the results of evaluation of two new dura
substitutes that have not been available for testing in the past. These dura substitutes should
serve as templates to support and guide dura regeneration while being slowly resorbed in the
rabbit. In order to compare our results with other studies in which the dura substitute was
sutured to the dura, we evaluated this dura substitute implant as a graft sutured to the rabbit’s
native dura.

**Materials and Methods**

**Implantation of Dura Substitutes**

Adult New Zealand White rabbits (8, 4kg) were sedated with Rabbit Mix and maintained
under anesthesia with halothane to effect (0.5-2%). The scalp was shaved and prepared with a
betadine scrub. Using sterile technique, a 5cm midline incision in the sagittal plane exposed
the calvarium which was stripped to bone with a periosteal elevator. Wheatland retractors
were used to maintain exposure for the duration of the operation. Access to the dural
membrane was through craniectomies approximately 1cm$^2$ located left to midline and
posterior to the coronal suture. The dura was elevated with a dura hook and an 8mm x 8mm
defect produced by removing the dura with angled iridectomy scissors. The dura substitute
was soaked in sterile saline for 5 minutes then placed over the dural defect with an
approximate 1mm overlap of the native dura. The dura substitutes were then sutured to the
native dura. The area was rinsed with saline and dabbed with sterile gauze. The bone flap was
replaced and tied into place using 2-0 silk ties through small holes drilled in the ends of the
bone flap. The periosteum was closed with 6-0 polyamide suture and the skin closed to
watertight with 4-0 nylon. The animals recovered under observation and were monitored
periodically for CSF leakage by observation of the skin incision for the presence of clear fluid. The animals were given Buprenorphine at .02-.05 mg/kg every 12 hours as needed for pain. The skin sutures were removed at 2 weeks.

**Harvest Procedure**

At 4 weeks, the rabbits were sacrificed and CSF samples were taken to assess white cell counts. In 2 rabbits per group (2A and 2B, 4 rabbits total), the craniotomy was reopened, documented photographically and assessed for leakage around the implant and adhesion to the overlying bone and underlying cortex. In 2 rabbits per group (2A and 2B, 4 rabbits total), the craniotomy sites were fixed by cardiac infusion with 10% buffered formalin. Heads were removed and skinned, and lower jaws were removed. Heads were then fixed in 10% buffered formalin, examined by a Veterinary Pathologist, trimmed, and decalcified. Then block sections containing the defects were prepared. Specimens were processed, embedded in paraffin, sectioned and stained with Hematoxylin and Eosin (H&E) and Mallory’s trichrome. Slides were assessed histologically in a blinded manner by a veterinary pathologist.

**Analysis**

1. **Gross Examination of Rabbit Dura Substitutes for Strength and Adhesion**
   At 4 weeks, 4 rabbits (2A and 2B) were used for gross evaluation of the dura substitutes from 3 aspects: strength of dura substitute, adhesion to bone, and adhesion to cortex. Rabbits were sedated with Rabbit Mix, midline incisions were made over the scalp and the calvaria exposed using a periosteal elevator. The scar in the bone from the original surgery was located and a Dremel tool fitted with a 1mm burr was used to carefully drill out an oval section following the outline of the scar. With a small spatula, the bone flap was carefully lifted while assessing adhesion to the underlying tissues. The defects were cleaned with sterile saline and gauze. Once the craniotomies were reopened and photographed, dura substitutes were taken out and assessed for strength and adhesion to the cortex and bone. These assessments were scored qualitatively as follows. Adhesion to bone: 0-none, 1-barely adherent, 2-normal, 3-more adherent than normal, 4-very adherent. Adhesion to cortex: 0-no adherence to underlying cortex, 1-adherent to the cortex but separable without causing macroscopic injury, 2-adherent to the cortex causing tearing of cortical vessels on elevation, 3-adherent with tearing of the cerebral cortex on elevation of the bone flap. Strength of dura substitutes was assessed as: 1-no resistance to pulling, 2-low resistance to pulling, 3-high resistance to pulling, 4-very high resistance to pulling, 5-extremely high resistance to pulling (normal).

2. **Gross Histological Examination of Rabbit Dura Substitutes**
   At 4 weeks, 4 rabbits (2A and 2B) were sedated with Rabbit Mix and prepared for histological analysis. Buffered formalin (10%) was slowly administrated by cardiac infusion to immediately fix the craniectomy sites. Then the rabbit heads were removed, skin and muscle peeled, lower jaws removed, surgical sites photographed and the specimens submerged in 10% buffered formalin for 72h. These processed samples were delivered to the Veterinary Pathologist who sectioned the brain en bloc with the cranium, examined it grossly
for evidence of hemorrhage or infection, and made coronal cuts through the brain to assess ventricles for signs of hydrocephalus, subdural hemorrhage or infection.

3. Microscopic Examination of the Histology of the Implants
Following gross evaluation of the dura repair, sections (2cm x 2cm) including dura substitutes with overlying bone and underlying brain (0.5cm cortex) were blocked and stored in formalin. The tissues were then decalcified, processed, and embedded in paraffin so the plane of the sections was vertical in relation to the meninges and cortex. The samples were microtomed to make 5µ sections and stained in H&E and Mallory’s trichrome and then assessed. For the control, which was taken from the non-operated side of rabbit 3, scores for all variables were 0. Histology was assessed in a semiquantitative (0 = none, 1 = mild, 2 = moderate, 3 =severe) blinded fashion. The stained slides were evaluated in 7 categories: adhesion formation to underlying structures by observing the amount of connective tissue, anchorage to dura by examining tight junctions, resorption/replacement by host tissue by observing fibroblast invasion and reformation of dura, vascularization by quantifying the number of blood vessels/area, hemorrhage by counting the number of red cells not in blood vessels, infection by observing the presence of bacteria, and cellular response to dural substitutes by scoring inflammation: number of polymorphonuclear leukocytes (PMNs), extent of foreign body response: giant cells.

4. Examination of Cerebral Spinal Fluid (CSF)
At 4 weeks, 4 rabbits were sedated with Rabbit Mix and had CSF drawn from the cisterna magna to assess the white cell count.

Results

Gross Examination of Rabbit Dura Substitutes for Strength and Adhesion
Table 1 summarizes the results of gross evaluation of the dura substitutes. Compared to the virgin implants which both had strengths of 5 (maximum—could not be ripped apart) following 5 minutes soaking in saline, all dura substitutes were weak following implantation and could be easily ripped apart. Both implants (A and B) had maximum adhesion to bone (4). Only one implant (R2) grossly appeared to be adhered to the brain cortex.

Table 1: Gross Evaluation of Dura Substitutes

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Implant</th>
<th>Strength of Implant</th>
<th>Adhesion to Bone</th>
<th>Adhesion to Cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>B</td>
<td>2</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>R2</td>
<td>A</td>
<td>1-2</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>R5</td>
<td>A</td>
<td>1</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>R6</td>
<td>B</td>
<td>1-2</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 1 shows gross observation of dura repair at 4 weeks. At sacrifice, healing of the bone plugs to the bone defects was partial (one side) to complete (all sides). The implants covered the dural defects well but all adhered to the bone plugs so holes were left in the dura
substitutes when the bone plugs were removed (Figure 1A). Implant material could be seen on the underside of all bone plugs upon removal (Figure 1B). All rabbits except R6 showed some integration of the dura substitutes with the native dura at 4 weeks (Figure 1A). Implant A showed a yellow material lacking structure underlying the implant when it was removed for strength testing (Figure 1C). No CSF leakage was observed in any rabbit and there were no signs of infection.

Figure 1: Gross Observation of Dura Repair at 4 Weeks
1A. A hole in the dura substitute was caused when the overlying bone flap was removed. The dura substitute was somewhat anchored to the native dura. The black suture was used to attach the bone flap to the skull.
1B. Dura substitute adhered to the underside of the bone flap.
1C. Yellow material lacking structure was seen underlying the implant.

Gross Histological Examination of Rabbit Dura Substitutes
Table 2 and Figure 2 summarize the results of gross histological examination of rabbit dura substitutes by a Veterinary Pathologist. One rabbit (R3) displayed subcutaneous hemorrhage dorsal to the cranium which should not have affected the results. All 4 rabbits, with both implants A and B, displayed adhesions between the implant, cerebral cortex, and calvarium.
and mild to moderate focal encephalomacia (softening of the brain due to degenerative changes) subjacent to the implants (Figure 2B). Adhesions and encephalomacia were not seen on the contralateral side that was not implanted (Figure 2A). However, no rabbit displayed subdural hemorrhage, infection, or hydrocephalus (Table 2).

Table 2: Gross Examination of Implanted Dural Substitutes

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Implant</th>
<th>Subdural Hemorrhage</th>
<th>Infection</th>
<th>Hydrocephalus</th>
</tr>
</thead>
<tbody>
<tr>
<td>R3</td>
<td>A</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>R4</td>
<td>B</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>R7</td>
<td>A</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>R8</td>
<td>B</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 2: Coronal Sections of Skull with Inclusion of Dural Implant Site

2A. Section of rabbit cranium without implant (unoperated control side) Adhesions and encephalomacia were not seen.

2B. Section of rabbit cranium with dural implant. All 4 rabbits, with both implants A and B, displayed adhesions between the implant, cerebral cortex, and calvarium and mild to moderate focal encephalomacia subjacent to the implants.

**Histology of the Implants by Microscopic Examination**

Table 3 and Figure 3 summarize the microscopic examination of the histology of the implants. Histologically, adhesion of the implants to the brain cortex was scored 1.75 for implant A and 1.25 for implant B, which agrees with the observations by the Veterinary Pathologist. Anchorage of implants A and B to native dura was scored 1.5 histologically, which agrees with the gross adhesion findings. Resorption of the dura substitutes was 2 for A and 1 for B, showing a slight difference in the rate at which they were broken down in the body. Fibroblast invasion of the dura was 1 for both implants. Replacement of the dura substitute was minimal for implant A (0.5) and 0 for implant B. There was no vascularization of the implants, no hemorrhage observed aside from one subcutaneous hemorrhage observed grossly by the Veterinary Pathologist, and no evidence of infection in A or B. Inflammation
was scored 2.5 for implant A and 1.5 for implant B. Finally, there was no foreign body response observed against the implants.

Table 3 Histology of the Implants by Microscopic Examination

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Implant A</th>
<th>Implant B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R3C</td>
<td>R7</td>
<td>R3</td>
</tr>
<tr>
<td>1) Adhesion of dura substitute to brain (amount of connective tissue)</td>
<td>0</td>
<td>2</td>
<td>1.5</td>
</tr>
<tr>
<td>2) Anchorage of dura substitute to dura (0 = none, 3 = strong)</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>3) Resorption of dura substitute (0 = none, 3 = none remaining)</td>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>4) Fibroblast invasion of dura</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5) Replacement of dura substitute with host tissue</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>6) Vascularization (# of blood vessels/area)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7) Hemorrhage (# of blood cells not in vessels)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8) Infection (presence of bacteria)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9) Inflammation (# PMNs)</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>10) Foreign body response (# macrophages and giant cells)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

0 = none, 1 = mild, 2 = moderate, 3 = severe

After coronal sectioning of each skull with inclusion of implant sites, the tissues were placed in cassettes for further decalcification and tissue processing. All slides processed showed some degree of mechanical disruption of tissues, almost unavoidable in dissection of brain-cranium with implants. Figure 3A, representative of most slides, shows adhesion between the implant, cerebral cortex, and calvarium which could be assessed by observation of the mating threads of tissue on opposing surfaces. Figure 3B, representing all slides, shows chronic inflammation characterized by presence of lymphocytes, plasma cells and monocytes. In Figure 3C, host fibroblasts were seen on the collagen bundles of the implant material in all sections, but no “new” collagen was observed other than expected fibrosis around the periphery of the implant material.
Figure 3: Microscopic Examination of Histology of Dura Substitutes Implanted in Vivo

3A  Implant showing adhesion to brain (H&E). The adhesions between the implant and brain are minimally disrupted by the mechanical handling of tissues in preparation. The dark “specks” in the implant are inflammatory cells.

3B  Implant material with typical infiltration by inflammatory cells (H&E).

3C  Bone-periosteum-dura with suture, showing expected fibrosis, thickening/scarring, around the suture material (H&E).

Examination of Cerebral Spinal Fluid

It was extremely difficult to retrieve any CSF from the rabbits without contamination from blood. All specimens except two (R4 and R5) showed high white blood cell counts resulting from visible blood contamination. R4 had 613 cells/ microliter and R5 had 48 cells/ microliter. No conclusions about white blood cells (WBC) in the blood could be drawn from the limited data. The rabbits exhibited no signs of CSF infection.

Discussion

An ideal dura substitute should be able to: 1) prevent CSF leakage, 2) serve as a scaffold for the regrowth of native dura, and 3) be biocompatible with the host tissue. In addition, an ideal dura substitute should not adhere to the overlying bone flap or the underlying brain cortex, which could obstruct CSF flow and cause other problems. Overall, dural implants A and B appear ill-suited in their present forms for dura substitution for several reasons. Gross examination of the dura substitutes for strength showed that all implants had lost much of their original strength following implantation for 4 weeks and could be easily ripped apart. Microscopic examination of the resorption of the dura substitutes at 4 weeks showed that the implants ranged from somewhat to severely resorbed. Consequently, at 4 weeks, when the
native dura is not fully repaired, dura substitutes A and B might not be strong enough to prevent CSF leakage and protect the underlying brain. Second, both gross and microscopic examinations show that these dura substitutes were ill-suited to serve as scaffolds for replacement with native tissue. In microscopic examination of the histology of the implants, there was minimal anchorage of the dura substitute to the native dura, minimal fibroblast invasion of the dura substitutes, and minimal replacement of the dura substitutes with host tissue. Consequently, dura substitutes A and B may not ideally aid regrowth of native dura. Third, microscopic examination shows chronic inflammation characterized by the presence of lymphocytes, plasma cells, and monocytes. This pattern is most consistent with rejection of the implant material by the host. Thus, dura substitutes A and B are not biocompatible with the host. Fourth, both dura substitutes A and B resulted in maximal adhesion of the implants to the overlying bone and some exhibited adhesion to the underlying brain cortex. These adhesions may result in such severe consequences as obstruction of CSF flow and midline shift. In conclusion, dura implants A and B do not serve as ideal dura substitutes because they cause tissue rejection and abnormal adhesion to the brain and skull; and they do not promote rapid regeneration of and integration with native dura to close the defects.

References


