Comparative Analysis of Cerafix® Dura Substitute and DuraMatrix™ Collagen Dura Substitute Membrane in a Large Animal Model of Dural Repair

Matthew R. MacEwan, PhD¹; Tamas Kovacs, MS¹; Wilson Z. Ray, MD²

¹Acura Surgical, Inc. St. Louis, MO, 63132, USA.
²Department of Neurosurgery, Washington University School of Medicine, St. Louis, MO 63110, USA.

Email: macewan@acera-surgical.com

ABSTRACT: Background: Dura substitutes are commonly required to repair the dura mater during routine neurological procedures. Biologic materials composed of xenogenic collagen represent the most prevalent dura substitute, yet often incite undesirable tissue responses that impair wound healing. New materials are therefore needed to overcome the shortcomings of existing products and facilitate effective and reliable repair of native dura. The aim of the present study was to compare the performance of Cerafix® Dura Substitute, a novel non-biologic dura substitute, to DuraMatrix™ Collagen Matrix, a crosslinked bovine collagen dura substitute, as a means of facilitating dural repair.

Methods: The biocompatibility and efficacy of Cerafix® was compared to DuraMatrix™ in a rabbit duraplasty model. Bilateral dura defects were repaired with either material and secured with non-tension sutures. Animals were monitored post-operatively for neurological sequela and cerebrospinal fluid leak. Repair sites were explanted 4 weeks after implantation and evaluated by histopathology to assess neurulation, cortical adhesion, implant resorption, local inflammation, and tissue response.

Results: Both Cerafix® and DuraMatrix™ were effective in repairing dura defects and preventing cerebrospinal fluid leakage post-operatively. Histopathology revealed increased neurulation and reduced cortical adhesion in defects repaired with Cerafix® versus site repaired with DuraMatrix™. Histological analysis further demonstrated that DuraMatrix™ induced a greater inflammatory response than Cerafix®, with greater infiltration of inflammatory cells in DuraMatrix™ implants at the terminal timepoint. Furthermore, DuraMatrix™ induced a greater fibrous capsule thickness compared to Cerafix®.

Conclusions: Cerafix® and DuraMatrix™ demonstrated effective repair of induced dura defects and successfully prevented CSF leakage without infection or damage to underlying brain tissue. Cerafix® exhibited increased neurulation, reduced cortical adhesions, and progressive resorption compared to DuraMatrix™. Cerafix® further demonstrated less inflammation, irritation, and fibrosis than DuraMatrix™. Cerafix® thereby provides a unique non-biologic option in dural repair procedures, and offer reduced risk of inflammation and adhesions commonly associated with traditional xenogenic collagen products.

1. Introduction

Neurosurgical procedures commonly result in the perforation or removal of the watertight fibrous membrane surrounding the brain known as the dura mater. In all of these cases, the tissue barrier surrounding the brain must be repaired in a watertight manner in order to prevent damage to cortical tissues and leakage of cerebrospinal fluid. Dura substitutes are therefore needed to repair dural defects, reestablish the barrier that encloses the cerebrospinal space, and prevent cerebrospinal fluid (CSF) leakage and infection.

Numerous materials are currently in use as dura substitutes, including autograft, allograft, xenograft, and non-biologic synthetic materials. An ideal dura substitute should adequately restore the continuity of the dura mater and prevent CSF leak while minimizing infection. The mechanical properties of the material should facilitate suturing and/or tacking, yet also mimic the compliance of natural dura to allow ease of draping over delicate cortical tissues. Furthermore, an ideal dura substitute will minimize
local tissue inflammation and preferably encourage the infiltration of cells and vasculature to expedite the reconstruction of native dura without inducing undesired outcomes of fibrosis or cortical adhesions.

Autografts materials utilized in dura repair are commonly acquired from a patient’s own pericranium or facia latae. These tissues are desirable due to their minimal inflammatory response and their similarity to native dura. However, the use of these grafts is limited by the poor availability and host-site morbidity of the autograft material. Alternatively, human tissue is commonly utilized in the form of allografts, which are obtained from cadaveric dura (e.g. Lyodura™). This tissue can be collected, sterilized, and stored to provide greater availability of graft material to repair large dura defects. However, significant risk of disease transmission limits the use of allografts in contemporary neurosurgical settings. [1]

Xenograft materials are also commonly utilized as dura substitute products. Xenogenic materials are derived from bovine or porcine sources and are available in the form of decellularized tissues of the pericardium, small intestinal submucosa, and dermis (e.g. Lyoplant™, Tutopatch™, Dura-guard™, Durasis™, and Durepair™) or in the form of processed materials synthesized from collagen-rich sources such as the bovine Achilles tendon (ex. Duraform™, DuraMatrix™, and DuraGen™). Like allografts, xenografts have an inherent risk of zoonotic disease transmission and furthermore have the potential to incite allergic and inflammatory reactions. [2] Many biologic grafts have the advantage of being fully remodeled, whereby the natural components of the graft (e.g. collagen) recruit cell infiltration and angiogenesis that participate in the restructuring of the graft material. However, the rate at which a biologic graft is remodeled and resorbed is not well controlled, such that graft degradation can occur prematurely. This mismatch between graft resorption and native tissue regeneration can result in thin, weak tissue in the dura defect. [3] The mechanical properties of xenograft materials also vary greatly due to differences in material processing such as crosslinking and protein denaturation. [3] Select products have limited mechanical strength as to only be suitable for use as onlay grafts without the option of suturing (e.g. DuraGen™). Other xenograft materials provide the tear resistance and tensile strength required for suturing (e.g. Dura-Guard™, Durepair™, and DuraMatrix™).

DuraMatrix™, for example, is a commonly used dura substitute for dural defect repair. This bovine-derived collagen material is crosslinked to provide the mechanical strength necessary for suture repair of a dural defect. This manipulation of the mechanical properties causes undesirable effects in the handling of the material, leading to a dura substitute with decreased compliance. Furthermore, the crosslinking of DuraMatrix™ has been shown to interfere with the degradation expected of its biologic collagen composition, leading to prolonged presence at the implant site with poorly defined material resorption. [4] The effect of crosslinking to retard the resorption of xenograft collagen materials is likely two-fold: first, by preventing the migration of host cells into the material and second, by interfering with the mechanism of degradation for native collagen. [5] For biologically derived dura substitutes, desirable mechanical properties for suturability and desirable resorption properties for tissue remodeling are often mutually exclusive. [3]

Despite the range of existing dura substitute materials available in contemporary neurosurgical clinics, there remains a need for a dura substitute that offers improved handling characteristics, mechanical properties, and safety compared to biologically derived grafts. Non-biologic synthetic materials have been explored to overcome the limitations of biologic grafts, whereby material strength, resorption, and safety can be controlled with much greater precision. Preclude™ dura substitute, for example, is an expanded polytetrafluoroethylene film. This non-degradable graft can provide a long-term barrier to fulfill its desired function, but its permanent presence in the body often leads to fibrosis that may interfere with the proximal cortex and surrounding tissues. [6] Ethisorb™ is an alternative synthetic graft formed from a composite of a polylactic 910/polydioxanone fleece and polydioxanone film that is fully resorbable following neoduralization. [6] Despite these offerings, tissue response to synthetic grafts has yet to be optimized. Synthetic grafts also fall short in their approximation of the mechanical properties of the dura mater, such that these materials often have poor handling that complicates their clinical use. Based on the shortcomings
of the current clinically available materials, there remains a need for an improved resorbable non-biologic dura substitute that provides better handling and ease of use and improves the local tissue response during reconstruction of the native dura.

Cerafix® Dura Substitute is a novel non-biologic dura substitute designed to provide optimal strength, handling, and suturability, while reducing local inflammation to provide improved wound healing and dura regeneration (Fig. 1). Cerafix® is a non-woven material synthesized by electrospinning of biodegradable poly(lactic-co-glycolic acid)/polydioxanone, which creates an architecture that is reminiscent of native extracellular matrix. [7] This method of synthesis creates a material that is mechanically strong, while providing the look and feel of native dura. The architecture of this non-biologic graft furthermore supports tissue ingrowth and neoduralization with minimal inflammation. Cerafix® thereby provides a novel solution to dura repair, improving upon the performance of existing graft materials. The following study was designed to evaluate the performance of a commercially available xenogenic dura substitute product comparable to the Cerafix® Dura Substitute.

2. Materials and Methods

2.1. Study Design

Ten female New Zealand White rabbits (5.0-5.5 mo, Western Oregon Rabbit Company) were randomized into two groups (I,II) of five animals each (n=5). Group I served as the positive control as all animals underwent bilateral craniotomy and dural resection followed by bilateral surgical repair of the induced dural defects utilizing commercially-available DuraMatrix™ Collagen Dura Substitute Membrane (Stryker, Inc. Kalamazoo, MI). Group II served as an experimental group as all animals underwent bilateral craniotomy and dural resection followed by bilateral surgical repair of the induced dural defects utilizing fully resorbable non-biologic Cerafix® Dura Substitute (Acera Surgical, Inc. Saint Louis, MO). All animals underwent daily / weekly behavioral assessment and examination for signs of neurotoxicity, neurological sequelae, CSF leakage, and infection. Four weeks post-operatively all animals were euthanized and repair sites, including proximal skull and underlying cortical tissue, were explanted for histological and histopathological analysis. All animal procedures were performed in strict accordance with guidelines set by the Animal Welfare Act (AWA), the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), and Institutional Animal Care and Use Committee (IACUC) of the University of Utah.

2.2. Surgical Procedure: Bilateral Craniotomy

Prior to surgery, all animals were administered butorphanol, acepromazine, cefazolin, and dexamethasone, as well as a transdermal fentanyl patch for prophylactic analgesia. All animals were anesthetized via ketamine and diazepam, administered intravenously via catheterization of the marginal ear vein, and maintained through the duration of the surgery via isoflurane. The cranium was then aseptically prepared and sterilized from the frontal ridge to the occiput. All hair was removed and the surgical site was prepared with povidone iodine and isopropyl alcohol. A 6cm midline sagittal incision was then made extending through the scalp and the underlying periosteum. The periosteum was then elevated and
retracted. Bilateral bone flaps were then created on either side of the skull utilizing a high-speed neurosurgical drill fitted with a matchstick bit. Resulting bone flaps measuring approximately 10mm x 12mm were then elevated and removed exposing the underlying dura mater. The dura mater was incised bilaterally utilizing a micro-dissection blade and two circular dural defects each approximately 8mm x 10mm were created under microdissection.

2.3. Surgical Procedure: Dural Repair

Induced dural defects were subsequently repaired with either gold-standard xenogenic collagen matrix (DuraMatrix™) or fully resorbable non-biologic dura substitute material (Cerafix®) (Fig. 2). DuraMatrix™ Collagen Dura Substitute Membrane is a biologic, xenogenic graft material composed of type I collagen derived from bovine Achilles tendon. DuraMatrix™ collagen graft material is highly crosslinked resulting in a firm, woven, suturable implant material suitable for use in dural repair. DuraMatrix™ was provided sterile and stored at room temperature prior to use. Cerafix® Dura Substitute is a fully-resorbable non-biologic graft material composed of electrospun poly(lactic-co-glycolic acid) and polydioxanone. Cerafix® Dura Substitute possesses a unique non-woven architecture resulting in a compliant, flexible, and suturable implant material indicated for use in the repair of the dura mater. Cerafix® was provided sterile and stored at room temperature prior to use.

Prior to implantation, both DuraMatrix™ and Cerafix® graft materials were hydrated in sterile saline according to their respective instructions for use. Hydrated graft materials was then placed on the surgical field and trimmed to fit each dural defect. The size and shape of the graft material was selected to achieve at least a 2mm overlap with the adjacent dura mater around the circumference of the defect. Hydrated grafts were then draped onto the dural defect to maximize contact between the graft material and the underlying dura and promote watertight closure. Graft materials were then secured to the native dura utilizing four interrupted, non-tension sutures (7-0 PDS) spaced equidistant around the circumference of the defect. Graft materials were implanted such that each animal received either two DuraMatrix™ implants (n=5 animals) or two Cerafix® implants (n=5 animals). Following repair of induced dural defects, each surgical site was irrigated and closed in two layers (periosteum / muscle, skin). Excised bone flaps were not replaced during closure.

Following surgery all animals were recovered prior to reintroduction into the general housing facility. Butorphanol was administered as a post-surgical analgesic in addition to the fentanyl transdermal patch. Post-operatively all animals were observed daily and evaluated weekly for behavioral signs of neurotoxicity (posture, pupillary light reflex, limb placement, proprioception reflex, corneal reflex, gait), indications of CSF leakage, and change in body weight.

2.4. Tissue Harvesting / CSF Evaluation

All animals were humanely euthanized 4 weeks post-operatively. CSF was collected for physiochemical analysis by inserting a needle into the cisterna magna and aspirating 1-2ml of fluid which was then placed in cold-storage. Following CSF collection, the skull, brain, and implant sites were excised en bloc and fixed in neutral buffered formalin. Draining lymph nodes were similarly explanted and fixed in neutral buffered formalin. CSF fluid was sent Logan Regional Hospital (Logan,
UT) for physiochemical analysis. CSF was analyzed for cellular content (white blood cells, neutrophils, lymphocytes, monocytes/macrophages, eosinophils, basophils, lining cells, red blood cells) as well as glucose and protein levels.

2.5. Histological / Histopathological Analysis

Explanted skulls and peripheral lymph nodes were embedded in epoxy resin, blocked, and sectioned. Sections of the implant site (including neodural tissue and adjacent skull / brain) were stained with Luxol Fast Blue and Hematoxylin & Eosin (H&E) to visualize and evaluate the general health of neodural tissue, cortical tissue, and myelin. Sections of the implant site were also immunostained for Glial Fibrillary Acidic Protein (GFAP) to visualize local glial cells/astrocytes and evaluate the inflammatory response at the implant site. Sections of the lymph nodes were stained only with Hematoxylin & Eosin (H&E). Representative photomicrographs were then obtained utilizing light microscopy under a 40X optical objective and a Nanozoomer automated slide scanner provided by the Hope Center, Washington University School of Medicine.

The local tissue response to implanted dura substitute materials was quantified via microscopic scoring of neovascularization, vascularization of the tissue within the implant site, fibrosis, adhesions of the implant to the pia mater, and neoduralization. Fibrous capsule thickness (in µm) was averaged between three measurements in each implant site. If the presence of implant was not well defined, the thickness of fibrous tissue at the implant site was reported. Inflammation at the implant site was quantified by microscopically scoring the degree of infiltration of polymorphonuclear cells, lymphocytes, plasma cells, eosinophils, macrophages, and multinucleated giant cells into the implant field. Necrosis was scored as the severity of nuclear cellular debris from inflammatory cell death.

3. Results

3.1. Intraoperative / Postoperative Performance of Dura Substitute Materials

Both biologic and non-biologic dura substitute materials were successfully utilized to repair induced bilateral dural defects created in female New Zealand White rabbits. Intraoperative observations demonstrated that both commercially-available collagen-based DuraMatrix™ grafts and fully-resorbable synthetic Cerafix® grafts possessed suitable properties for effective dural repair. Upon surgical implantation Cerafix® implants were noted to be less thick and more flexible / compliant than DuraMatrix™ implants. Cerafix® materials were also observed to better conform to underlying native dura and were more easily sutured in place compared to DuraMatrix™.

Post-operatively all animals survived to the terminal time point and all animals exhibited normal behavior, neurological function and general health. Regular examination of the implant site confirmed that 0/10 implant sites containing DuraMatrix™ and 0/10 implant sites containing Cerafix® exhibited signs of CSF leakage or focal implant site infection during the course of the study. Post-mortem examination of the repair sites further confirmed the absence of CSF leaks and pseudomeningocele in all animals on study. Post-operative observation demonstrated that both Cerafix® and DuraMatrix™ were efficacious in repairing dural defects and preventing CSF leakage.

3.2. Analysis of Cerebrospinal Fluid / Sentinel Lymph Nodes

Cellular and physiochemical analysis of CSF collected from animals undergoing dural repair utilizing DuraMatrix™ and Cerafix® grafts was conducted in order to identify potential signs of neurotoxicity, inflammation, and/or infection resulting from implant materials. Complete blood counts and protein analysis conducted on collected CSF appeared normal in all animals implanted with both DuraMatrix™ and Cerafix® grafts. Negative findings in CSF analysis suggest that neither implant material induced neurotoxic or inflammatory responses in regional cortical tissue. Histological analysis of sentinel
lymph nodes was conducted in order to further examine the inflammatory and foreign body response to the dural substitute implants. Animals implanted with both DuraMatrix™ and Cerafix® materials exhibited normal appearing lymph nodes upon H&E staining suggesting no regional inflammatory or foreign body response to the grafts.

### 3.3. Histological / Histopathological Analysis of Implant Sites

Histological and histopathological analysis of surgical repair sites was conducted in order to qualitatively and quantitatively evaluate the efficacy of various dura substitute materials and the tissue / inflammatory response to the implanted grafts. Qualitative analysis of representative sections of defect sites repaired with either DuraMatrix™ or Cerafix® demonstrate significant differences in the efficacy of the implanted material (Fig. 3). Coronal sections obtained from defect sites repaired with collagen-based DuraMatrix™ demonstrated poor cellular infiltration and incorporation into the graft material. Sections further demonstrated frequent fibrous adhesions or connective tissue bridging the implanted DuraMatrix™ implants and the underlying cortical tissue. Qualitative observations further demonstrated frequent incomplete neoduralization across the cortical surface of the DuraMatrix™ grafts. In comparison, qualitative analysis of representative histological sections obtained from defect sites repaired with fully-resorbable Cerafix® material demonstrated increased cellular infiltration and lower incidence of fibrous cortical adhesions. Coronal sections further demonstrated more complete neoduralization across the cortical surface of Cerafix® grafts. Noted differences in tissue response to the implanted materials further related to the state of graft resorption at the time of explantation. At 4 weeks post-operatively, DuraMatrix™ implants demonstrated minimal cellular infiltration and resorption, while Cerafix® implants demonstrated marked cellular infiltration and resorption (Fig. 3).

Quantitative scoring of histologic sections provided additional comparison of the tissue level reaction to both dura substitute devices. Microscopic scoring of histopathological examinations of the implant site revealed significant differences in the inflammatory and tissue-level responses to Cerafix®, as compared

![Figure 3: Hematoxylin & Eosin-stained sections obtained from defects repaired with (A,C) Cerafix® Dura Substitute and (B,D) DuraMatrix™ Collagen Dura Substitute Membrane 4 weeks post-operatively.](image-url)
to DuraMatrix™ (Fig. 4). Cerafix® implants were observed to recruit a reduced number of inflammatory cells (e.g. monocytes and lymphocytes) compared to DuraMatrix™ grafts. Cerafix® materials also exhibited less fibrosis and lower fibrous capsule thicknesses compared to DuraMatrix™. Histopathological scoring of inflammation and tissue response further indicated that Cerafix® exhibited a lower inflammatory response, and was therefore classified as non-irritant, compared to DuraMatrix™.

Quantitative histopathological analysis also confirmed qualitative observation of graft performance in vivo. Microscopic scoring demonstrated robust neoduralization and modest neovascularization of Cerafix® implants in all animals (Fig. 4). Histopathological analysis also demonstrated lower levels of fibrosis and low rates of cortical adhesions associated with the Cerafix® implants. In contrast, quantitative histopathological analysis demonstrated significantly lower neoduralization and greater incidence of cortical adhesions associated with the DuraMatrix™ graft. Neovascularization was also evident in implant sites receiving DuraMatrix™, albeit at a reduced occurrence in comparison to those receiving Cerafix®. Quantitative analysis further confirmed observations of modest resorption of Cerafix® implants at 4 weeks post-operatively, largely as a result of phagocytosis of resorbable materials via infiltrating macrophages. Comparatively, minimal resorption or cellular infiltration of DuraMatrix™ was observed, coinciding with increased levels of fibrosis proximal to the implant.

Histopathological evaluation of underlying and proximal cortical tissue was further performed to examine the effect of the surgical procedure and the implanted graft material on local neural tissue. Local brain tissue appeared normal in H&E, LFB, and GFAP sections in the majority of animals implanted with Cerafix® and with DuraMatrix™. Mild astrocytosis, neuronal necrosis, and microgliosis were observed in four animals across both groups and were attributed to the surgical procedure rather than to either the Cerafix® or DuraMatrix™ materials.

4. Discussion

The present study offers a comparative analysis of a novel fully-resorbable non-biologic dura substitute (Cerafix® Dura Substitute) and a clinical gold-standard biologic dura substitute (DuraMatrix™ Collagen Dura Substitute Membrane) in a bilateral rabbit duraplasty model. Both materials demonstrated
effective repair of induced dural defects and prevention of CSF leakage without damaging proximal neural
tissue. This functional comparison demonstrates equivalent performance of the Cerafix® material with
gold-standard collagen-matrices widely used in contemporary neurosurgical clinics. Histopathological
analysis of the implant site 4 weeks post-operatively revealed, however, that the performance of Cerafix®
and DuraMatrix™ were not equivalent when considering local inflammatory and tissue-level responses
elicited at the site of implantation. Cerafix® exhibited distinct advantages in local tissue response including
reduced fibrosis / fibrous capsule formation and decreased cortical adhesions compared to DuraMatrix™
(Figures 3A-B). Furthermore, Cerafix® induced greater neoduralization than DuraMatrix™ at the implant
site, and in some cases Cerafix® supported complete neoduralization of the defect by the time of
explantation (Figure 3C).

The difference in tissue response to Cerafix® and DuraMatrix™ is likely influenced by differences
in the composition of the implants and, specifically, differences in the resorbable nature of the materials. DuraMatrix™
did not appear to undergo significant resorption 4 weeks after implantation, but rather was
associated with minimal cellular / tissue infiltration and significant fibrous capsule around the acellular,
crosslinked collagen material. Thus, although DuraMatrix™ is composed of biologically-derived animal-based collagen, the biological response to the implanted material is unlike what may be expected of native protein. DuraMatrix™, despite its biologic composition, exhibits an in vivo response significantly
divergent to that of native or fresh tissue.

Alternatively, the synthetic Cerafix® implant demonstrated modest resorption in parallel with
increased cellular infiltration of the material. Particularly, resorbing elements of the Cerafix® implant were
observed to be localized within macrophages that had infiltrated the implant site. This observation confirms
the resorbable and transient nature of the Cerafix® material. The synthetic electrospun material utilized in
the construction of the Cerafix® graft provided an environment in which cells could migrate and which
could be broken down to allow subsequent remodeling of the tissue. Fully-resorbable constructs such as
the Cerafix® implant may possess multiple advantages over long-term or permanent implants in that the
material serves as an acute barrier and scaffold for new tissue formation yet resorbs following tissue
regeneration precluding undue chronic reactions to the implanted material. Furthermore, the lack of animal-derived, xenogenic, or allogenic constituents may effectively reduce the incidence of allergic or
inflammatory responses to the implanted dura substitute material commonly associated with existing
biologic graft materials.

The lack of resorption of the implanted DuraMatrix™ graft is likely an effect of the post-processing
utilized in the construction of the biologic material. The crosslinking of bovine collagen required to provide
the mechanical strength necessary for intraoperative use and suturability simultaneously affects the biologic
and structural elements of the material. As demonstrated in this study, fully-resorbable synthetic dura
substitutes can provide adequate mechanical strength for suturability, optimal handling and compliance, as
well as reliable resorption that encourages tissue remodeling in the form of neoduralization. Cerafix® is
unique, however, in that the non-biologic dura substitute also exhibits reduced inflammation, decreased
fibrosis, and fewer adhesions to the pia mater than gold-standard biologic dura substitutes presently in use
in neurosurgical clinics. The non-woven architecture, created by electrospinning, may be attributed with an
improved tissue response, as compared to alternative synthetic dura substitutes. Furthermore, this
 mechanism of synthesis provides a material with superior handling and drapability as compared to
alternatives with reduced compliance. Cerafix® thereby offer a unique and attractive option in dural repair
procedures that provides ease of handling, efficacy, and biocompatibility, ultimately leading to improved
dural repair.

5. Conclusions

Cerafix® is a fully-resorbable, non-biologic dura substitute that offers a unique combination of
mechanical strength for suturability and compliance for ease of handling. The non-woven architecture of
Cerafix® permits cellular infiltration and supports full resorption of the implant material while encouraging
regeneration of native dura. Cerafix® effectively closed dura defects equivalent to a gold-standard biologic
dura substitute (DuraMatrix™) and induced a superior local tissue response characterized by decreased inflammation and increased neoduralization. Cerafix® thereby offers significant advantages over existing dura substitutes that may lead to improved clinical outcomes in multiple neurosurgical settings.

6. References


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