Pre-Clinical Evaluation of Collagen Dura Substitutes in a Rabbit Duraplasty Model: DuraMatrix® Suturable

ABSTRACT

Rationale: The purpose of this study was to evaluate whether a bovine dermis based membrane (DuraMatrix® Suturable, Collagen Matrix Inc.) is substantially equivalent to a currently marketed dura substitute control device (Durepair®, TEI Biosciences) using a rabbit duraplasty model.

Methods: DuraMatrix® Suturable and Durepair® were implanted according to an established protocol using standard surgical methods. At pre-determined time points (6 and 12 weeks), animals were euthanized, surgical sites and explanted implants were analyzed macroscopically. Tissue specimens were prepared for histological evaluation according to established grading systems. The adhesion of dura implants to the cortex of the brain was evaluated only at the 12 week time point. The resorption and new tissue replacement of the dura implants were analyzed at the 6 and 12 week time points.

Results: All animals survived the procedures, displayed appropriate weight gain, and were healthy over the course of the study. The following criteria were compared: cerebral spinal fluid (CSF) leakage, local tissue response, integration of the implanted sample to the dura, adhesion formation to surrounding tissue, hemorrhage, changes to the cortex, signs of infection, cellular changes within the cortex, implant resorption and new collagen deposition. Substantial equivalence was observed from DuraMatrix® Suturable compared to Durepair® according to the parameters studied.

Conclusion: DuraMatrix® Suturable was comparable to Durepair® as a collagen based dura substitute membrane based on clinical, gross morphological, and histological data at 6 and 12 week time points after implantation in a rabbit duraplasty model. Both DuraMatrix® Suturable and Durepair® are biocompatible without significant tissue reaction. Overall, it can be concluded that DuraMatrix® Suturable is substantially equivalent to Durepair® as collagen dura substitute membrane in duraplasty procedures.

INTRODUCTION

The dura mater functions to contain cerebrospinal fluid (CSF) which provides a mechanical barrier to protect and interact with the central nervous system. In order to minimize post-operative complications, the neurosurgeon requires a water-tight seal for the primary closure of procedures related to the skull base and spine. While autologous tissue transplantation or the suturing of the native dura were once considered the standard of care, the use of collagen and synthetic materials has become the preferred methodology due to better patient outcomes and ease of use for the clinician. [1-6] The dura is composed primarily of collagen. Many of the current dura replacement options are harvested from allogeneic tissues sources[7], xenogeneic tissue sources[8-10], or synthetic polymer sheets[11-12]. However, the most commonly used dural substitutes are collagen based due to the benefits of the overall biocompatibility, the architectural similarity to the native structure, and the mechanical functionality to withstand the forces exerted in the neurosurgical setting. [13-17]

DuraMatrix® Suturable is a substitute dura membrane made of highly purified bovine dermis collagen. The indicated use of DuraMatrix® Suturable is as a graft material for the repair of dura mater. The conformable implant's ease of handling allows it to be shaped to the contours of the brain. It also possesses high mechanical strength to avoid suture pull-out during surgery. DuraMatrix® Suturable has a similar thickness to the native dura, which facilitates healing by balancing the *in vivo* resorption of the implant and the regeneration of host tissue. [18]

METHODS

Animal Model

Twenty-six male and female New Zealand white rabbits were used in this study. A single 10 x 10 mm craniotomy defect was created over the midline of the skull of each rabbit and stored in sterile saline. An 8 x 8 mm defect was then created in the dura. DuraMatrix® Suturable or Durepair® was sutured into each defect according to the study protocol. The bone flap was then placed, and the surgical site was sutured closed with non-resorbable suture. Animals were observed daily for general health. and for CSF leakage over the first 14 days.

At the end of each pre-determined time point (6 and 12 weeks), animals were anesthetized, CSF collected by needle insertion into the cisterna magna to assess white blood cell counts, and euthanized. The

surgical sites were explanted intact for histological analysis. The samples were fixed in 10% neutral buffered formalin, stained according to standardized techniques, and analyzed by an independent pathologist via light microscopy. At the 12 week time point, a sub-group of the animals (n = 4 for each treatment group) had the defect sites exposed, the skull was elevated, and adhesion formation was scored according to an established scoring system as outlined in Table 1.

Table 1: Adhesion Scores

Score	Description
0	No adhesion to underlying cortex
1	Adherent to cortex, but separable without causing macroscopic injury
2	Adherent to cortex causing tearing of cortical vessels on elevation
3	Adherent with tearing of the cerebral cortex on elevation of the bone flap

Histology

H&E stained tissue sections were evaluated for local tissue response, hemorrhage, cellular changes within the cortex and signs of infection following the scoring criteria described in the International Organization for Standardization (ISO) 10993, Part 6 (0=normal, 1=minimal, 2=mild, 3=moderate, and 4=severe).

Local Tissue Response

Microscopic analysis included the following: evaluation of the inflammatory response; changes outside the brain; changes associated with the surrounding implant material (infiltration with lymphocytes, monocytes, macrophages, plasma cells, some multinucleated cells and vascularization). Within the cortex, inflammation and other changes underlying the implant were analyzed in the context of neutrophils, gliosis, astrocytosis, edema, hemosiderosis, and macrophage increase. Hemorrhage was defined as the presence of free extravascular blood within or surrounding the implant or within the underlying neurophil.

Interpretation of histological scoring for local tissue response, hemorrhage, vascularization, and neutrophil response were added together to determine a total cellular score for each specimen. The scores were averaged based on the total number of specimens for each group and compared according to Table 2.

Table 2: Histological Scores

Score	Description
0.0 up to 2.9	Non-irritant
3.0 up to 8.9	Slight irritant
9.0 up to 15.0	Moderate irritant
>15	Severe irritant

GFAP Scoring

Slides stained via immunohistochemistry (IHC) were evaluated for the amount of Glial Fibrillary Acidic Protein (GFAP) associated with each implant. GFAP staining demonstrates the presence of astrocytes, which are cerebral and spinal cells that support the repair of brain and spinal function following injury. The incidence and severity of the lesions were scored following the scoring criteria: 0=normal, 1=minimal, 2=mild, 3=moderate, and 4=severe as described in the International Organization for standardization (ISO) 10993, Part 6. The scores were averaged based on the total number of specimens for each group.

Implant Resorption and New Collagen Deposition

Stained tissue sections (H&E and Masson's Trichrome) were evaluated for implant resorption and new collagen deposition. Samples were semi-quantitatively scored according to Table 3.

Table 3. Grading Scores for Implant Resorption and New Collagen Deposition

Score	Resorption: % of Implant Material Present	New Collagen: % Present
0	None	None
1	<10	<10
2	11-25	11-25
3	26-50	26-50
4	51-75	51-75
5	76-100	76-100

RESULTS

Clinical Observations

None of the animals had signs of CSF leakage after duraplasty with either implant material. Three animals (one DuraMatrix® Suturable and two Durepair®) were diagnosed with a lack of appetite during the study. This was not related to the implant material and was likely due to the surgical

procedure. All the rabbits survived the procedure and gained weight over 12 weeks.

Gross Observations

All animals appeared macroscopically normal at necropsy. Three animals (one DuraMatrix® Suturable and two Durepair®) were noted during necropsy as having hematomas near the operative site. One of the two animals with Durepair® did have extensive bleeding at surgery which likely contributed to the finding.

At the 12 week time point, minor adhesions were observed for two out of the four animals in the DuraMatrix® Suturable group and one out of four for the Durepair® group. The adhesions for both groups were categorized with a score of 1 (see Table 1 for description).

Microscopic/Histological Analysis

Six Weeks

The histological findings at six weeks were very similar for both implants. Each implant appeared to be well adhered to both the skull and the meningeal surface. There was some mild to moderate multifocal chronic inflammation associated with all samples. The inflammation was composed of multifocal accumulations of lymphocytes and monocytes, primarily macrophages. There was some light fibrosis and a limited amount of neovascularization. The underlying brain was intact, with minimal mononuclear inflammation, vascular congestion and increased prominence of capillaries, hemosiderin, and increases in astrocytes, indicative of tissue response. There was no sign of infection and no sign of significant injury to the neurophil. Table 4 summarizes the histopathology scoring for the 6 week time point for both groups.

At 6 weeks, the mean score for the total cellular response for DuraMatrix® Suturable was 6.2 ± 0.4 and 6.6 ± 0.5 for Durepair®. Both implants are categorized to be slight irritants at the 6 week time point. Overall, these observations at six weeks would not be classified as significant abnormal microscopic findings. Representative histological micrographs for DuraMatrix® Suturable and Durepair® at 6 weeks are shown in Figures 1-4 and 5-8, respectively.

Twelve Weeks

For both implants, the histological findings at twelve weeks were less severe than those observed at six weeks; indicating a tolerance of both implants and continued repair and healing. Table 5 summarizes the scoring for the 12 week time point.

At 12 weeks, the mean score for the cellular response for DuraMatrix® Suturable was 2.8 ± 2.0 and 4.5 ± 2.1 for Durepair®. DuraMatrix® Suturable was reduced to a non-irritant at 12 weeks while the Durepair® remained categorized as a slight irritant. Representative histological micrographs for DuraMatrix® Suturable and Durepair® at 12 weeks are shown in Figures 9-12 and 13-16, respectively.

Table 4: 6 Week Histopathology Score (Average of n=5)

Group	Local Tissue Response	Vascularization	Hemorrhage	Neurophil Cellular Response	Total Cellular Response Score
DuraMatrix® Suturable	2	1	1	2.2	6.2± 0.4
Durepair®	2.4	1	1	2.2	6.6 ± 0.5

Table 5: 12 Week Histopathology Score (Average of n=8)

Group	Local Tissue Response	Vascularization	Hemorrhage	Neurophil Cellular Response	Total Cellular Response Score
DuraMatrix® Suturable	1.125	0.5	0.125	0.75	2.8 ± 1.9
Durepair®	1.75	0.75	0.5	1.5	4.5 ± 2.1

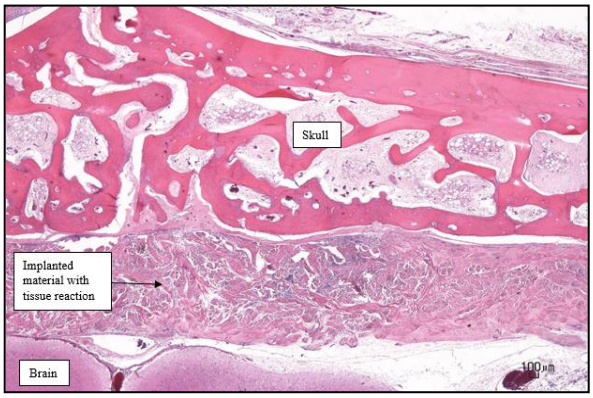

Figure 1: 6 week DuraMatrix® Suturable (20x, H&E stain)

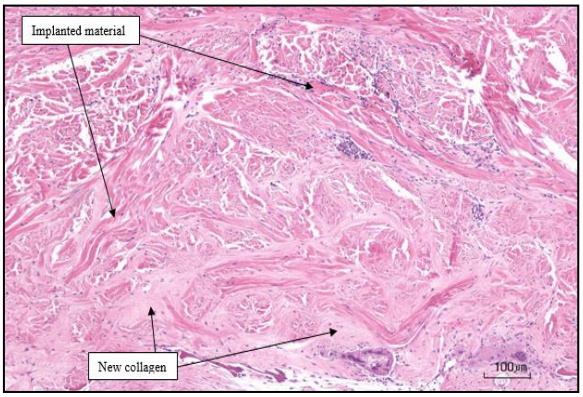

Figure 2: 6 week DuraMatrix® Suturable (100x, H&E Stain)

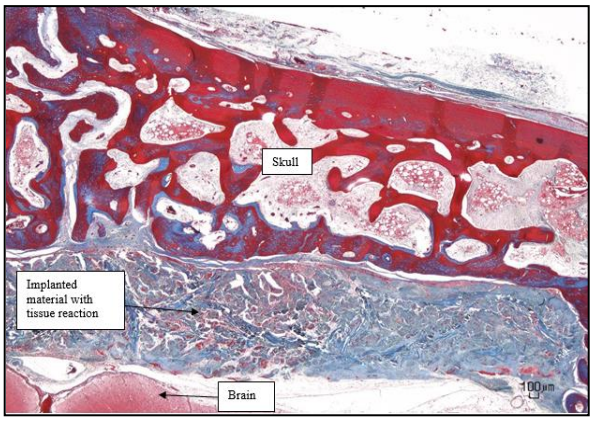

Figure 3: 6 week DuraMatrix® Suturable (20x, Trichrome)

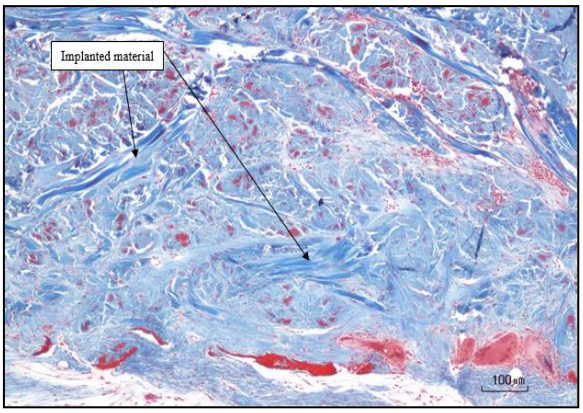

Figure 4: 6 week DuraMatrix® Suturable (100x, Trichrome)

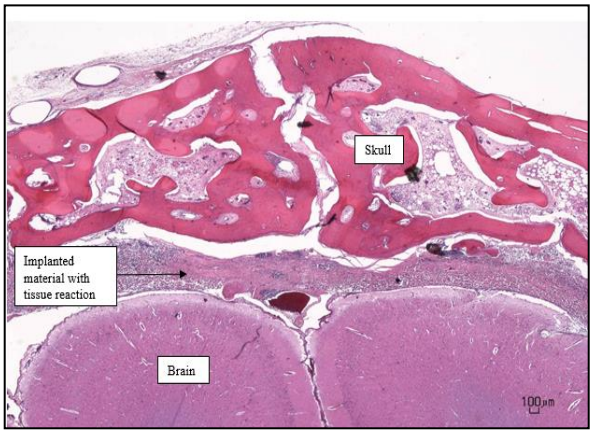

Figure 5: 6 week Durepair® (20x, H&E stain)

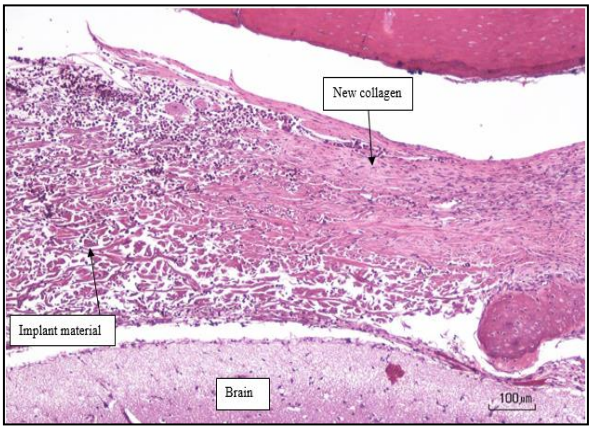

Figure 6: 6 week Durepair® (100x , H&E stain)

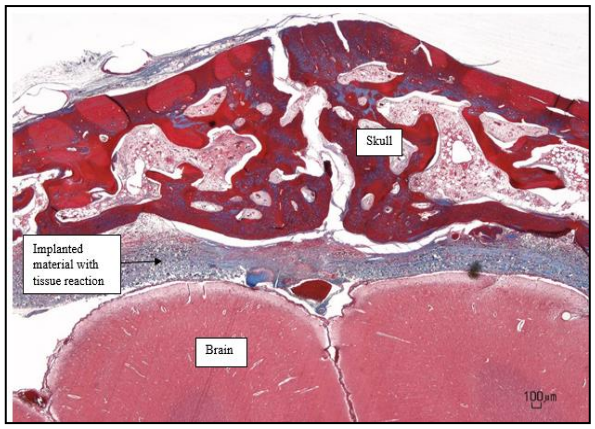

Figure 7: 6 week Durepair® (20x, Trichrome)

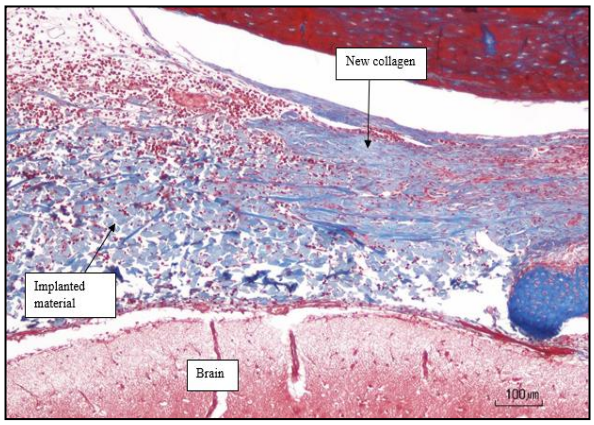

Figure 8: 6 week Durepair® Suturable (100x, Trichrome)

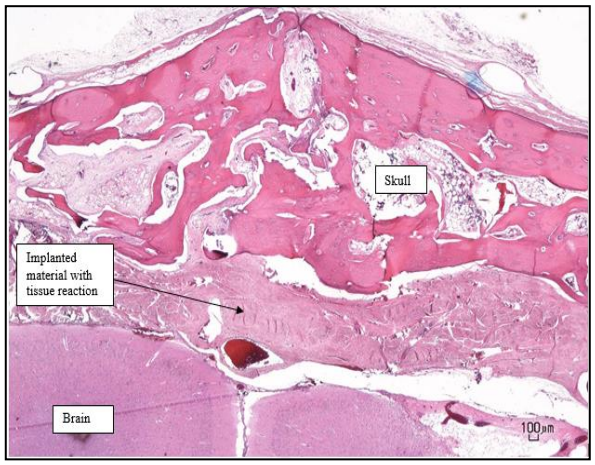

Figure 9: 12 week DuraMatrix® Suturable (20x, H&E)

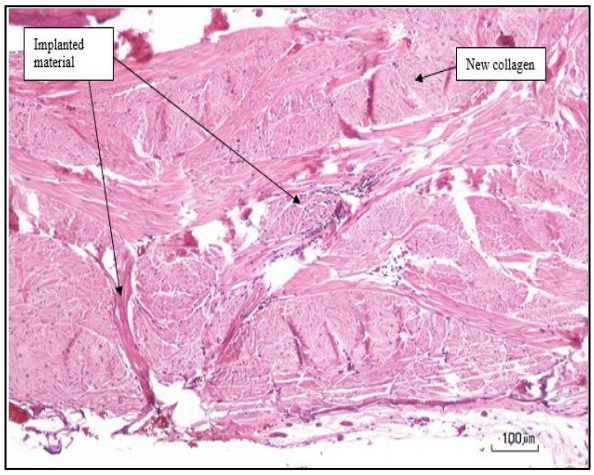

Figure 10: 12 week DuraMatrix® Suturable (100x, H&E)

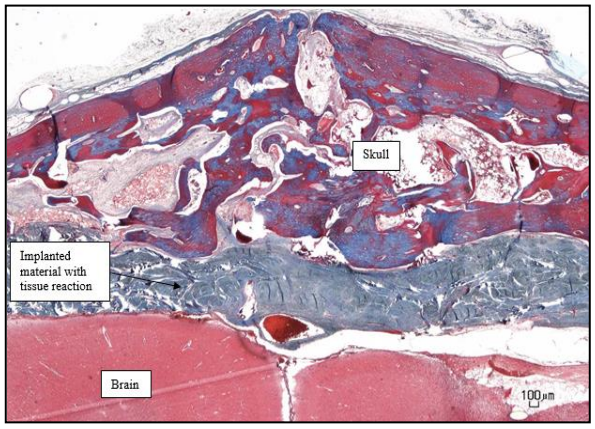

Figure 11: 12 week DuraMatrix® Suturable (20x Trichrome)

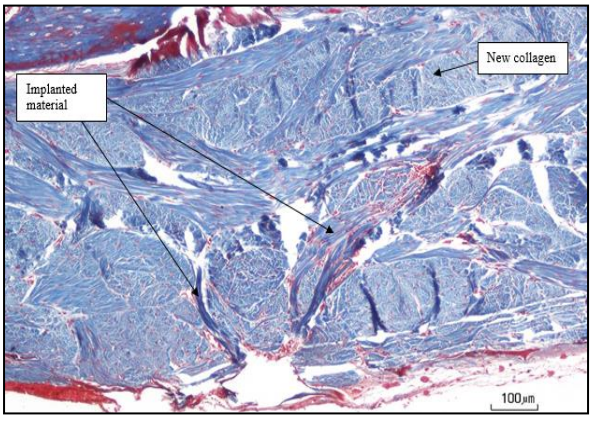

Figure 12: 12 week DuraMatrix® Suturable (100x Trichrome)

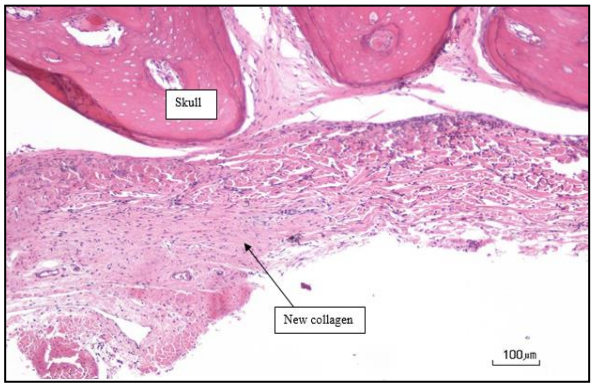

Figure 13: 12 week Durepair® (20x H&E)

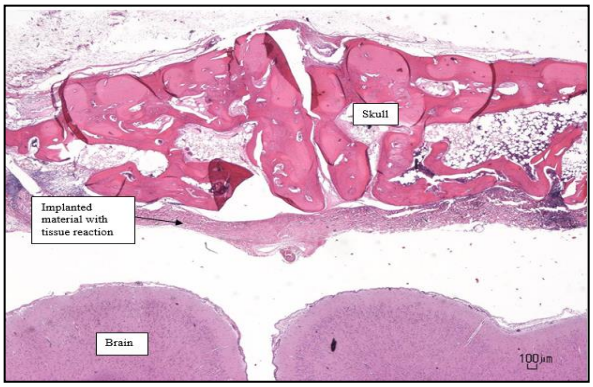

Figure 14: 12 week Durepair® (100x H&E)

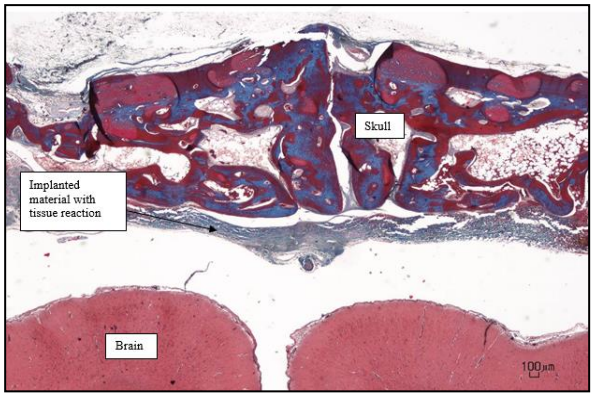

Figure 15: 12 week Durepair® (20x Trichrome)

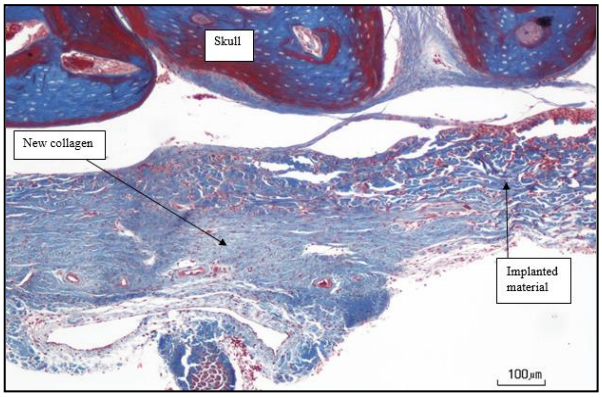

Figure 16: 12 week Durepair® (100x Trichrome)

GFAP – Stained Tissues

For both implant groups, GFAP staining demonstrated the presence of astrocytes in brain tissue at 6 and 12 weeks. Table 6 shows the Mean ± STD based on the total number of specimens for each group at each time point. There were increases in astrocytes subjacent to the dural implant sites indicative of mild inflammation, tissue repair, and reaction to the implants, which was resolving over time. However, astrocyte proliferation was consistentbetween groups and there did not appear to be any significant or irreversible damage to the neural tissue underlying the implants.

Table 6: GFAP Scores (Mean and SD *n=8, **n=12)

	GFAP Scores	
	6 weeks*	12 weeks**
DuraMatrix® Suturable	3.4 ± 0.9	2.9 ± 0.9
Durepair®	3.0 ± 1.0	1.9 ± 0.7

Implant Resorption and New Collagen Deposition

The resorption of the implants and the deposition of new collagen over time were semi-quantitatively scored according to the scoring system as shown in Table 3.

Figures 17 and 18 show the results of percent implant remaining and new collagen deposition, respectively, over the duration of the study. It can be seen at the 6 week time point there were larger amounts of the DuraMatrix® Suturable remaining and accompanied with a larger amount of new collagen deposition than the Durepair®. At 12 weeks, it was observed that there was approximately 26-50% of DuraMatrix® Suturable remaining at the defect site. The resorption of DuraMatrix® Suturable was accompanied by 26-50% of new collagen infiltrated into the implant and surrounding host tissue. At 12 weeks, there was only 11-25% implant remaining at the defect site for Durepair®. This was accompanied by 26-50% new collagen infiltrated into the Durepair® and the surrounding tissue, similar to the DuraMatrix® Suturable.

As a first order approximation, the total resorption time, defined as ≤ 5% implant remaining, is approximately 38-40 weeks for DuraMatrix® Suturable and approximately 20 weeks for the Durepair® (Figure 17). Resorption of the implants was accompanied by new collagen deposition (Figure 18).

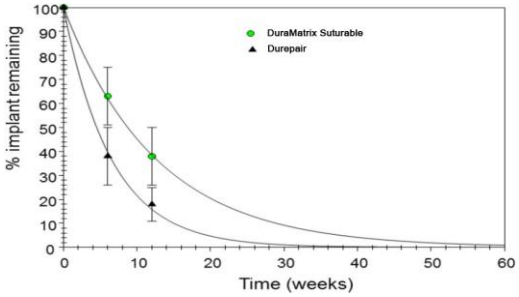

Figure 17: Percent Implant Remaining

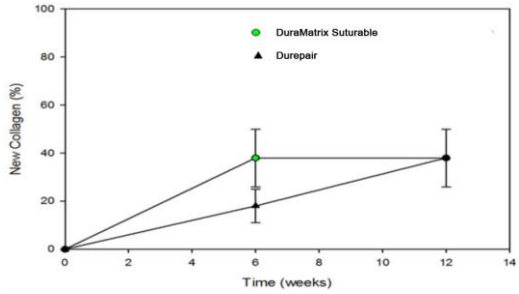

Figure 18: Percent New Collagen Deposition

CONCLUSION

This study demonstrated the safety and efficacy of DuraMatrix® Suturable as a collagen dura substitute membrane in a rabbit duraplasty model compared to a current commercially available dura repair implant, Durepair®.

Efficacy was demonstrated macroscopically and histologically at 6 and 12 weeks. All the rabbits survived the procedure and gained weight over the 12 weeks after the duraplasty procedure. The application of both implants appeared to prevent CSF leakage in all animals.

Microscopically, both the DuraMatrix® Suturable and Durepair® were present at 6 and 12 weeks. Cellular responses to the implants were assessed histologically at these time points and determined that all of the groups had similar inflammatory response, indicative of normal tissue repair response. There were no safety issues with regards to inflammatory response or negative cellular changes at the implant site. Both DuraMatrix® Suturable and Durepair® were resorbed over time and aided in new collagen deposition.

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18. Lab Reports on file with Collagen Matrix, Inc.

This study was sponsored by and directed by Collagen Matrix, Inc.

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