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Research Article

Comparison of The Histopathologic Outcome of Three Different Allograft Used For The Repair of Spinal Dural Defect in Rats

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Summary

Purpose: Repairing of the duramater is one of the major factor that effects the mortality and morbidity of patients after neurosurgical approaches. The gold standard for repairing of duramater is watertight suture or duraplasty with autografts such as pericranium and/or temporal fascia. Sometimes edges of the dura mater generally are shrunken and the watertight suture of the dura becomes impossible especially in emergency conditions. In the present study, we aimed to determine the most effective artificial dural graft in experimental dural defect in rats.

Materials and Methods: Twenty eights wistar albino rats weight ranging from 280-320 grams and equal numbers of male and female were used. The animals were divided into four groups. Control (n=7 Group-1), collagen matrix graft (n=7 Group-2), cellulose graft (n=7 Group-3) and teflon graft (n=7 Group-4). Rats were sacrificed after 30 days and their damaged dura were removed and sections were taken. All histological preparations examined using light microscope. Histological analysis focused on fibroblastic activation, new capillary formation, inflammatory reaction, foreign body reaction and capsule formation and results were compared.

Results: While fibroblastic activation was observed most frequently in teflon graft group, new capillary formation, inflammatory reactions and capsule formation were most frequently seen in cellulose grafts group.

Conclusion: This animal model for artificial dural grafts suggest that cellulose was the most effective dural substitute for repairing of defective dura.

Key words: Capsule formation, Cellulose, Collagen matrix, Fibroblastic activation, Inflammatory reaction, New capillary formation, Teflon

Ratlarda Spinal Dura Defekt Tamirinde Kullanılan Üç Farklı Allogreftin Histopatolojik Sonuçlarının Karşılaştırılması

Özet

Amaç: Duramater tamiri nöroşirürjikal yaklaşımlardan sonra mortalite ve morbiditeyi etkileyen en önemli faktörlerden biridir. Altın standart duramaterin, su geçirmez şekilde sütüre edilmesi veya perikranium, temporal fasya gibi otogreftlerle duraplasti yapılmasıdır. Bu işlem özellikle acil durumlarda dura kenarlarının kısalması nedeniyle mümkün

olmayabilir. Çalışmamızda ratlarda deneysel dura defekti yaparak en etkili yapay dura greftini belirlemeyi amaçladık.
Materyal ve Metod: Ağırlıkları 280-320 gr arasında değişen, eşit sayıda erkek ve dişi toplam 28 adet wistar albino cinsi rat kullanıldı. Hayvanlar, kontrol (n=7 Grup-1), kollojen matriks greft (n=7 Grup-2), selülöz greft (n=7 Grup-3) ve teflon greft (n=7 Grup-4) olmak üzere 4 gruba ayrıldı. Ratlar 30 gün sonra kesilip hasarlı dura mater kısımları çıkarıldı ve kesitleri alındı. Tüm histopatolojik kesitler ışık mikroskobu kullanılarak yapıldı. Histolojik çalışmada fibroblastik aktivite, yeni kapiller oluşumu, inflamatuar reaksiyon, yabancı cisim reaksiyonu ve kapsül formasyonu değerlendirilip sonuçlar karşılaştırıldı.
Sonuç: Fibroblastik aktivite en fazla teflon grubunda görülürken yeni kapiller oluşumu, inflamatuar reaksiyon ve kapsül oluşumu en fazla sellüloz grubunda görüldü. Karar: Bu deneysel model, selülözün hasarlı duramater tamirinde en etkili yapay dura grefti olduğunu göstermiştir.

Anahtar Kelimeler: Kapsül formasyonu, Selülöz, Kollojen matriks, Fibroblastik aktivite, İnlamatuar reaksiyon, Yeni kapiller oluşum, Teflon

INTRODUCTION

In surgical operations such as, spinal tumor, trauma meningocele, myelomeningocele, lipoma, and tethered cord dura may be opened as intentional duratomies or as incidental tears and finally, irreparable dural defects may occur and can lead to a cerebrospinal fluid (CSF) fistula which arises due to an improper closure of the dura. Continuous CSF leakage increases the risk of infections at rates reported to be as high as 10-50%[21]. Thus, the watertight closure of the dura during operation is very important for minimizing the risk of CSF fistula and infection. However, watertight dural closure is not always possible and autologous and/or cadaveric grafts might be used. Not only autologous and/or cadaveric grafts also some experimental animal studies reported successful results of nonhuman-originated artificial dural substitutes for covering of dural defects. Ideally, an artificial graft should not trigger a foreign body reaction. It must increase the number of new capillary networks with migration of fibroblasts, also capsule formation should be considered in treated area. In the present study, three different artificial dural grafts were used to find the most effective graft hat can be used as a physiological barrier.

MATERIAL AND METHODS

This study was conducted at the Ege Universtiy Faculty of Medicine after approval by the Ethics Committee (18.02.2010 and 20.03.2010). The number of animals was limited by the Ethics Committee. Pathological specimens were obtained from 28 rats weighing 280-320 g and investigated by a neuropathologist. All animals were housed in cages at a temperature of 21-22°C under a daily 12/12-h light/dark cycle. All animals were allowed free access to food and water during the research. They were examined by a microbiologist daily for possible sign of surgical or systemic infection.

All animals were anaesthetized with Ketamine Hydrochloride (35-50 mg/kg/ intraperitoneally) and Xylene (5-10 mg/kg intraperitoneally). Rats were fixed in supine position and cefazolin sodium was applied subcutaneously for antibiotic prophylaxis. Using routine aseptic conditions 3 cm midline skin incision was made to allow access to the lower thoracic to midlumbar vertebrae. Laminectomy in 2 cm was performed by using rongeur and high-speed drill. Midline duratomy was performed on each rat by using microscissors. The rats were randomized to three different dural groups and a control group. In Group 1 (n = 7), no additional

process was done. In Group 2 (n = 7), artificial dural grafts, including a collagen matrix (DuraGen-Integra US), were placed under the dural margins. In Group 3 (n = 7), the same procedures were performed by using cellulose fiber dural graft (DuRepair, Medtronic US), and Group 4 (n = 7) included polytetrafluoroethylene (teflon) (Dural substituted-GoreTex US). During the study, 1 rat from each group was excluded due to wound infections and abscesses within the skin and lamina and study was completed by adding new one rat to each group. All animals were maintained in housing for 1 month. At the end of first month, rats were sacrificed and the duraplasty region with surrounding healthy dura was excised. Tissue samples were passed through the tissue tracking device using formaldehyde for fixation and then decontaminated by submersion for 6 hours. The samples were dehydrated for 1 hour using 50% ethylalcohol and then placed in xylol for 2 hours until they became transparent. As a last step, tissues were paraffinized and sectioned at a thickness of 5 µm using a microtome. Microscopic examinations were performed under an Olympus microscope using 100× magnification and hematoxylin and eosin staining. In each group five parameters were studied. 1-) fibroblastic activity, 2-) inflammatory reaction (inflammatory cell density), 3-) capsule formation, 4-) foreign body reaction and 5-) capillary network production. Fibroblastic activity is predicated on the density of fibroblasts and blocking CSF leakage is a positive factor. Inflammatory cell reactions trigger fibroblastic tissue reactions and increasing adhesion is a positive factor. Capsule formation gives rise to a blockage of CSF transmission by coating the duraplasty surface and is a positive factor and new capillary network formation increases blood flow to the graft tissue and quickens the healing process with an increase of migration of inflammatory cells and fibroblasts to the environment, which is also a positive factor. On the other hand the foreign body reaction is the only negative factor in this study.

Each parameter was scored qualitatively as follows:

- (-) None
- - (+) Focal and minimal
- - (++) Diffuse and moderate
- - (+++) Diffuse and marked

Statistical analyses were conducted by the Biostatistics Faculty of Ege University using the SPSS software (ver. 14 for Windows). Datas were compared and statistically analyzed by using Kruskal Wallis test. A p value of <0.05 was accepted as statistically significant.

RESULTS

In all groups histopathological preperations were stained with hematoxylin-eosin and examined under light microscopy (Figure-1).

Groups and histopathological parameters are shown in Table 1.

Three groups with dural substitutes were first compared with the control group then compared in each other. On the basis of the fibroblastic reaction, no activity was found in the control group whereas activity was high in cellulose and teflon groups (Figure-2).

The fibroblastic activity was greatest in teflon group. The values were compared using Kruskal-Wallis test and there were statistically significant differences between control group and collagen- cellulose-teflon groups. (P values were p=0,007, p=0,001 and p=0,003 respectively). Also there were statistically significant differences between collagen group and cellulose-teflon groups. (P values were p=0,015 and p=0,031). On the other hand there was no statistically significant differences between cellulose and teflon groups.

Comparison of inflammatory reaction and new capillary production among all groups are shown in Figure-3 and Figure-4.

The inflammatory reaction was greatest in cellulose group. The values were compared using Kruskal-Wallis test and there were statistically significant differences between control and cellulose-teflon groups (P values were p=0,005 and p=0,032 respectively). Also there was statistically significant differences between collagen and cellulose groups (p=0,01).

New capillary production were similar in all cases and there were no statistically significant differences between all groups.

Capsule formation was not seen in the collagen group. It was found in five samples in the cellulose group and in three samples in the teflon group (Figure-5). Also in all groups we did not determine foreign body reaction.

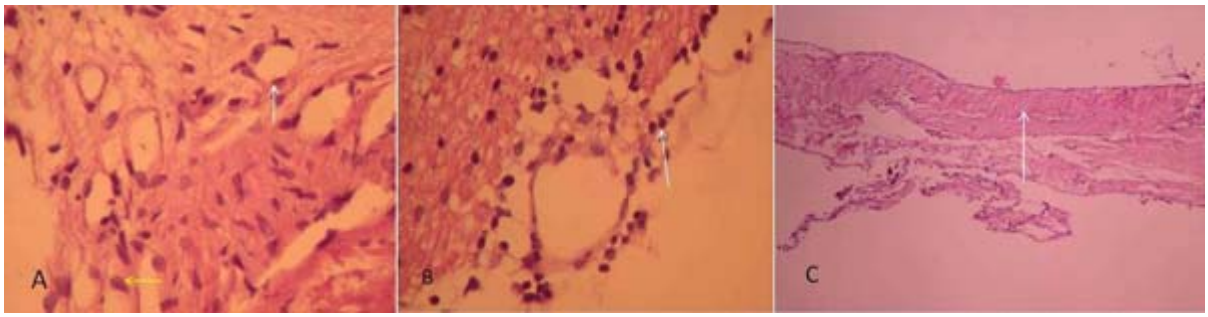

Figure 1: *A-White arrow shows new capillary production and yellow arrow shows fibroblast cell under 400x magnification. B-White arrow shows lymphocyte cell under 400x magnification and C-White arrow shows capsular formation under 100x magnification*

Table-1: Histopathological parameters' scores are shown for each group.

	Fibroblastic activity				**New Capillary Production**				**Foreign Body Reaction**				**Inflammatory Reaction**				**Capsule Formation**			
No.subj	**G1**	**G2**	**G3**	**G4**	**G1**	**G2**	**G3**	**G4**	**G1**	**G2**	**G3**	**G4**	**G1**	**G2**	**G3**	**G4**	**G1**	**G2**	**G3**	**G4**
1	+	+	+++	-	+	++	+	-	-	-	-	-	+	+	+	+	-	+	+	-
2	+	++	-	-	++	++	++	++	-	-	-	-	+	+	-	-	-	+	-	-
3	-	++	++	-	++	++	+	+++	-	-	-	-	-	++	+	-	-	+	-	-
4	+	++	++	-	+	++	++	++	-	-	-	-	-	+	+++	-	-	+	+	-
5	+	++	+	-	++	++	+	++	-	-	-	-	-	+	+	-	-	+	-	-
6	-	+	+++	-	-	+	-	-	-	-	-	-	+	+++	+	+	-	-	-	-
7	+	+	++	-	+	+	+	+	-	-	-	-	-	++	+	-	-	-	-	-

G1: Control G2:Collagen G3:Cellulose G4: Teflon

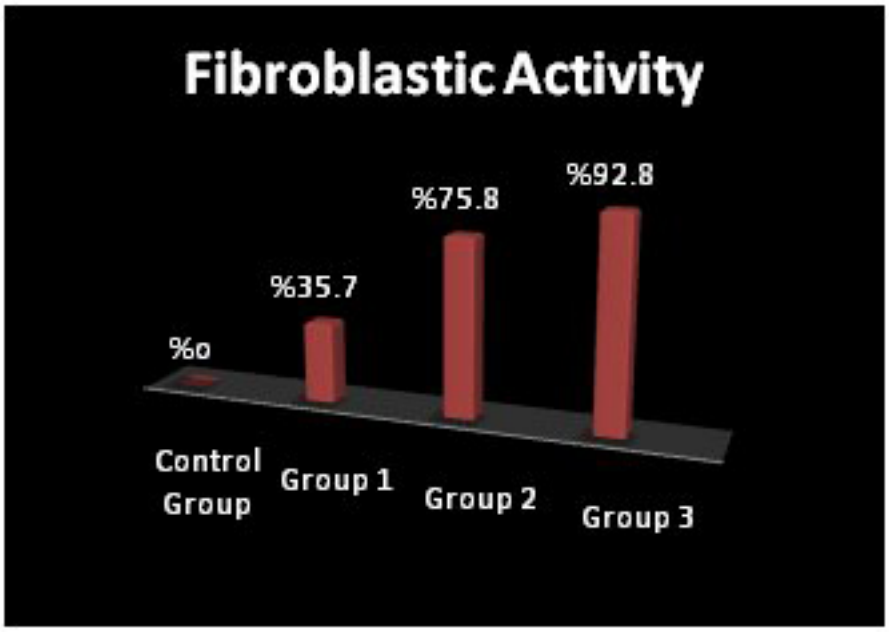

Figure 2: *Comparison of fibroblast activity among all groups. Group-1: Collagen, Group-2: Cellulose, Group-3: Teflon*

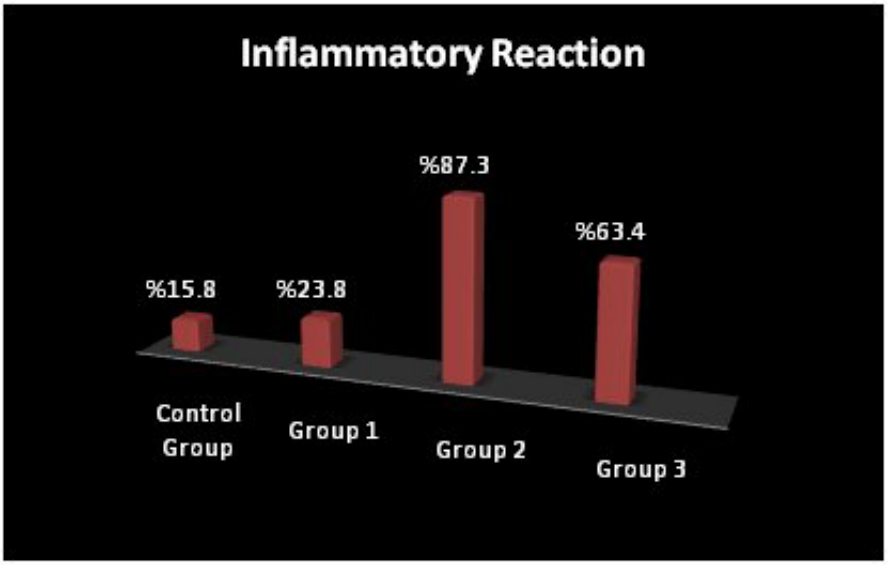

Figure 3: *Comparison of inflammatory reaction among all groups. Group-1: Collagen, Group-2: Cellulose, Group-3: Teflon*

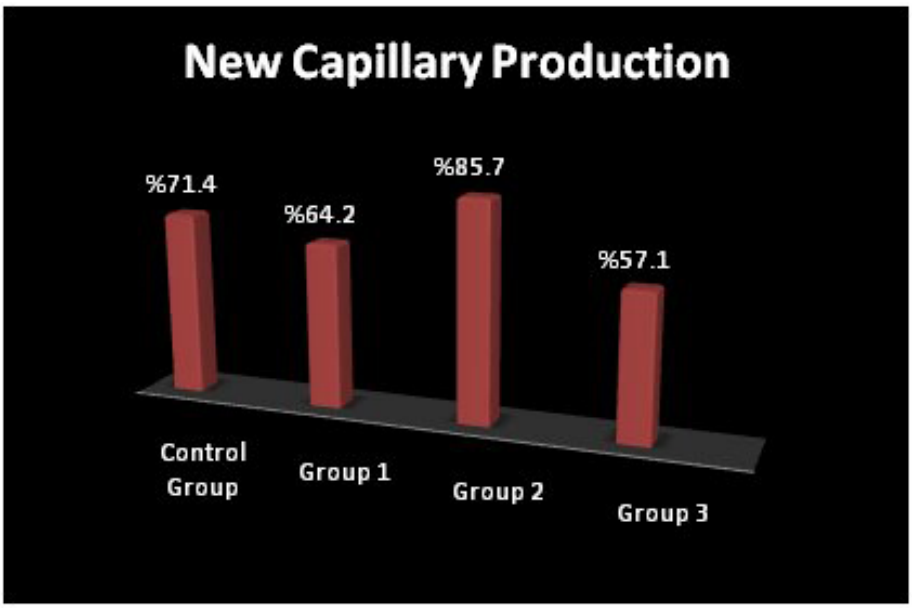

Figure 4: *Comparison of new capillary production among all groups. Group-1: Collagen, Group-2: Cellulose, Group-3: Teflon*

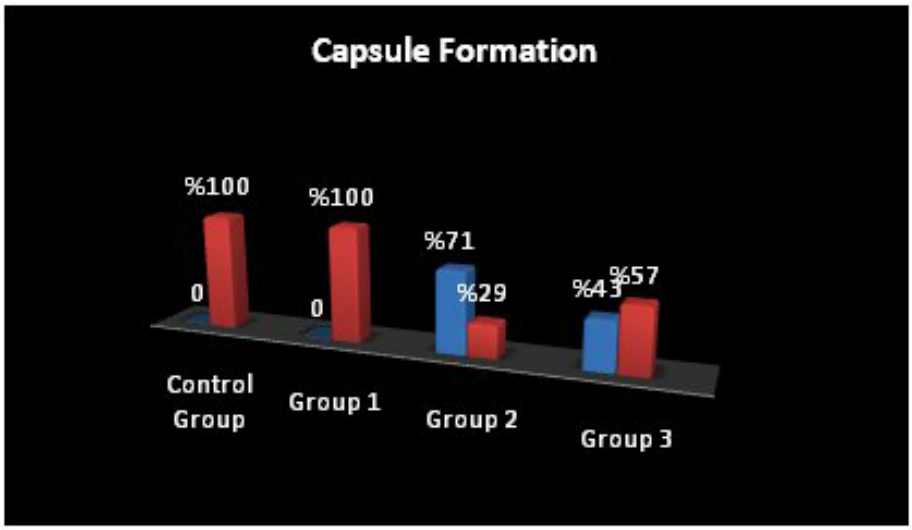

Figure 5: *The distribution of capsule formation among all groups. Blue: Positive for capsule formation. Red: Negative for capsule formation. While capsule formation was 29% (n=2) in cellulose group, this was 57% (n=5) in teflon group.*

DISCUSSION

The first duraplasty was performed in 1895 by Abbe using a rubber sheet[14,20]. Two years later, Beach et al. covered dural defects using a golden folio and soon thereafter Freeman et al. produced a dural graft from the membrane of an egg[14]. Following these initial procedures, many types of materials and procedures have been used for dural repair, including autografts, allografts, xenografts, and synthetic grafts. In many procedures, fascia lata, temporalis fascia, and pericranium have been used as autografts, while metallic folio, orlon, Vinyon N, and Silastic tubing have been used to cover the spinal cord or brain tissue as artificial grafts[14]. In 1958, cadaveric human dura mater was introduced as an alternative to autografts but reports on the transmission

of a prion pathology, Creutzfelt-Jacob disease, limited its use. This and other problems with autografts and cadaveric grafts have motivated researchers to create new synthetic materials instead.

Collagen, which is widely present in the human body as well as in nature generally, was used effectively as the first artificial dural graft material. Collagen-originated artificial grafts were first used in the clinical setting in 1980 following several successful experimental models. The first animal report was published in 1990 and detailed laboratory studies have continued during the 2000s[2,6,18]. Chaplin et al.[3] reported the formation of a cellular matrix at the end of the 1st, 3rd, and 6th months after use of dural grafts in Yucatan minipigs. Using an in vitro model for dural healing with collagen-coated wells, Zhou et al.[23] demonstrated that the cellular migration of fibroblasts from the dural defect margin may be an important mechanism involved in wound healing. Çetin et al.[5] studied a posterior fossa dural defect in New Zealand white rabbits, which was covered with collagen matrix and sutured. These findings showed the presence of a fibrous membrane, which was indistinguishable from the dura, good fibroblastic proliferation, and no foreign body reaction. Similarly, good vascular and inflammatory cell reactions in the collagen group were found by Maher et al.[9]. Three clinical studies by Narotam et al.[14,15,16] focused on collagen use in cranial, spinal, and posterior fossa craniotomy cases; all results were uneventful. Fibroblastic activity and inflammatory reactions were less than cellulose and teflon groups in our study.

Cellulose is an organic structure, $(C_6H_{10}O_5)n$, and includes D glucose and polysaccharides. It is the one of the most important organic materials naturally and represents 33% of plant structural material. It is also broadly used industrially, especially in the production of paper, cartons, and cardboard . In animal studies, the negative aspects of cellulose were assessed using biosynthetic cellulose when a parenchymal injury was present. Iannotti et al.[7] and Mello et al.[11] found this to be a progressive secondary injury due to the adhesion of cellulose to the cortical area on the parenchymal injury side. However, a clinical study including decompressive craniectomy cases showed just the opposite and dural healing was uneventful[9]. In our study inflammatory reaction was the greatest in cellulose group. Also new capillary production and fibroblastic activity were seen in high level by cellulose graft.

Polytetrafluoroethylene (teflon-PTFE) is a recent dural material. The human body rarely rejects PTFE and its non-stick effect is superior. Some artificial body organs or structures are produced from PTFE, including vascular grafts, knitted fabrics for aneurysm treatments, heart valves, aortic implants, shunts (in hemodialysis equipment), bone cartilage and ligament replacements, sutures, and tissue replacement patches[13]. Few animal studies have been conducted using PTFE as an artificial dura substitute. Islam et al.[8] noted that dural grafts made from PTFE produced a superficial cortical friction injury and a CSF fistula developed in an experimental study. In contrast to this study, many clinical observations of PTFE dura grafts have shown few complications[1,4,12,19,22]. Three different substances, collagen, PTFE, and vicryl-coated mesh, were compared by Park et al.[17] who noted arachnoid adhesion and an inflammatory reaction. PTFE was found to be the most reliable material in that study. Our study showed while fibroblastic activity was seen mostly by using teflon graft, new capillary production was vice versa.

CONCLUSION

Within all three groups, cellulose was found to be the most effective dural substitute. This graft showed the densest fibroblastic activity, moderate levels of

new capillary networks, and the greatest inflammatory reaction. Additionally, capsule formation was found to be higher than the other two groups. This study is one of few reported studies comparing different artificial dural substitutes (collagen, cellulose, teflon) in an experimental setting. Further studies focused on artificial dural grafts and tethered neural tissue are necessary for the safe use of these materials, especially in pediatric spina bifida cases.

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REFERENCES

1. *Attenello F J, McGirt M J, Garces-Ambrossi G L, Chaichana, et al. Childs Nerv Syst. 25(2):183-90*
2. *Brown R, Malcolm Jr. Cellulose. Cellulose Molecular and Structural Biology. S:92-94, 2007*
3. *Chaplin J M, Costantino P D, Wolpoe M E, et al. Use of an acellular dermal allograft for dural replacement. Neurosurg . 45(2):320-7, 1999*
4. *Chappell E T, Pare l ,Salehpour M, et al. Dura substitute applied as a nonwatertight underlay graf for craniotomies. Surg Neurologic. 71(1):126-9, 2009*
5. *Çetin B, Şengül G, Tüzün Y, et al. Suitability of collagen matrix as a dural graft in the experimental posterıor fossa dura mater defect.Turkish Neurosurg . Vol:16,No:1,9-13, 2006*
6. *Flippi R, Schworz M, Woth D, et al, Bovine Pericardium for Duraplasty. Neurosurgıcal Rev. 24-103:107, 2001*
7. *Iannotti C, Zhang Y P, shields L B, et al. Dural repair reduces connective tissue scar invasion and cystic cavity formation after acute spinal cord laceratin in adult rats. J of Neurotrauma. 23(6):853-65, 2006*
8. *Islam S,Ogane K, Ohkuma H, et al. Usefulness of acellular dermal graft as a dural substitute. Surgical Neurology. 61(3):297-302, 2004*
9. *Güresir E, Vatter H, Schuss P, et al. Rapid closure technique in decompressive craniectomy. J of Neurosurg. 29, 2010*
10. *Maher C O, Anderson R E, McClelland R L, et al. Evaluation of novel propylene oxide-treated collagen materials as a dural substitute. J of Neurosurg. 99(6):1070-6, 2003*
11. *Mello L R, Feltrin L T, Fontes Neto P T, et al, Duraplasty with biosynthetic cellulose. J of Neurosurg. 86(1):143-50, 1997*
12. *Messing-Jünger A M, Ibanez J, Calbucci F, et al. Effectiveness and hadling characteristics of a three –layer polymer dura substitute. J of Neurosurg. 105(6):853-8, 2006*
13. *Nakagawa S, Hayashi T, Anegawa S, et al. Postoperative infection after duraplasty with expanded polytetrafluorothylene sheet. Neurol Med Chir (Tokyo). 43(3):120-4, 2003*
14. *Narotam PK, Van Dellen JR, Bhoola KD A clinicopathological study of collagen sponge as a dural graft in neurosurgery J of Neurosurg . 82(3):406-1, 1995*
15. *Narotam P K, Reddy K, Fewer D, et al. Collagen matrix duraplasty for cranial and spinal surgery: a clinical and imaging study. J of Neurosurg. 106(1):45-51, 2007*
16. *Narotam PK, Qiao F, Nathoo N. Collagen matrix duraplasty for posterıor surgery: evaluation of surgical technique in 52 adult patients.Clinical article. J of Neurosurg. 111(2):380-6, 2009*
17. *Park Y K , Tator C H . Prevention of arachnoiditis and postoperative tethering of the spinal cord with Gore-Tex surgical membrane. Neurosurg. 42(4):813-23, 1998*
18. *Roberts Royston M Serendipity: Accidental Discoveries in Science. polytetrafluororthylene; John Wiley and Sons; New York . 1989.*
19. *Samuels R, Attenello F J,Garces Ambrossi G L, et al. Incedence of symptomatic retethering*

after surgical management of pediatric tethered cord syndrome with or without duraplasty. Childs Nerv. Syst. 25(9):1058-9, 2009

20. *Stendel R, Danne M, Fiss I, et al. Efficacy and safety of a collagen matrix for cranial and spinal dural reconstruction using different fixation techniques. J of Neurosurg. 109(2):215-21, 2008*
21. *Stone A J, Castillo M, Noelon Band, et al. Evoluation of CSF leaks: High-resolution CT compared with contrast enhanced CT and Radionuclide cisternography. AJNR. 20:706-712, 1999*
22. *Yamamura K,Sakata K,Yamamoto I. Experimental study of water leakage in duraplasty using EPTEF sheet as an artificial dura mater. No Shinkei Geka. 27(9):825-9, 1999*
23. *Zhou F, Chen G, Zhang JM, et al. An in vitro culturing model for rabbit dural cells. Ann Clinical Lab Sci. 36(3):341-4, 2006*