Iraqi Journal of Veterinary Sciences

www.vetmedmosul.com

Using lyophilized bovine pericardium and acellular ovine esophageal mucosa to repair cerebral dura mater defect in dogs

A.M. Saiegh[1], O.H. Al-Hyani[2] and K.W. Alheyali[3]

[1]Department of Community Health, College Health and Medical Technology/Shekhan, Duhok Polytechnic University, Duhok, [2]Department of Surgery and Theriogenology, College of Veterinary Medicine, University of Mosul, [3]Ibn Sina Teaching Hospital, Directory of Health in Ninevah, Mosul, Iraq

Article information

Article history:
Received 26 July, 2023
Accepted 16 September, 2023
Published online 08 February, 2024

Keywords:
Dura mater defect
Close of dural defect
Xenografted tissues

Correspondence:
O.H. Al-Hyani
osamahazim854@yahoo.com

Abstract

This article assessed the ability of lyophilized bovine pericardium and acellular ovine esophageal mucosa with fibrin glue to repair induced cerebral dural defects in dogs. Eighteen adult male dogs were used and divided randomly into two groups. A square defect about (1x 1cm) was created in all animals in the dura mater. In the first group, the defect was closed with lyophilized bovine pericardium, while the defect was grafted with acellular ovine esophageal mucosa in the second group. The results were assessed by monitoring the clinical signs post-operation, the gross and histopathological changes, cerebrospinal fluid pressure with a level of glucose and protein, and total cell count of cerebral spinal fluid on the 15th, 30th, and 60th postoperative days. All animals were still alive without the appearance of any abnormal nervous signs after the operation. Grossly, in both groups, the craniectomy site was occluded completely, and no cerebrospinal fluid leaks were noticed postoperatively. Also, the dura mater of the host was connected with grafted subjects internally. The histopathological changes revealed connective tissue formation, infiltrations of inflammatory cells, and angiogenesis. The rapid formation of connective tissue and angiogenesis with little or no infiltration of inflammatory cells and total cell count were shown in the first rather than the second group. The cerebrospinal fluid pressure, glucose level, and protein appeared within the normal range in both groups study periods. It concluded; that both xenografted tissues with fibrin glue could be used to repair dural defects in dogs.

DOI: 10.33899/ijvs.2023.142111.3159,

Introduction

The meninges are membranes that enclose or surround the brain and spinal cord. The meninges consist of three layers: dura mater, arachnoid mater, and pia mater (1,2). Dura mater is a stout membrane composed of irregular, dense connective tissue that encloses the brain and spinal cord. It is regarded as the outer layer of the meninges and envelops the arachnoid mater. The dura mater consists of two layers, called lamellae. The first is a superficial or periosteal layer, which forms the inner periosteum of the skull, and the second is a deep or meningeal layer (3). The arachnoid mater is a fibrous tissue with a spider web; therefore, this name was applied. The Pia mater is a delicate inner layer of the meninges. It is surrounding the brain and spinal cord (4). The space between the arachnoid and pia mater is called subarachnoid space (5), which contains CSF, major blood vessels, and cisterns. The cisterns are considered enlarged pockets of CSF created by separating the arachnoid from the pia mater (6). Like other tissues, meninges may be affected due to different causes, such as injury, cancer, or inflammation (7). The defect or tearing of the dura may have occurred due to injury or during head surgery. However, suturing of the dura was indicated for minor size defects, but

sometimes the suturing may also create small dural tears (8). Therefore, a patch or graft is indicated in case of sizable dural defect or tear. Fat or fibrin glue may be a sealant to reinforce the repair (9). The grafted materials that are used to close the dural defect are either autologous tissue grafts, such as periosteal flaps, or allografts, as in lyophilized cadaveric dural grafts, or xenografts, such as using bovine pericardium and submucosa of porcine small intestinal (10). The xenogeneic graft material can be used for different conditions, but these materials have some adverse effects, such as tissue reaction. Therefore, these materials were applied under the decellularization process to remove any adverse effects, especially tissue and immunological reactions during tissue healing (11,12). The decellularization process can performed by using physical (13), chemical (14), or enzymatic (15).

Therefore, this article aims to evaluate the capacity of lyophilized bovine pericardium and acellular ovine esophageal mucosa as xenografted tissues with fibrin glue instead of suturing to repair induced cerebral dura mater defect in dogs.

Materials and methods

Animals

This experimental work, used eighteen adult local breed male dogs aged 1-2 years with an average weight of 25±1.8 kg. The animals were divided into equal groups. The animals were examined clinically to ensure their safety from diseases, especially those related to the nervous system. The animals were kept in specific cages in the animal house at the College of Veterinary Medicine, University of Mosul.

Ethical approve

The research was approved by the Ethics Committee of the Faculty of the College of Veterinary Medicine, University of Mosul. No. UM.VET.2023.003.

Anesthesia

Atropine sulfate at dose 0.05 mg/kg. was also used as pre-anesthetic drug, and it gives subcutaneously. A mixture of 10% ketamine HCL at 10 mg/kg and 2% xylazine at 2 mg/kg as a protocol was used to induce general anesthesia in all animals, where the mixture was injected intramuscularly (16).

Surgical procedure

The animals fasted for about 12 hours before the surgical operation from food and water intake as a routine regime. A craniectomy was indicated to perform cerebral dura mater defect after general anesthesia. In this surgical operation, a piece of the parietal bone of the skull should be removed without returning it to the normal position in the skull immediately after the operation. However, after a skin incision of a V shape the forehead of the animal, the incised skin was reflected caudally to expose the subcutaneous tissue. Then, the subcutaneous tissue was incised and reflected laterally to expose the temporalis muscle. The temporalis muscle was incised along the sagittal suture, dissected with blunt scissors, and expanded by a wound retractor to facilitate exposure of the parietal bone. Then, the parietal bone was excised as a square shape using an electric saw. The excised piece of parietal bone was elevated using a bone chisel to show the dura mater. The craniectomy site was located between the lambdoid and coronary suture of the skull. The hemorrhage during craniectomy was controlled by bone wax. After exposure to the dura mater, an induced defect, a square shape of about (1x1cm.), was created on the dura mater of the host (Figure 1). The cerebral spinal fluid was leaked immediately after incising the dura mater. The induced dura mater defect was closed in the first group with a piece of lyophilized bovine pericardium obtained, ready, and made by Braun company (17) (Figure 2), while in the second group, a piece of the acellular ovine esophageal mucosa, which was previously prepared, was used to close the site of the dural defect (Figure 3). In both groups, the fibrin glue (Baxer Healthcare SA, 8010 Zurich, Switzerland, Belgium) was used to seal the xenografted tissues with dura mater of the host instead of suturing where the glue was applied as a few drops along the edges of each grafted piece of tissue. Then, the craniectomy site was closed routinely by suturing the temporalis muscle and subcutaneous tissue, respectively, with polyglactin suture using a simple continuous pattern and skin with silk using a simple interrupted pattern.

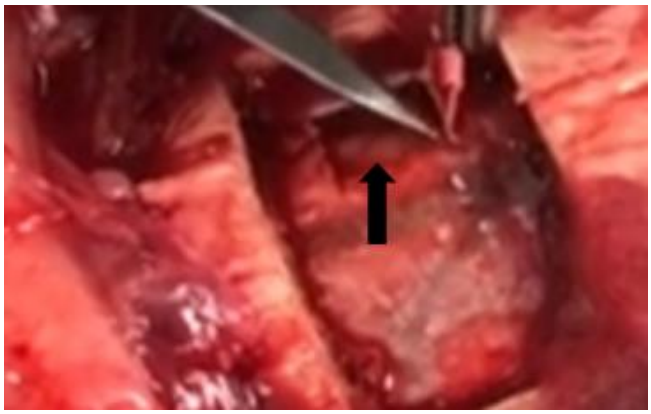

Figure 1: Cerebral dural defect.

Postoperative care

All the experimental animals were injected with systemic antibiotics and analgesics postoperatively using penicillin-streptomycin (1ml /10 kg. and metalgen 1ml/ day respectively) for seven days after the surgical operation. Also, the operation site was dressing daily with topical spreading of oxytetracycline spray until the complete healing of the skin wound.

Figure 2: Ready product of lyophilized bovine pericardium.

Figure 3: The prepared a cellular ovine esophageal mucosa.

Assessment of dural defect healing

The follow-up examinations include; monitoring the clinical signs postoperatively, estimation of CSF pressure, and analysis of CSF total cell count with CSF glucose and protein level in both groups on the15th, 30th, and 60th postoperative days. Specimens of the graft area with the cortex of the brain were obtained at the 15th, 30th, and 60th days after euthanizing three animals of each group in each period for macroscopic observation, and histopathological evaluation where the samples fixed in 10% formalin and stained with hematoxylin and eosin (H&E) stain (18). The histological sections were scored according to the following criteria [1] Intensity of connective tissue, [2] Intensity of angiogenesis, and [3] Intensity of inflammatory reaction (Table 1).

The histopathological scores were analyzed statistically by a pathologist using the Kruskal-Wallis One Way Analysis of Variance on Ranks test and used Pairwise Multiple Comparison Procedures (Tukey Test) at $P \leq 0.05$. We used the Sigma Plot software program for statistical analysis. Also, the statistical analysis of CSF protein, and glucose level with total cell count was dependent.

Collection of cerebral spinal fluid

This study, collected CSF under general anesthesia from the cerebellomedullary cistern at the back of the animal head. The main landmarks depended collecting cerebrospinal fluid from the cistern, including the external occipital protuberance, the cranial aspect of the dorsal spine of the axis, and the transverse processes of the atlas. However, after induction of general anesthesia, the back of the neck was shaved and sterilized. Then, a spinal needle is inserted at the base of the skull, and into the spinal column, penetrating the dura mater and arachnoid membranes to the subarachnoid space. Then, CSF will escape or leak through a spinal needle into a sterile plain tube. About 5 ml was collected to make CSF analysis (20).

Table 1: Showing the histopathological scoring (19)

Criteria	0	1	2	3	4
Connective tissue	Absent	Discrete	Moderate	Intense	Complete
Angiogenesis	Absent	Discrete	Moderate	Intense	Complete
Severity of inflammation	Absent	Few	Few	Moderate	Sever

Estimation of cerebral spinal fluid pressure

Generally, the best methods to diagnose any CSF leaks were accomplished by magnetic resonance cisternography (MRCG) and computerized tomography myelography (CTMG). Due to none of these techniques being available, monitoring of CSF pressure by using a catheter as the modified technique was depended to ensure there were no CSF leaks where a small, flexible tube was placed and fixed on the calculated ruler and connected with a spinal needle after inserting it in the subarachnoid space. The animal must be placed on one side and one level. Additionally, the tube used to collect CSF should be placed vertically on the animal and spinal needle and left for a short time to track changes in the CSF pressure.

Cerebral spinal fluid analysis

The main components of the CSF adopted during the analysis of CSF to evaluate the efficiency of each type of grafted tissue used in this work include the protein and glucose levels with an estimation of the total cell count.

Estimation of CSF glucose

1 ml of CSF was taken from the collected sample of CSF and put in a sterile glass tube. Then, a glass tube that contained the CSF sample was placed inside a device spin 120. The reagent used in this study to estimate the level of CSF glucose is called, glucose spin reagent. The reaction between the sample of CSF and glucose spin reagent will provide the data for analysis.

Estimation of CSF protein

The device used in this work to estimate the level of CSF protein was the device Fujifilm. However, 1 ml of CSF was put in the Eppendrof tube. Then, a disposable fuji dri-chem slide and auto tips with Eppendrof tube containing the sample of CSF were put in the Fujifilm device. The chemical reaction between the CSF sample and the fuji dri-chem slide will be given as the analysis result.

Estimation of CSF total cell count

A hemocytometer chamber was used to estimate the total cell count of CSF. Each chamber was composed of 5 mini chambers in a square shape, four peripheral and one central. To read white cells (lymphocyte, monocyte, neutrophils, and eosinophils), 25nmicrons of the CSF sample were applied on one peripheral mini chamber in addition to its 75 microns of new methylene blue. Then, the sample was left for 10 minutes to fix, and then the hemocytometer chamber was washed with tap water and the sample to dry. After that, 25 microns of oily drop was applied on the chamber with putting cover slide. The results were recorded under a microscope using a lens with power (100x). While, the central mini chamber was used to read only the red cells. The methylene blue was not used (only the CSF sample).

Decellularization of ovine esophageal mucosa

The esophagus was obtained from a local ovine breed after being slaughtered in a Mosul abattoir. About 10 cm lengths were removed from the central region of the esophagus. Then, the fresh esophagus was washed several times by rinsing in phosphate-buffered saline containing antibiotics. The muscularis layer of the esophagus was removed mechanically by dissection with a scalpel. Then, the remaining layers were washed in phosphate buffer saline (PBS) and penicillin/streptomycin. The esophagus tissue was placed in 5% sodium dodecyl sulfate (SDS) for seven days at room temperature while agitated by an orbital shaker at 300 rpm. After that, the tissues were then washed in PBS for 24 h, followed by 24 h incubation at 37ºC. Finally, decellularized tissues were stored in PBS containing penicillin/streptomycin at 4◦C for no more than four weeks (21) (Figures 4 and 5).

Results

Clinical signs postoperatively

In all animals, the site of operation showed swelling postoperatively. The swelling disappeared at the end of the first-week post-operation. Generally, all animals lived with complete recovery without any health complications, especially the appearance of abnormal nervous signs such as epilepsy, muscle tremors, abnormal herring, or visions. Additionally, the animals ate and drank water normally, and no change was shown in their behaviors.

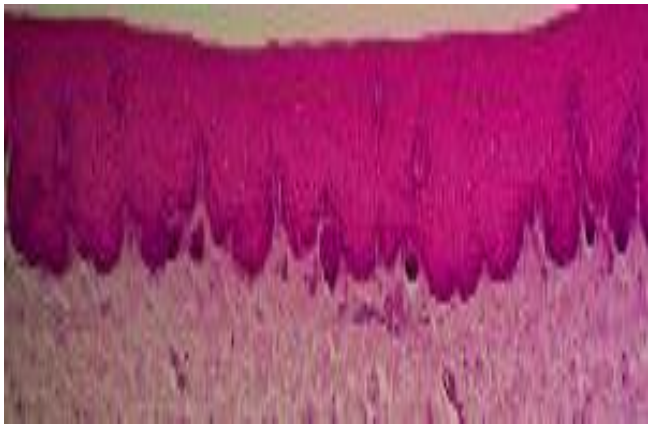

Figure 4: Normal esophageal tissue (H&E.100X).

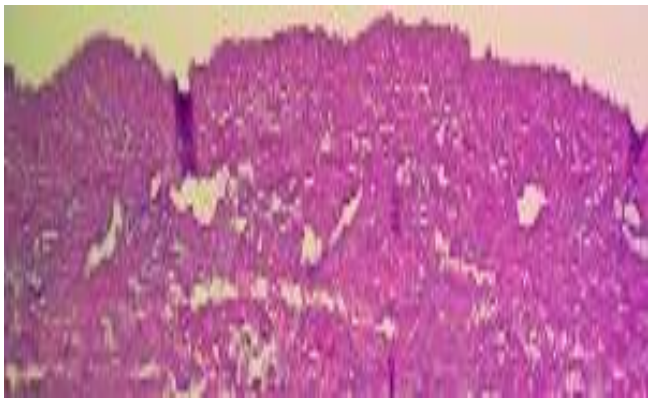

Figure 5: Acellular esophageal tissue (H&E.100X).

Gross changes

In all animals, the craniectomy site was occluded completely with granulation tissue during all study periods and did not diagnose cerebral spinal fluid leaks. The dural defect size was reduced internally at the end of the study in both groups, where the xenografted tissue adhered with the dura mater of the host completely (Figures 6-9).

Histopathological changes

In the first group, the histological section at the grafting site after the 15th postoperative day was revealed more connective tissue proliferation and angiogenesis with few infiltrations of inflammatory cells (Figure 10). On the 30th postoperative day, the connective tissue is more maturated with very few infiltrations of inflammatory cells and angiogenesis with deposition of eosinophilic pretentious material within connective tissue. At the 60th postoperative day, the histopathological changes were represented by increased connective tissue maturation (Figure 11). The normal histological architecture of the brain cortex section during the study period was shown (Figure 12).

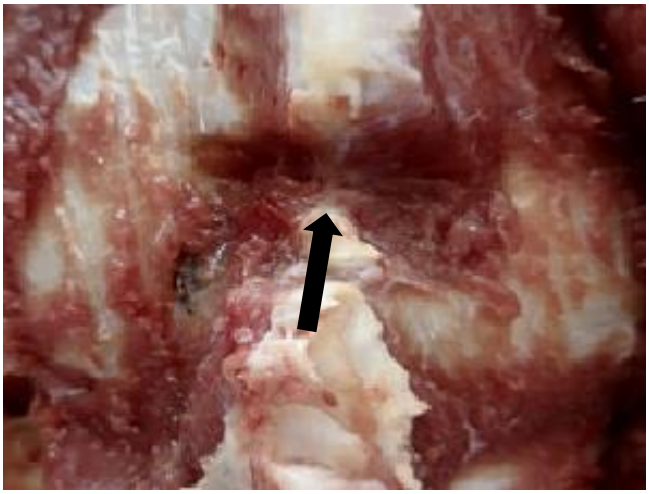

Figure 6: Show closure of the operation site on 60th day post-surgery in group one.

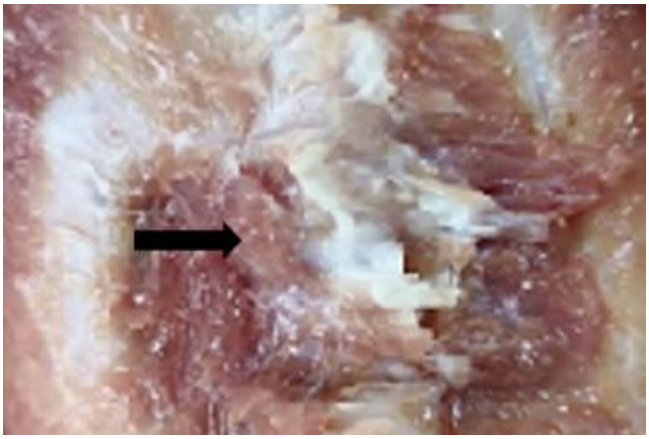

Figure 7: Show closure of the operation site on 60th day post-surgery in group two.

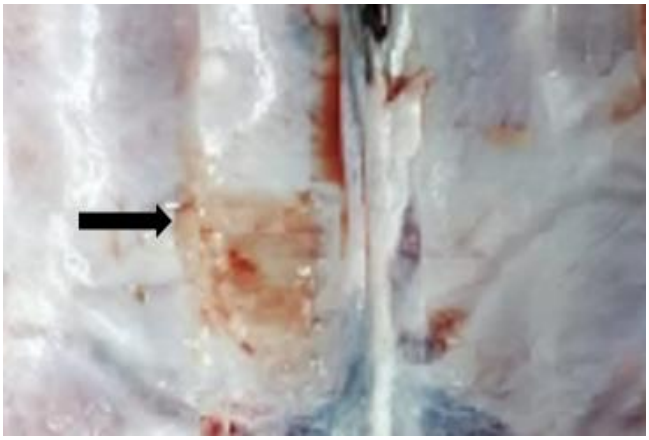

Figure 8: Show closure of the dural defect at the 60th day post-surgery in group one.

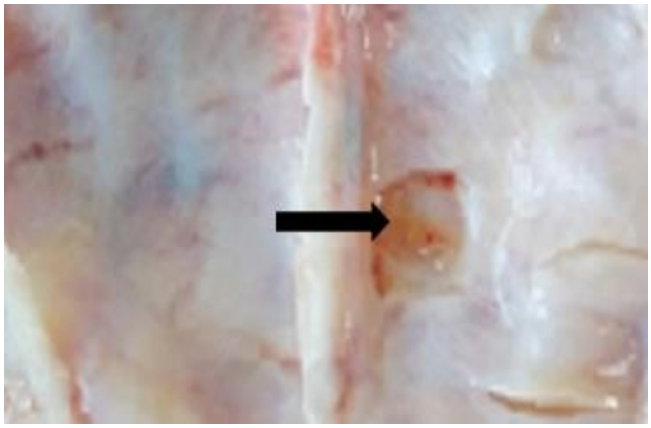

Figure 9: Show closure of the dural defect at the 60th day post-surgery in group two.

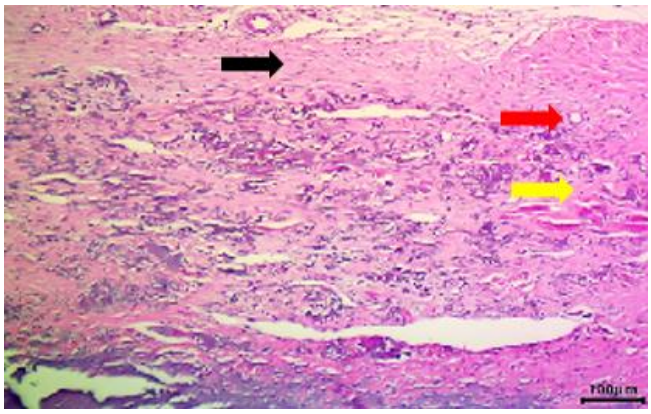

Figure 10: The micrograph on the 15th day after the operation in group one shows connective tissue (black arrow), new blood vessels (red arrow), and a few infiltrations of inflammatory cells (yellow arrow). (H&E.100X).

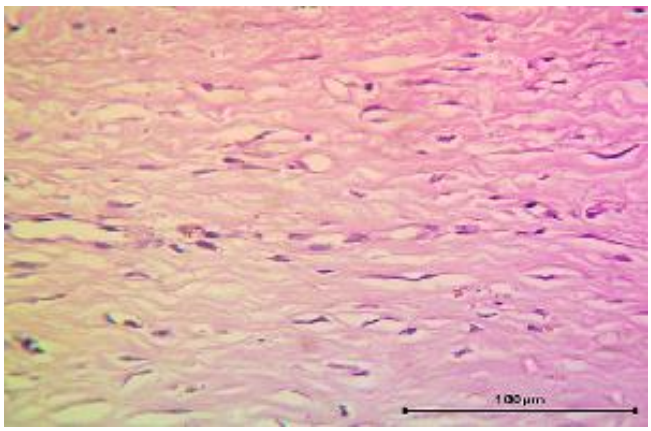

Figure 11: Micrograph on the 60th day after operation in group one shows mature connective tissue without inflammatory cells. (H&E.400X).

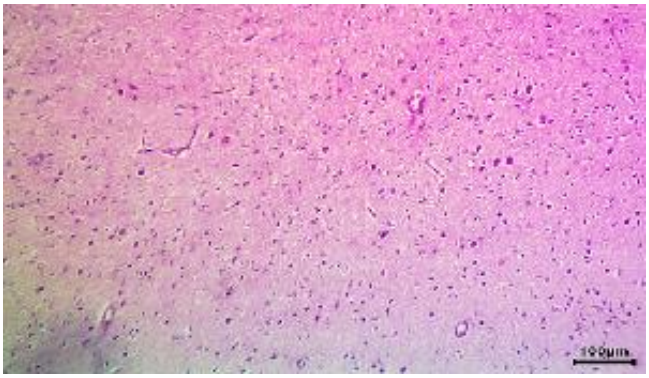

Figure 12: Micrograph on the 60th day after operation in group one shows normal brain cortex (H&E.100X).

In the second group, the histological section at the grafting site after the 15th postoperative day revealed the proliferation of connective tissue, severe inflammatory cell infiltration, and new blood vessels (Figure 13). On the 30th postoperative day, the histopathological sections were characterized by connective tissue maturation, decreased inflammatory cell infiltration, and mild angiogenesis. On the 60th postoperative day, connective tissue had more maturation with little infiltration of inflammatory cells and little angiogenesis (Figure 14). The normal histological architecture of the brain cortex section during the period of the study was shown (Figure 15).

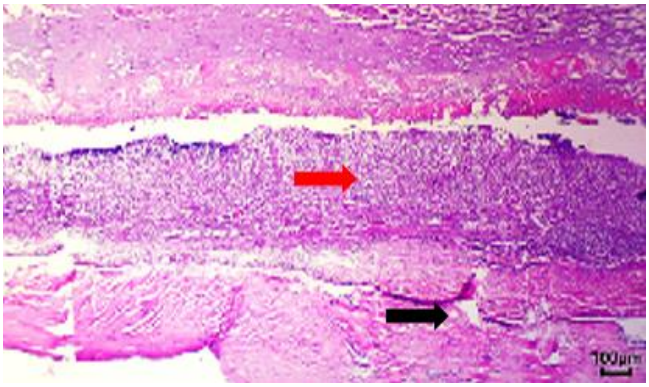

Figure 13: Micrograph at 15th postoperative days in group two shows proliferation of connective tissue (black arrow) and severe infiltration of inflammatory cells (red arrow). (H&E.40X).

The statistical analysis of the histopathological scoring

The statistical analysis of the intensity of connective was revealed no any significant difference at P≤0.05 between both groups (Table 2). The statistical analysis of the intensity of angiogenesis was revealed significant deference at P≤0.05 in group one rather than group two at 30th day post operation (Table 3). The statistical analysis of the intensity of inflammatory was revealed significant deferens at P≤0.05 in group one rather than group two at 15th and 60th day after operation (Table 4).

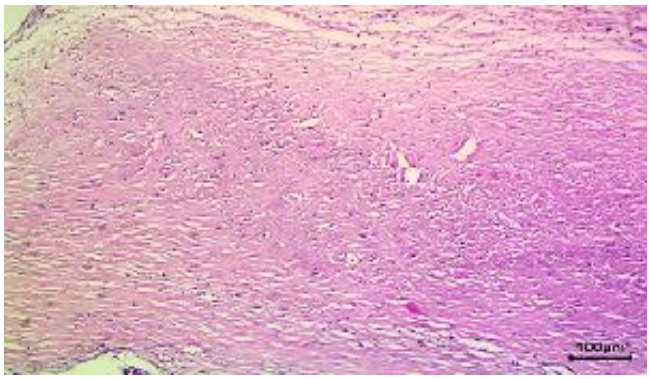

Figure 14: Micrograph at 60th postoperative days in group two shows mature connective tissue (H&E.100X).

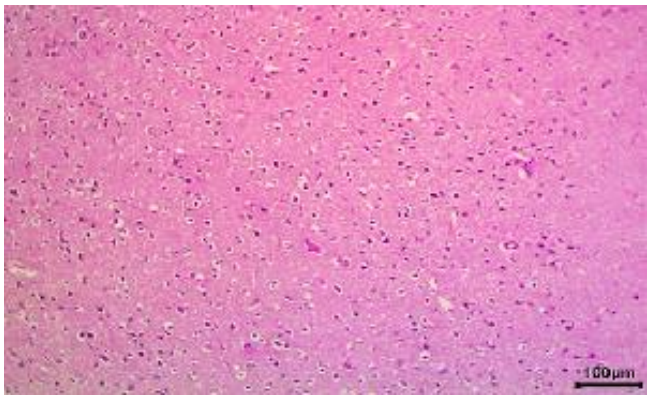

Figure 15: Micrograph on the 60th day after operation in group two shows normal brain cortex (H&E.100X).

Table 2: Intensity of connective tissue scores

Groups	15th P.O.Ds. / n=9 / Median	30th P.O.Ds. / n=6 / Median	60th P.O.Ds. / n=3 / Median
G1	3	3	4
G2	2	4	4

Data expressed as Median of the scores (Kruskal-Wallis test). The difference letters mean there are significant differences between groups at P≤0.05.

Table 3: Intensity of angiogenesis scores

Groups	15th P.O.Ds. / n=9 / Median	30th P.O.Ds. / n=6 / Median	60th P.O.Ds. / n=3 / Median
G1	4	3A	2
G2	4	2	1

Data expressed as Median of the scores (Kruskal-Wallis test). The difference letters mean there are significant differences between groups at P≤0.05.

Table 4: Intensity of inflammatory response scores

Groups	15th P.O.Ds. / n=9 / Median	30th P.O.Ds. / n=6 / Median	60th P.O.Ds. / n=3 / Median
G1	1 B	1	0 B
G2	3	2	1

Data expressed as Median of the scores (Kruskal-Wallis test). The difference letters mean there are significant differences between groups at P≤0.05.

The statistical analysis of cerebrospinal fluid analysis with pressure

The cerebrospinal fluid pressure in all animals during all periods of the study, in addition to the level of protein and glucose, appeared within the normal range, and there is no significant difference at P≤0.05 (Tables 5-7). However, there is a significant difference at P≤0.05 in the total cell count in group one than group two on the 30th postoperative day (Table 8).

Table 5: Cerebral spinal fluid pressure

Groups	CSF pressure (mm Hg)±St. E.			
	Before operation (N=9)	15th P.O.Ds. (N=9)	30th P.O.Ds. (N=6)	60th P.O.Ds. (N=3)
G1	10.8±0.67 A	10.7±0.40 A	10.6±0.33 A	10.3±0.88 A
G2	12.1±0.97 A	11.0±0.40 A	10.6±0.55 A	10.0±0.57 A

The difference letters mean there are significant differences between groups at P≤0.05.

Table 6: Cerebral spinal fluid protein

Groups	CSF protein (mg/dl)±St. E.			
	Before operation (N=9)	15th P.O.Ds. (N=9)	30th P.O.Ds. (N=6)	60th P.O.Ds. (N=3)
G1	28.1±1.9 A	31±0.3 A	30.8±0.4 A	30.3±0.3 A
G2	28.3±2 A	32.8±0.6 A	32.8±0.7 A	30.6±0.6 A

The difference letters mean there are significant differences between groups at P≤0.05.

Table 7: Cerebral spinal fluid glucose

Groups	CSF glucose (mg/dl)±St. E.			
	Before operation (N=9)	15th P.O.Ds. (N=9)	30th P.O.Ds. (N=6)	60th P.O.Ds. (N=3)
G1	63±4.2 A	62±1.7 A	62.1±1.1 A	60.6±0.6 A
G2	63.7±4.2 A	69.1±3.6 A	65.1±1.1 A	61.3±0.8 A

The difference letters mean there are significant differences between groups at P≤0.05.

Table 8: The total cell counts

Groups	CSF cells count (%)±St. E.			
	Before operation (N=9)	15th P.O.Ds. (N=9)	30th P.O.Ds. (N=6)	60th P.O.Ds. (N=3)
G1	0.8±0.2 A	1.7±0.2 A	1.1±0.1 B	0.0±0.0 A
G2	0.8±0.3 A	2.3±0.4 A	3±0.4 A	0.6±0.3 A

The difference letters mean there are significant differences between groups at P≤0.05.

Discussion

Several methods and materials used to repair dura defect or tear due to many causes, such as injury, congenital deformities, and tumors, have been applied to clinical uses, but the best choice of materials to use in repairing dura remains unclear. However, dural substitutes are common methods for dural repair using materials to replace the damaged or resected dura mater (22-25). Many studies Fontana *et al.* (26) and Van Calenbergh *et al.* (27) revealed that the closure of the dura with different biomaterials was regarded as very important to prevent the appearance of some major complications such as cerebrospinal fluid leaks, brain herniation, infections, adhesions, and scarring of the brain cortex. However, this work used two xenografted tissue types: lyophilized bovine pericardium and acellular ovine esophageal mucosa. Some authors Cobb *et al.* (11) showed the grafted subjects used to repair dural defects, either autologous tissue grafts such as fascia of femoris or allografts as in lyophilized cadaveric dural grafts or xenografts such as using bovine pericardium and submucosa of porcine small intestinal. Generally, the fixation of the grafted materials on the site of the dural defect was accomplished either by suturing or by using glues (28-30),

but sometimes, in duraplasty, the dural defect cannot be closed. In this work, suturing was not used because was time-consuming, and in addition, no thread has all perfect qualities such as being easy to handle, less tissue reaction and, non-allergenic, and not promoting infection (31). Therefore, using fibrin glue instead of suturing provided good and smooth fixation between the grafted materials and host dura mater, whereas, some authors Sharma *et al.* (32) said the glues provided a smooth seal to the wound edge with significantly fewer complications. Other authors Eckersberger *et al.* (33) and Fundaro *et al.* (34) showed that fibrin glue can be used successfully in different surgical procedures because it has an effective hemostatic agent and a potent tissue glue to adhere to tissue surfaces even if the moisture is present. In addition, fibrin glue does not increase the risk of thromboembolic complications or interfere with wound healing (35). Also, it has no adverse pathological or immunological effect when used in dogs (36).

Repairing dural defects after neurosurgical operation by suturing, glues, or grafted materials is essential because of the imperfect closure to the dural defect, cerebrospinal fluid will leak from the punctured dura, and several complications may occur, such as meningitis and epidural abscess (37,38). However, using lyophilized bovine pericardium and acellular ovine esophageal mucosa in this article to close dural defect provided excellent results in the healing process where both xenografted tissues have some characteristics such as less inflammatory reactions, not rejected, strong to provide watertight seal without tearing and easy to applicate where the authors' Bernd *et al.* (23), Pogorielov *et al.* (39), Grotenhuis (40), Biroli *et al.* (41), and Yamada *et al.* (42) mentioned the grafted materials which used for grafting must have some essential characteristics such as they should be improved tissues regeneration and resorb, provide a good scaffold for the formation of neodura, little inflammatory reactions, watertight seal, easy to applicate and less costly.

In this study, both xenografted tissues provided relatively good rebuilding for dural defects, and prevented complications, such as cerebral spinal fluid leaks and abnormal nervous signs. The dogs, after operation, showed a normal level of health and still lived with complete recovery during all periods of the study, and this may be due to the ability of grafted materials to heal the dural defect without any complications, especially leaks of cerebrospinal fluid, brain tissue infection or damage where previous study reported Klekamp (43) the clinical improvement was appeared in patients after duraplasty regardless to the kind of grafted materials whether it is autologous or non- autologous subjects.

In both groups, the gross features revealed complete healing where each grafted subject was adhered to and interlocked with the dura mater of the host internally. In addition, the site of craniectomy externally was occluded completely with granulation tissue, and no cerebrospinal fluid leaks or fistula was noticed. These good results may be due to the effectiveness of both grafted tissue with fibrin glue where Borgesen and Vang (44), and Bosacco *et al.* (45) showed any collection to the cerebrospinal fluid subcutaneously may prevent appropriate wound healing and develop to infection, wound dehiscence, and cutaneous fistula. In addition, the formation of pseudo meningocele leads to abnormal neurological signs (45). Also, other authors De almeida *et al.* (46), Nishihira and McCaffrey (47) and Siedentop *et al.* (48) mentioned that the fibrin sealant can be used alone or with sutures and dural substitutes to provide correct closure to the dural defect and prevent cerebral spinal fluid leaks and other complications.

Although, some researchers reported that the most common complications, especially occurrence of aseptic meningitis, infection wound, and leak of cerebrospinal fluid, appeared during use non-autologous materials (49,50), such as during duraplasty with polytetrafluoroethylene (51), but in the present study the healing process at the site of the dural defect in both groups was relatively excellent due to the positive effect of fibrin glue with grafted materials which are used in this study.

The healing process is a complex mechanism, starting with the coagulation phase, which occurs after a few minutes from injury and includes hemostasis and platelet aggregation. Then, the inflammatory phase, where the inflammatory cells begin to migrate to the site of a wound. The infiltration of inflammatory cells occurs due to the release of cytokines or the presence of prosthetic subjects, which regarded as foreign material. Then, the proliferation phase when the fibroblasts migrate and proliferate with the deposition of collagen and the formation of granulation tissue with neovascularization. This phase takes several weeks after injury, according to the size of the wound damage. In the end, the wound remodeling phase will start, consisting of collagen fiber reorganization lasting for several months (52). Microscopically, in both groups, the histological features were represented by the proliferation of connective tissue, infiltration of inflammatory cells, and angiogenesis, but less infiltration of inflammatory cells with more angiogenesis was shown in group one rather than in group two. However, both grafted materials were interlocked with the dura mater of the host. This was mentioned by Shi *et al.* (53), where the grafted materials that used to close the dural defect in dogs should have as much as possible to facilitate the invasion of host cells and gradually biodegraded and replaced by endogenous tissue relatively equal to the host dura mater without developing any abnormal pathological feature.

The use of lyophilized bovine pericardium and acellular ovine esophageal mucosa in this study gave excellent results, and this agreed with Zhang *et al.* (54), who showed the xenogeneic materials are relatively good infection-resistance with properties like dura mater of the host. Also, many studies Bejjani and Zabramski (55), Narotam *et al.* (56), and Tatsui *et al.* (57) showed that the bovine pericardium,

submucosa of pig small intestines, and processed collagen matrices can be used as a xenogeneic graft material. However, these materials have some adverse effects, such as dissolution of graft tissue, encapsulation, foreign body reaction, scarring, and adhesion formation. Therefore, the materials used in this work as xenografts were applied under the decellularization process to remove any adverse effects during the repair of the dural defect, especially tissue and immunological reactions where some researchers Wallis *et al.* (58), and Lim *et al.* (59) said there are several methods used to decellularize the tissues either physically or chemically and the main goal to use this protocols to provide non-immunogenic tissues that can be used without need to any immunosuppressive medication. Other authors Colaco and Atala (60), and Guyette *et al.* (61) mentioned that the process of tissue decellularization could be done with physical, chemical, and enzymatic agents to change a tissue from cellularity to cellularity through the removal of cellular components of tissue and leaving the noncellular extracellular matrix (ECM) for therapeutic uses. This process can be applied to tissues or organs such as pericardium (62). Many studies Cheung *et al.* (63), and Khor (64) mentioned that collagen tissues are characterized by fast degeneration and want to be stabilized by specific agents to reach original integrity and eliminate their antigenic properties to be used as biomaterials. The use of acellular ovine esophageal mucosa and lyophilized bovine pericardium as decellularized tissues to close induced dural defects in dogs produced excellent results where these implanted materials were interlocked, interwoven, and adhesive with dura mater of dog, and covered with connective tissue and invasive with newly blood vessels. These results agree with Macchiarini *et al.* (65), and Conconi *et al.* (66), who reported that the remaining extracellular matrix of the decellularized tissue could provide a micro-architecture for adhesion, cell proliferation, remodeling, differentiation, and angiogenesis. Another study Filippi *et al.* (67) reported that using bovine pericardium as a xenograft was characterized by flexibility and efficiency to suture and handle, safe, and less costly with positive effects for duraplasty as a dural substitute. The pericardium is a biological tissue used for tissue engineering such as constructing a tracheal defect (68). Because of the critical or restricted utilization of bovine pericardium as a xenograft in some clinical practices due to the possibility of rejection (69), lyophilized bovine pericardium was used in this study. The lyophilized bovine pericardium was characterized by rapid application, easy to handle, less tissue reaction and infiltration of inflammatory cells, rapid and more induction for angiogenesis and fibroblast proliferation, and these features resemble (70) mentioned the Lyoplant® is characterized by several advantages such as simple, quick to applicate, prevent cerebral spinal leak due to high liquid tightness. Therefore, these characteristics make it excellent choice to treat dural tearing and defects (71).

In both groups, the percentage of protein and glucose was located within the normal range after implantation of the different types of biomaterials, and there was no significant difference between both groups statistically except in the total cell counts in group one at 30 postoperative days. However, any increase or decrease in the protein or glucose of cerebral spinal fluids was indicated in the presence of leaks, infection, bleeding, and inflammation (72).

Conclusions

We could use lyophilized bovine pericardium and acellular ovine esophageal mucosa as xenografted tissues with fibrin glue to close and repair cerebral dura mater defect in dogs with priority to lyophilized bovine pericardium.

Acknowledgments

The authors thank the college of Veterinary Medicine, University of Mosul, Mosul, Iraq.

Conflict of Interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

References

1. Abbott J, Patabendige A, Dolman E, Yusof R, Begley J. Structure and function of the blood-brain barrier. Neurobiol Dis. 2010;37(1):13-25. DOI: 10.1016/j.nbd.2009.07.030
2. Kevin J, Hester M, John G. Diagnostic radiology and ultrasonography of the dog and cat. 5th ed. USA: Elsevier; 2010. 447-541 p.
3. Gagan R, Tholpady, Sunil S, Ogle, Roy C. Cellular dynamics and tissue interactions of the dura mater during head development. Embryo Today. 2007;81(4):297-304. DOI: 10.1002/bdrc.20104
4. Nadia J, Anne M, Iben L, Maiken N. The glymphatic system: A beginner's guide. Neurochem Res. 2015;40(12):2583-2599. DOI: 10.1007/s11064-015-1581-6
5. Altafulla J, Bordes S, Jenkins S, Litvack Z, Iwanaga J, Loukas M, Tubbs S. The basal subarachnoid cisterns: Surgical and anatomical considerations. World Neurosurg. 2019;129:190-199. DOI: 10.1016/j.wneu.2019.05.087
6. Papaiconomou C, Bozanovic-Sosic R, Zakharov A, Johnston M. Does neonatal cerebrospinal fluid absorption occur via arachnoid projections or extracranial lymphatics, Am J Physiol Regul Integr Comp Physiol. 2002;283(4):869-876. DOI: 10.1152/ajpregu.00173.2002
7. Harmony P, Garges M, Anthony C, Michael P, Brian K. Neonatal meningitis: What is the correlation among cerebrospinal fluid cultures, blood cultures, and cerebrospinal fluid parameters. Pediatr 2006;117(4);1094. DOI: 10.1542/peds.2005-1132
8. Mansour A, Ahmed A, Ahmad M. Posterior and postero-lateral incidental durotomy during lumbar spine surgery: Primary repair versus augmented primary repair. Egypt J Neurosurg. 2021;36(26):2-6. [available at]
9. Massimo M, Filippo M, Stefano F, Maria A, Luca R, Alessandro R, Antonino R. The use of surgical sealants in the repair of dural tears during non-instrumented spinal surgery. Eur Spine J. 2013;23(8):1761-1766. DOI: 10.1007/s00586-013-3138-1
10. Yu F, Wu F, Zhou R, Guo L, Zhang J, Tao D. Current developments indural repair: A focused review on new methods and materials. Front Biosci. 2013;18(4):1335-1343. DOI: 10.2741/4182

11. Cobb A, Badylak F, Janas W, Simmons-Byrd A, Boop A. Porcine small intestinal submucosa as a dural substitute. Surg Neurol. 1999;51(1):99-104. DOI: 10.1016/s0090-3019(97)00475-8
12. Knopp U, Christmann F, Reusche E, Sepehrnia A. A new collagen biomatrix of equine origin versus a cadaveric dura graft for the repair of dural defects--a comparative animal experimental study. Acta Neurochir. 2005;147(8):877-887. DOI: 10.1007/s00701-005-0552-0
13. Flynn E. The use of decellularized adipose tissue to provide an inductive microenvironment for the adipogenic differentiation of human adipose-derived stem cells. Biomater. 2010;31(17):4715-4724. DOI: 10.1016/j.biomaterials.2010.02.046
14. Guyette JP, Gilpin SE, Charest JM, Tapias LF, Ren X, Ott HC. Perfusion decellularization of whole organs. Nat Protoc. 2014;9(6):1451-1468. DOI: 10.1038/nprot.2014.097
15. Baptista PM, Orlando G, Mirmalek-Sani SH, Siddiqui M, Atala A, Soker S. Whole organ decellularization-a tool for bioscaffold fabrication and organ bioengineering. Ann Int Conf IEEE Eng Med Biol Soc. 2009:6526-6529. DOI: 10.1109/iembs.2009.5333145
16. Al-Hyani OH. Histological comparison between histoacryl and suturing to close lung wounds in dogs. Iraqi J Vet Sci. 2023;37(3):751-758.DOI: 10.33899/ijvs.2023.136382.2578
17. Aesculap, Inc.- a B. Braun company. DOC1118-RevA-Lyoplant-Onlay-Brochure. 2013. https://www.aesculapusa.com/.
18. Taha AN, Ismail H KH. The amelioration of vitamin E on histological changes of rabbit's brain treated with zinc oxide nanoparticles. Iraqi J Vet Sci. 2022;37(1):95-104. DOI: 10.33899/ijvs.2022.133599.2265
19. Sultana J, Molla MR, Kamal M, Shahldullah M, Begum F, Bashar MA. Histological differences in wound healing in maxillofacial region in patients with or without risk factors. Bangladesh J Pathol. 2009;24(1):3-8. DOI: 10.3329/bjpath.v24i1.2874
20. Koch BC, Daniels LO, Thomsen LT, Nielsen MM, Berendt M, Gredal H. Collection of cerebrospinal fluid into EDTA versus plain tubes does not affect the standard analysis in dogs. Acta Vet Scand. 2019;61(23):2-7 DOI: 10.1186/s13028-019-0457-1
21. Richard A, Herwig A, Markus G, Amulya K. Decellularized ovine esophageal mucosa for esophageal tissue engineering. Technol Health Care. 2012;20(3):215-223. DOI: 10.3233/thc-2012-0672
22. Gazzeri R, Neroni M, Alfieri A, Galarza M, Faiola A, Esposito S, Giordano M. Transparent equine collagen biomatrix as dural repair. A prospective clinical study. Acta Neurochir. 2009;151(5):537-543. DOI: 10.1007/s00701-009-0290-9
23. Bernd E, Kunze C, Freier T. Poly(3-hydroxybutyrate) (PHB)patches for covering anterior skull base defects-an animal study with minipigs. Acta Otolaryngol. 2009;129(9):1010-1017. DOI: 10.1080/00016480802552493
24. Esposito F, Cappabianca P, Fusco M. Collagen-only biomatrix as a novel dural substitute. Examination of the efficacy, safety, and outcome: clinical experience on a series of 208 patients. Clin Neurol Neurosurg. 2008;110(4):343-351. DOI: 10.1016/j.clineuro.2007.12.016
25. Wang F, Guo F, Ying J. Multilayer scaffold of electrospun plapcl-collagen nanofibers as a dural substitute. J Biomed Mater Res B Appl Biomater. 2013;101(8):1359-1366. DOI: 10.1002/jbm.b.32953
26. Fontana R, Talamonti G, D'Angelo V, Arena O, Monte V, Collice M. Spontaneous haematoma as unusual complication of silastic dural substitute. Report of 2 cases. Acta Neurochir. 1992;115(1-2):64-66. DOI: 10.1007/bf01400594
27. Van Calenbergh F, Quintens E, Sciot R, Van Loon J, Goffin J, Plets C. The use of vicryl collagen as a dura substitute: A clinical review of 78 surgical cases. Acta Neurochir. 1997;139(2):120-123. DOI: 10.1007/bf02747191
28. Danish F, Samdani A, Hanna A, Storm P, Sutton L. Experience with acellular human dura and bovine collagen matrix for duraplasty after posterior fossa decompression for Chiari malformations. J. Neurosurg. 2006;104(1):16-20. DOI: 10.3171/ped.2006.104.1.16
29. Vanaclocha V, Saiz-Sapena N. Duraplasty with freeze-dried cadaveric dura versus occipital pericranium for chiari type I malformation: Comparative study. Acta Neurochir. 1997;139(2):112-119. DOI: 10.1007/bf02747190
30. Lam C, Penumaka A, Chen C, Fischer G Kasper M. Fibrin sealant augmentation with autologous pericranium for duraplasty after suboccipital decompression in chiari 1 patients: A case series. Surg Neurol Int. 2013;4:1-6. DOI: 10.4103/2152-7806.106262
31. Forseth M, O'Grady K, Toriumi M. The current status of cyanoacrylate and fibrin tissue adhesives. J Long Term Eff Med Implants. 1992;2(4):221-233. [available at]
32. Sharma A, Kaur R, Kumar S, Gupta P, Pandav S, Patnaik B. Gupta A. Fibrin glue versus N-butyl-2-cyanoacrylate in corneal perforations. Ophthalmol. 2003;110(2):291-298. DOI: 10.1016/S0161-6420(02)01558-0
33. Eckersberger F, Moritz E, Wolner E. The application of fibrin sealant in tracheobronchial surgery. In: Schlag G, Redl H, editors. Fibrin sealant in operative medicine-thoracic surgery-cardiovascular surgery. Berlin: Springer-Verlag; 1986. 82-88 p. DOI: 10.1007/978-3-642-71633-1_11
34. Fundaro, P, Velardi R, Santoli C. Fibrin adhesive: Clinical application in coronary artery bypass graft surgery. Texas Heart Inst J. 1985;12(3):275-278. [available at]
35. Jakob H, Campbell D, Qiu K, Pick R, Replogle L. Evaluation of fibrin sealing for cardiovascular surgery. Circulation. 1984;70(3):138-146. [available at]
36. Bouvy M, Rosin E, Frishmeyer J. Evaluation of bovine fibrin sealant in the dog. J Invest Surg. 1993;6(3):241-250. DOI: 10.3109/08941939309141615
37. Koo J, Adamson R, Wagner F. A new cause of chronic meningitis: Infected lumbar pseudo meningocele. Am J Med. 1989;86(1):103-104. DOI: 10.1016/0002-9343(89)90238-6
38. Verner F, Musher M. Spinal epidural abscess. Med Clin North Am. 1985;69(2):375-384. DOI: 10.1016/s0025-7125(16)31049-5
39. Pogorielov M, Kravtsova A, Reilly C. Experimental evaluation of new chitin-chitosan graft for duraplasty. J Mater Sci Mater Med. 2017;28(2):34. DOI: 10.1007/s10856-017-5845-3
40. Grotenhuis A. Costs of postoperative cerebrospinal fluid leakage: 1-year, retrospective analysis of 412 consecutive nontrauma cases. Surg Neurol. 2005;64(6):490-494. DOI: 10.1016/j.surneu.2005.03.041
41. Biroli F, Fusco M, Bani G, Bani G, Signorelli A, de Divitiis O. Novel equine collagen-only dural substitute. Neurosurg. 2008;62(1):273-274. DOI: 10.1227/01.neu.0000317404.31336.69
42. Yamada K, Miyamoto S, Nagata I, Kikuchi H, Ikada Y, Iwata H. Development of a dural substitute from synthetic bioabsorbable polymers. J Neurosurg. 1997;86(6):1012-1017. DOI: 10.3171/jns.1997.86.6.1012
43. Klekamp J. Surgical treatment of Chiari I malformation-analysis of intraoperative findings, complications, and outcome for 371 foramen magnum decompressions. Neurosurg. 2012;71(2):365-380. DOI: 10.1227/NEU.0b013e31825c3426
44. Borgesen E, Vang S. Extradural pseudocysts. A cause of pain after lumbar-disc operation. Acta Orthop Scand. 1973;44:12-20. [available at]
45. Bosacco J, Gardner J, Guille T. Evaluation and treatment of dural tears in lumbar spine surgery: A review. Clin Orthop Relat Res. 2001;389:238-247. DOI: 10.1097/00003086-200108000-00033
46. De almeida R, Ghotme K, Leong I, Drake J, James L, Witterick J. A new porcine skull base model: fibrin glue improves strength of cerebrospinal fluid leak repairs. Otolaryngol Head Neck Surg. 2009;141(2):184-189. DOI: 10.1016/j.otohns.2009.03.008
47. Nishihira S, McCaffrey V. The use of fibrin glue for the repair of experimental CSF rhinorrhea. Laryngoscope. 1988;98(6):625-627. DOI: 10.1288/00005537-198806000-00009
48. Siedentop H, Grady K, Park J, Bhattacharya T, Sanchez B. Fibrin sealant for treatment of cerebrospinal fluid leaks. Otol Neurotol. 1999;20(6):777-780. [available at]
49. Abla A, Link T, Fusco D, Wilson A, Sonntag K. Comparison of dural grafts in chiari decompression surgery: Review of the literature. J Craniovertebr. Junction Spine. 2010;1(1):29-37. DOI: 10.4103/0974-8237.65479
50. Farber H, McDowell M, Alhourani A, Agarwal N, Friedlander M. Duraplasty type as a predictor of meningitis and shunting after chiari I

decompression. World Neurosurg. 2018;118:778-783. DOI: 10.1016/j.wneu.2018.07.050
51. Vanaclocha V, Saiz-Sapena N. Duraplasty with freeze-dried cadaveric dura versus occipital pericranium for chiari type I malformation: Comparative study. Acta Neurochir. 1997;139(2):112-119. DOI: 10.1007/BF02747190
52. Usher C, Fries G, Ochsner I, Tuttle D. Marlex mesh. A new plastic mesh for repairing tissue defects. Clin Stud AMA Arch Surg. 1995;78(1):138-145. DOI: 10.1001/1959.04320010140023
53. Shi D, Ming-Wang L, Zhong-Zong Q, Qin-mei W, Hai-Yong H, Ying G, Zhong-He Y. A new modified dura mater implant: characteristics in recipient dogs. Br J Neurosurg. 2009;23(1):71-75. DOI: 10.1080/02688690802582638
54. Zhang L, Yang Z, Jiang W, Zeng T. Extensive duraplasty with autologous graft in decompressive craniectomy and subsequent early cranioplasty for severe head trauma. Chin J Traumatol. 2010;13(5):259-264. [available at]
55. Bejjani K, Zabramski J. Safety and efficacy of the porcine small intestinal submucosa dural substitute: results of a prospective multicenter study and literature review. J Neurosurg. 2007;106(6):1028-1033. DOI: 10.3171/jns.2007.106.6.1028
56. Narotam K, Van Dellen R, Bhoola D. A clinicopathological study of collagen sponge as a dural graft in neurosurgery. J Neurosurg. 1995;82(3):406-412. DOI: 10.3171/jns.1995.82.3.0406
57. Tatsui E, Martinez G, Li X, Pattany P, Levi D. Evaluation of dura gen in preventing peridural fibrosis in rabbits. Invited submission from the joint section meeting on disorders of the spine and peripheral nerves. J Neurosurg Spine. 2006;4(1):51-59. DOI: 10.3171/spi.2006.4.1.51
58. Wallis M, Borg D, Daly B, Deng B, Ballif A, Allen B. comparative assessment of detergent-based protocols for mouse lung de-cellularization and re-cellularization. Tissue Eng C Methods. 2012;18(6):420-432. DOI: 10.1089/ten.tec.2011.0567
59. Lim L, Jungebluth P, Ajalloueian F, Friedrich H, Gilevich I, Grinnemo H. Whole organ and tissue reconstruction in thoracic regenerative surgery. Mayo Clin Proc. 2013;88(10):1151-1166. DOI: 10.1016/j.mayocp.2013.03.011
60. Colaco M, Atala A. The future of transplant biology and surgery. Interdiscip Med. 2014;15:206-218. [available at]
61. Guyette P, Gilpin E, Charest M, Tapias F, Ren X, Ott C. Perfusion decellularization of whole organs. Nat Protoc. 2014;9(6):1451-1468. DOI: 10.1038/nprot.2014.097
62. Peter C, Thomas W, Gilbert, Stephen B. An overview of tissue and whole organ decellularization processes. Biomater. 2011;32(12):3233-3243. DOI: 10.1016/j.biomaterials.2011.01.057
63. Cheung T, Tong D, Perelman N, Ertl D, Nimni E. Mechanism of cross-linking of proteins by glutaraldehyde IV. In vitro and in vivo stability of a cross-linked collagen matrix. Connect Tissue Res. 1990;25(1):27-34. DOI: 10.3109/03008209009009810
64. Khor E. Methods for the treatment of collagenous tissues for bioprostheses. Biomater. 1997;18(2):95-105. DOI: 10.1016/s0142-9612(96)00106-8
65. Macchiarini P, Walles T, Biancosino C Mertsching H. First human transplantation of a bioengineered airway tissue. J Thorac Cardiovasc Surg. 2004;128(4):638-641. DOI: 10.1016/j.jtcvs.2004.02.042
66. Conconi T, DeCoppi P, DiLiddo R, Vigolo S, Zanon F, Parnigotto P. Tracheal matrices, obtained by a detergent-enzymatic method, support in vitro the adhesion of chondrocytes and tracheal epithelial cells. Transp Int. 2005;18(6):727-734. DOI: 10.1111/1432-2277.00082
67. Filippi M, Schwarz D, Voth R, Reisch P, Grunert P. Bovine pericardium for duraplasty: Clinical results in 32 patients. Neurosurg Rev. 2001;24(2):103-107. DOI: 10.1007/pl00012392
68. Al-Hyani OH, Al-Hasan AM. A comparison between two different biomaterials for treatment of tracheal defect in dogs. Iraqi J Vet Sci. 2019;33(2):317-327. DOI: 10.33899/ijvs.2019.162883
69. Platt J, DiSesa V, Gail D, Massicot-Fisher J. Recommendations of the national heart, lung, and blood institute heart and lung xenotransplantation working group. Circ. 2002;27;106(9):1043-7. DOI: 10.1161/01.0000031064.67525.28
70. Greifzu F, Breuskin D, Prajsnar A, Geletneky K, Bode F, Tuettenberg J, Oertel J, Stadie A. Assessment of the performance of Lyoplant® Onlay for duraplasty. An observational, multi-center Post Market Clinical Follow-up study. MedRxiv. 2022:2022-09. DOI: 10.1101/2022.09.08.22279716
71. Sekhar N, Mai C. Dural repair after craniotomy and the use of dural substitutes and duralsealants. World Neurosurg. 2013;79(3-4):440-442. DOI: 10.1016/j.wneu.2011.12.062
72. Vasilios A, Kenneth S, Julie B, Eugene B, Carl B. Repair of the dura mater with processed collagen devices. J Biomed Mater Res B Appl Biomater. 2007;83(2):580-8. DOI: 10.1002/jbm.b.30831

استخدام التامور البقري المجفف بالتجميد والغشاء المخاطي غير الخلوي لمريء الأغنام لإصلاح عيب الجافية الدماغية في الكلاب

عبد الله مزاحم الصائغ[١]، أسامة حازم الحياني[٢] و خالد وليد الحيالي[٣]

[١]قسم صحة المجتمع، كلية الصحة والتقنيات الطبية شيخان، جامعة دهوك التقنية، دهوك، [٢]فرع الجراحة وعلم تناسل الحيوان، كلية الطب البيطري، جامعة الموصل، [٣]مستشفى ابن سينا التعليمي، مديرية صحة نينوى، الموصل، العراق

الخلاصة

تم تصميم هذا البحث لمعرفة كفاءة التامور البقري المجفف بالتجميد والغشاء المخاطي غير الخلوي لمريء الأغنام كأنسجة مغايرة الطعم مع غراء الفيبرين لإصلاح عيب الجافية المستحدث في الكلاب. تم استخدام ثمانية عشر كلبا بالغًا في هذه الدراسة وتم تقسيمهم عشوائيًا إلى مجموعتين. في جميع الحيوانات تم عمل عيب مربع حوالي (١ × ١ سم) في الأم الجافية. في المجموعة الأولى تم إغلاق الأذى بالتامور البقري المجفف بالتجميد بينما في المجموعة الثانية تم إغلاق الأذى بقطعة من الغشاء المخاطي غير الخلوي لمريء الأغنام. تم تقييم النتائج من خلال مراقبة العلامات السريرية بعد العملية الجراحة. وكذلك تمت دراسة التغيرات العيانية والنسيجية المرضية وقياس ضغط السائل الشوكي الدماغي مع تحليل العد الكلي للخلايا ومستوى الجلوكوز والبروتين للسائل الشوكي الدماغي في اليوم ٦٠,٣٠,١٥ بعد العملية ولكلا المجموعتين. بقيت جميع الحيوانات على قيد الحياة بعد إجراء العملية دون حدوث أي مضاعفات خاصة المتعلقة بظهور العلامات العصبية غير الطبيعية. عيانيا تم انسداد موقع العملية في كلا المجموعتين تمامًا ولم يلاحظ أي تسرب للسائل الشوكي الدماغي بالإضافة إلى ذلك تم انسداد أذى الجافية داخليًا حيث اتصلت الأنسجة المطعمة تمامًا مع الأم الجافية للمضيف. تمثلت التغيرات النسيجية المرضية في تكوين النسيج الضام مع ارتشاح الخلايا الالتهابية وتكوين الأوعية الدموية. وظهر تكاثر الأنسجة الضامة وكثرة تكوين الأوعية الدموية وقلة أو انعدام ارتشاح الخلايا الالتهابية والعد الكلي للخلايا بشكل واضح في المجموعة الأولى عن الثانية. وظهر ضغط السائل الشوكي الدماغي، مستوى الجلوكوز والبروتين ضمن المعدل الطبيعي خلال جميع فترات الدراسة في كلا المجموعتين. بالاستنتاج يمكن استخدام كلا الأنسجة المطعمة مع غراء الفيبرين لإصلاح عيب الجافية في الكلاب.