

## A comparative Study of Two Techniques for Repairing of Tracheal Defect in Dogs.

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### Summary

The objective of this study was designed to evaluate the possibility of repairing tracheal cartilage defect in dogs. 18 local breed dogs of both sexes was used in this study, they are allocated into 2 equal groups. A tracheal defect was induced in the cervical part of the trachea as a window about 3cm x 2cm in diameter. The defect was closed in 1<sup>st</sup> group by using polypropylene mesh and bone cement substance, while in 2<sup>nd</sup> group polypropylene mesh with fresh auto- bone marrow. Post-operative study including, clinical observation, gross pathology and histopathological evaluation was performed in all animals. The most important postoperative clinical observation was represented by subcutaneous emphysema at the site of operation in the 2nd group animals, which gradually disappeared within few days. Otherwise no other important complications was reported in both groups during the period of the experiment. The gross pathological changes and biopsy collection for all animals was done at 15, 30, 60 postoperative days. The gross examination revealed complete closing of the induced tracheal defect in all operated animals and a mild adhesion with the surrounding tissues. In both groups, the histopathological features was represented by newly granulation tissue formation and areas of hyaline cartilage degeneration and necrosis. The cartilage regeneration was showed only in 2nd group through by formation of new cartilage cells. In conclusion, it can use both techniques for reconstruction of tracheal defect in dogs but the auto bone marrow group was regarded the best due to improvement of the healing process.

**Keywords:** Tracheal defect healing, Bone cement, Bone marrow, Polypropylene mesh.

### Introduction

Tracheal restoration is one of the biggest challenges and sophisticated thoracic surgery particularly when end-to-end anastomosis is impossible or after this procedure failed. Generally, the most important indications for tracheal restoration include neoplasm, tracheoesophageal fistula, trauma and injuries, congenital stenosis and tracheal collapse (1 and 2). The trachea should not be treated as a simple tube because the action of trachea as a canal for transfer the air, and it should put in consideration its anatomical and histological characters of mucosa, hyaline cartilage, and segmental blood supply make it more challenging to repair (3-5). The perfect synthetic materials (tracheal prosthesis) used for tracheal defect should be sealed, sufficient constancy, and well tolerated by the host. It should also cause minimal inflammatory

reactions but still be incorporated by the surrounding tissue (6). Several kind of prosthetic and tissue grafts have been applied to repair such defects but with limited success due to graft ischemia and immune rejection leading to failure or dehiscence of anastomosis and stenosis of the trachea (7-9).

Tracheal defects can be treat by a variety of methods with little success. The different tracheal substitutes and techniques of restoration were analyzed (10), who classified them in five categories: foreign materials, nonviable tissues, auto-genous tissues, tissue engineering, and tracheal transplantation. The use of prosthetic materials, tissue flaps, stents, auto grafts, or a mixture of these methods have been reported, but complications associated with these methods including migration, dislodgement, material degradation failure,

chronic bacterial infection, obstruction because of granulation tissue formation, stenosis, necrosis, anastomosis failure, long duration immunosuppression, lack of suitable donor source, decrease of adequate vascularization, and epithelium (11). A number types of synthetic or natural subjects were used to repair tracheal defects such as muscle flap, mesh patch fashioned from a nickel-titanium, Marlex mesh and prosthesis framework made from high-porous synthetic material lined with autogenous mucosa and biodegradable glues such as gelatin are used to improve an airtight attachment between host tissue and the prosthesis (12-14).

Polypropylene is amonofilament, macroporous mesh is taking consideration of researchers due to its infection resistance, hydrophobicity, inert nature and strong material (15). The high biocompatibility of polypropylene mesh and its low cost were the main cause for wide use of polypropylene in biological prostheses (16). The cement is an acrylic resin prepared from a mixture of Methylemetacrylate (MMA) liquid and the powder of Poly Methylemetacrylate (PMMA). Poly (methyl methacrylate), is a widely consumed biomaterial because its properties of biocompatibility, non-toxic and mechanical resistance (17). Bone marrow is a complex tissue comprised of hematopoietic precursors, their differentiated progeny, and a connective tissue network referred to as stroma (18). The (bone marrow-derived adult mesenchymal stem cells are involved in tracheal regeneration (19). The aim of this study was to evaluate the ability of polypropylene mesh with bone cement or with fresh auto-transplantation of bone marrow to repair tracheal defect in dogs.

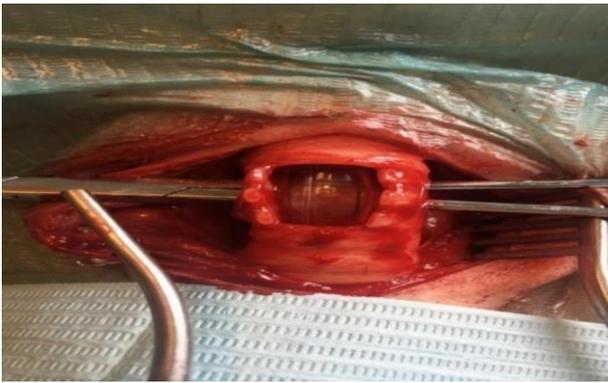
### **Materials and Methods**

18 local breed adult dogs of both sexes aged between (1-3) years were used in this study. The animals were divided into two equal groups. All operations were performed under general anesthesia using a mixture of ketamine HCL at 15 mg/kg and xylazine 2% at 5mg/kg B.W., given in a single syringe by I.M. route in the thigh muscles, then endotracheal intubation was applied. 10 cm surgical incision was made in the ventral aspect at mid 3rd of the neck through skin and subcutaneous tissue. The sternohayoidous

muscles were dissected bluntly to exposed the trachea. Then the trachea was isolated gently from the surrounding tissue to avoid any inadvertent damage to the related vital structures. In all animals about (3cm x 2cm) a window like defect was established in the trachea by removing ventral half of the cartilage of three tracheal rings (Fig. 1). In both groups, the defect of the trachea was covered by a suitable piece of polypropylene mesh (as a basement substance) and fixed with excised edges of trachea by few simple interrupted pattern stitches using nylon thread (Fig. 2).

In the G1, a thin layer of a soft bone cement (Argentina [www.laboratoriossl.com](http://www.laboratoriossl.com)) was spread on the mesh. The bone cement paste was prepared by mixing poly methylmetacrylate powder and methyl metacrylate liquid in a sterile flask. This material need several minutes to be consolidated (Fig. 3), then endo- tracheal tube was removed. The muscles of neck was closed with simple continuous pattern using cat gut while the skin was closed by simple interrupted pattern using silk.

In G2, a 2-3 ml of bone marrow was aspirated from the head of the femur of the same animal, and directly was spread on the mesh that covering the tracheal defect then the wound was left for several minutes to clot, after that the wound was closed (Fig. 4) like in G1. The experimental dogs had received penicillin streptomycin in a dose 10,000 I.U. and 10mg /kg B.W. respectively for 3post operative days by I.M. injection. The biopsy collection were performed in all groups at 15, 30 and 60 postoperative days. The biopsy was included the grafted material with one proximal and distal normal tracheal cartilage rings after euthanasia of animals. The biopsy was fixed in 10% buffered formalin solution, then processed and stained with hematoxycline and eosin stain as well as Masson's trichrome as a special stain.



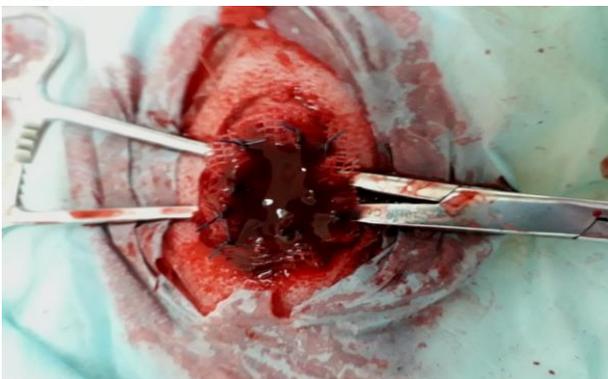
**Figure, 1:** Shows trachea of dog in which 2x3cm tracheal defect were created.



**Figure, 2:** Shows applied polypropylene mesh on tracheal defect of dog.



**Figure, 3:** Shows tracheal defect of dog in G1 were covered by polypropylene mesh and bone cement.



**Figure, 4:** Shows tracheal defect of dog in G2 were covered by polypropylene mesh and bone marrow.

## Results and Discussion

In both groups animals show slight swelling at the site of operation which gradually subsided within first week after operation. Post-operative subcutaneous emphysema was developed in two animals in G2, this emphysema had disappeared within few days after operation, while no subcutaneous emphysema was recorded in G1. This might be due to air leaks through the pores of mesh in G2 during respiration but its disappeared gradually because of defect closure by fibrous tissue formation and compression of surrounding tissue, while disappearance of emphysema in G1 might be due to seal the pores of mesh with bone cement substance which prevent air to leak. There was no clinical respiratory problem, cough or pneumonia in all animals, additionally there was no evidence of hoarseness and all animals start barking a few days after the operation .

In G1 and G2, adhesion with surrounding tissues at 15 postoperative day were seen. The adhesion was increased slightly in both groups after 30 postoperative day then its decreased at 60 postoperative day in G2 but in G1 hadn't subside and appeared as external hard mass. The formation of adhesion between the site of grafting and surrounding tissues in both groups were occurred as a result of inflammatory response to tissue injury and grafting materials (20). Mesh implantation will naturally generate an inflammatory response. A minimal response includes the formation of fibrosis around the prosthesis was showed; this response is generated with the best form of biocompatibility (20). The adhesion was relatively lesser in early stages in G1 rather than that seen in G2 and this might to due to poor anchorage of the host tissue on the bone cement (21), but at last stage of study, the adhesion was more noticed at site of grafting and this may be due to the irritable nature of methyl metacrylate, where the formed fibrous tissue was enclosed and bounded of it (22) and absence of liquid pocket (21). Also (23) had showed that the residue of monomer of PMMA can be leaks to out by diffusion from prosthesis and cause irritation to surrounding tissues .

In both groups, no stenosis or any excessive tissue formation was reported into the tracheal

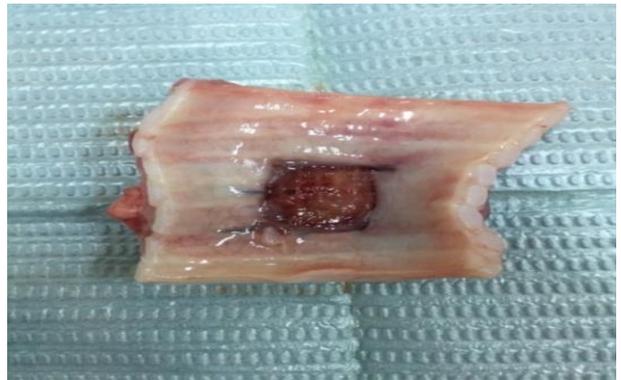
lumen, in addition good binding between the grafted material and trachea was seen (Fig. 5 and 6). The tracheal defect was still appear in the mucosal surface after 15 and 30 postoperative days in both groups (Fig.7-10), then the depression had disappeared relatively at 60 postoperative day in both groups (Fig. 11 and 12). Generally utilizing of prosthetic materials for long-segment tracheal reconstruction has been limited due to some conditions such as graft relocation, in growth of fibrous tissue, and stenosis (24). In both groups the tracheal lumen was still opened and did not occluded by granulation tissue formation. The polypropylene mesh was interlocked and connected intact with the host trachea via newly granulation tissue formation. The formation of collagen fibers with extension of it within the mesh and newly blood vessels formation after grafting was indicated to success of implantation to close the tracheal defect (25 and 26).



**Figure, 5:** Shows binding polypropylene mesh and bone cement with trachea of dog in G1 at 60 postoperative day.



**Figure, 6:** Shows binding polypropylene mesh and bone marrow with trachea of dog in G2 at 60 postoperative day.



**Figure, 7:** Shows tracheal defect of dog was still appeared at 15 postoperative day in G1 (polypropylene mesh and bone cement).



**Figure, 9:** Shows tracheal defect of dog was still appeared at 30 postoperative day in G1.



**Figure, 10:** Shows tracheal defect of dog was still appeared also at 30 postoperative day in G2.



**Figure, 11:** Shows disappearance tracheal defect of dog relatively at 60 postoperative day in G1.



**Figure, 12:** Shows disappearance tracheal defect of dog relatively at 60 postoperative day in G2.

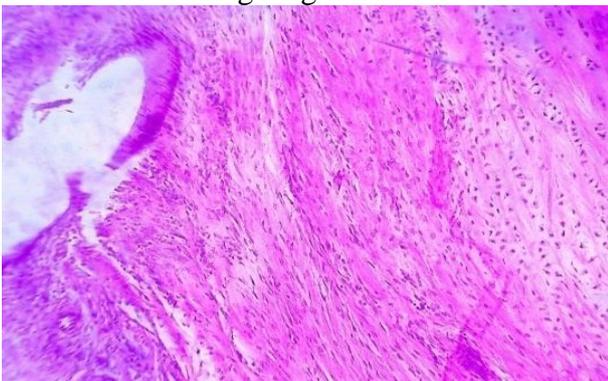
In both groups, the histopathological examination after 15th postoperative day showed new granulation tissue formation (collagen fibers, fibroblast and newly blood vessels) (Fig.13 and 14). Large number of new capillaries were observed in the site of implantation in G2. Staining with Masson's tircrom was revealed proliferation of collagen fibers in G1 and G2. The maturation of collagen fibers was increased at 30 and 60 postoperative days respectively in both groups also (Fig.15 and 16). The proliferation and maturation of collagen fibers formation and fibroblast was coincided with (27) that fibroblastic activity peaks one to two weeks post-wounding where the optimum quantity of fibroblasts needed for a successful integration of the mesh is achieved approximately two weeks after wounding. The presence of fibroblast is necessary for reconstruction of tracheal defect (28).

In G1, at 15 postoperative day, some sections showed infiltration of inflammatory cells in mucosal and sub mucosal layer of trachea with hyperplasia of epithelial lining cells (Fig.17). In G2, also there were infiltration of inflammatory cells in mucosal and sub mucosal layer of trachea. After 30 postoperative day in G1, there were infiltration of lymphocyte and plasma cells with presence of fibroblast in dense collagen fibers, while in G2 at 30 postoperative day, there were acute inflammatory process which characterized by infiltration of neutrophils. Chronic inflammatory process also was seen which characterized by formation of granulomatous reaction (Fig.18). In both groups, the inflammatory process at 60 postoperative day was seen in mucosal and sub mucosal layers,

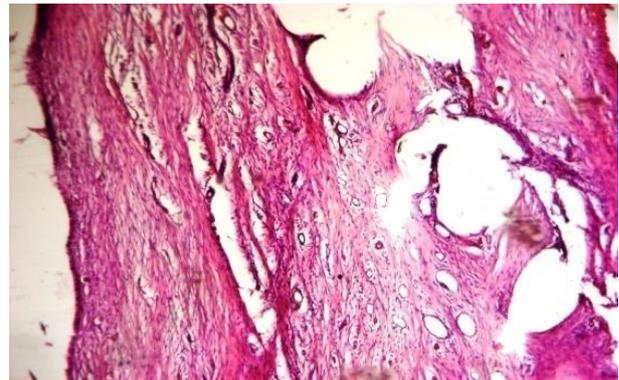
which characterized by infiltration of lymphocytes, macrophages and plasma cells and giant cells (Fig.19-21). The infiltration of mononuclear inflammatory cells (lymphocyte, macrophage, plasma cells and foreign body giant cells) was agreed with (29) whose said that such prosthetic materials may possibly cause allergic reaction and infection that lead to infiltration of mononuclear inflammatory cells. Some research revealed the use of polypropylene mesh was characterized by minimum tissue reaction when implanted even in infected and non-infected wound. In G1, the infiltration of inflammatory cells was lesser rather than G2 and this might be due to the bone cement substance has antibiotic effect. Other researchers (30) was showed, the MIC test demonstrated that the silver/PMMA nanofiber had enhanced antimicrobial efficacy compared to that of silver sulfadiazine and silver nitrate at the same silver concentration. A physical response triggers an acute inflammatory reaction, which involves the formation of giant cells and subsequently granulomas, meaning that the tissue is "tolerating" the mesh fairly well (20).

In G1 and G2, the site of cutting at 15 postoperative tracheal cartilage degeneration and necrosis (Fig.22 and 23). At 60 postoperative day, the microscopic examination in both groups was indicated the presence of degeneration, necrosis and sloughing of ciliary epithelial lining of mucosal layer with some region of tracheal hyaline cartilage necrosis. The occurrence of necrosis and degeneration of tracheal cartilage in some area at the site of cutting might be due to ischemia that result from interruption of blood supply innervations during removal tracheal segment. The developmental of epithelium of tracheal mucosa was not shown and this is regarded the major problems in tracheal prosthesis (31 and 32). The authors (33) was showed the injury to the outer layer of tracheal or larynx cartilage was characterized by regressive changes of the outer zone with loss of chondrocytes (necrosis) and loss of matrix proteoglycans possibly due to resorption by macrophages, and polymorphonuclear cells, eliminating enzymes and cytokines.

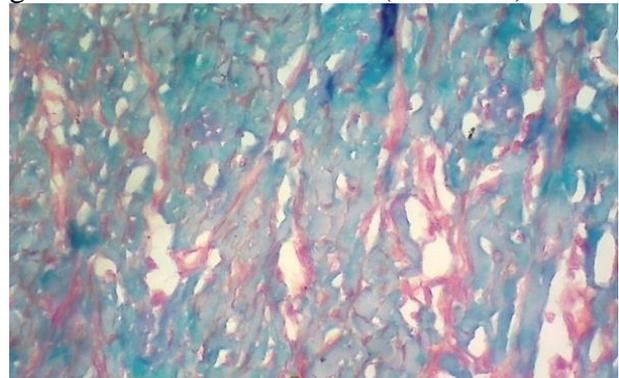
In G2 only at 30 and 60 postoperative day respectively, there were new cartilage regeneration represented by proliferation of chondrocytes (Fig.24), this findings revealed the ability to use bone marrow to repair the tracheal defect. In G1, disappearance of cartilage regeneration may be due to poor healing of cartilage because nature of blood circulation (34 and 35), in addition the production of heat resulted from polymerization of MMA powder with the liquid lead to some tissue defect or necrosis (36), although the cells that contact with the PMMA were growing naturally without alteration in their morphologies and natural processes of cell proliferation (37). In G2, the applied of fresh bone marrow on the mesh was improved the degree of healing through the formation and proliferation of collagen fibers, and regeneration of tracheal cartilage through proliferation of the chondrocytes. This features was in agreement with (38) who's founded the mesenchymal cells that isolated from bone marrow have ability to differentiate into different cells such as fibroblast and chondrocyte also the stem cells had the ability to form some connective tissue. In addition the main functions of stem/progenitor cells for the airway epithelium are epithelial homeostasis and the repair of defects in the airway wall (39). While (40) were showed, differentiation of the bone-marrow derived mesenchymal stem cells considered to have more potential for tracheal cartilage regeneration.



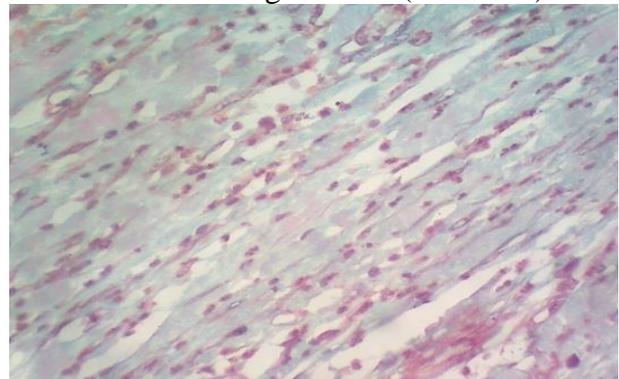
**Figure, 13:** Digital photomicrograph of tracheal defect at 15 postoperative day in G1 shows new granulation tissue formation (collagen fibers, fibroblast) (H&EX10).



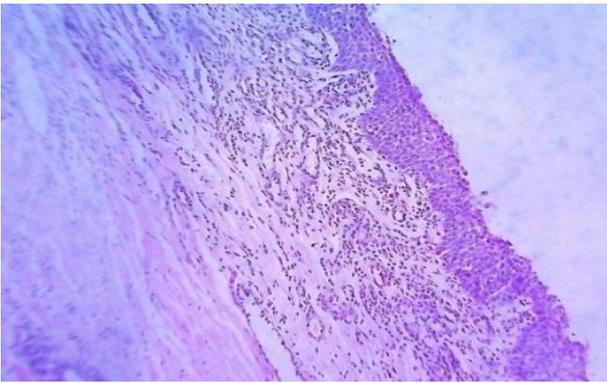
**Figure, 14:** Tracheal defect at 15 postoperative day in G2 shows new granulation tissue formation (H&EX10).



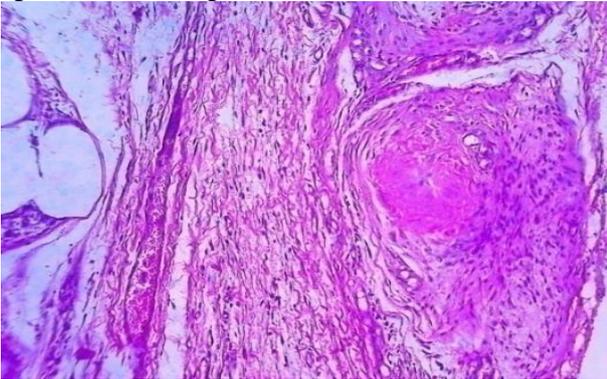
**Figure, 15:** Tracheal defect at 60 postoperative day in G1 with Masson's trichrom shows more maturation for collagen fibers (H&EX10).



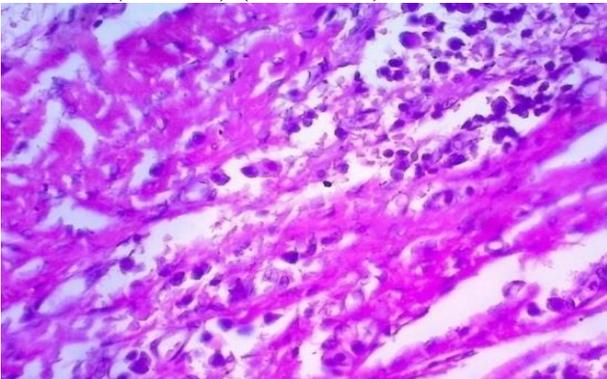
**Figure, 16:** Tracheal defect at 60 postoperative day in G2 with Masson's trichrom shows increases in maturation of collagen fibers (H&EX10).



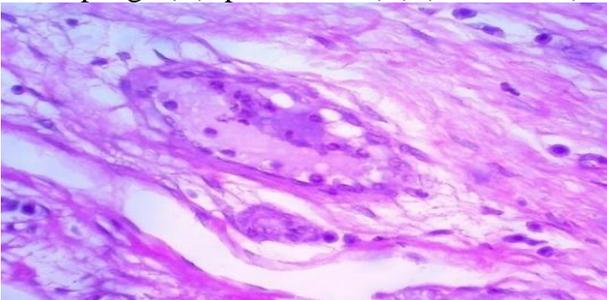
**Figure, 17:** Tracheal defect at 15 postoperative day in G1 shows infiltration of inflammatory cells (A) and hyperplasia of epithelium lining cells (B) ( H&E X).



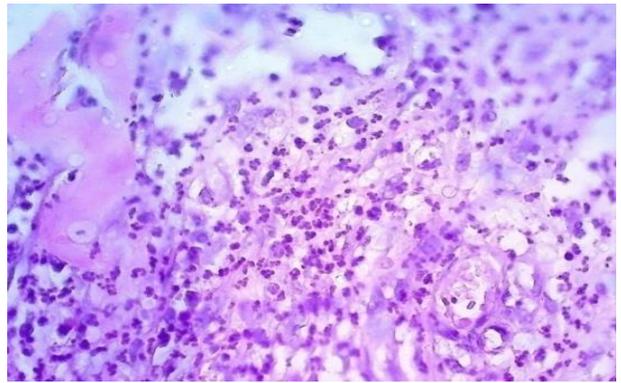
**Figure, 18:** Tracheal defect at 30 postoperative day in G2 shows granulomatous reaction (chronic) (H&EX10).



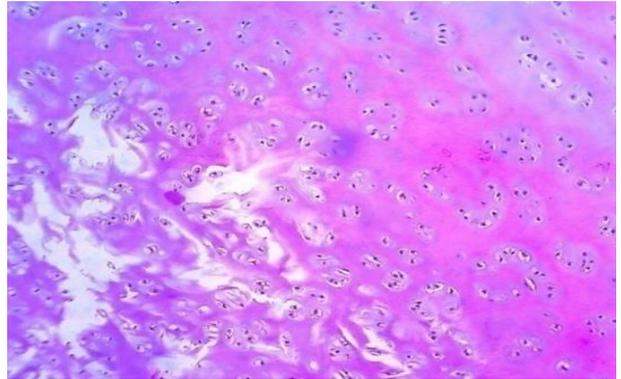
**Figure, 19:** Tracheal defect at 60 postoperative day in G1 infiltration of inflammatory cells (lymphocyte (A), macrophage (B), plasma cell(C) ( H&E X40).



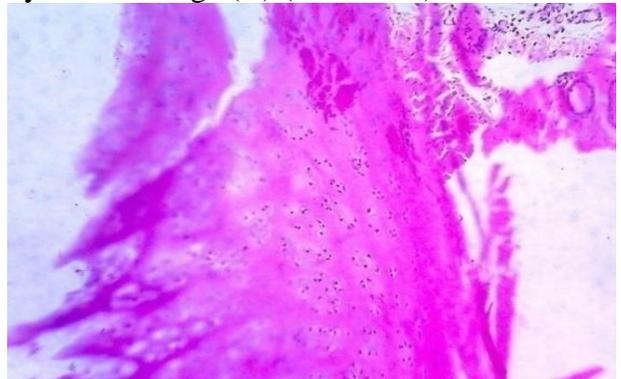
**Figure, 20:** Tracheal defect at 60 postoperative day in G1 giant cells (A) (H&E X40).



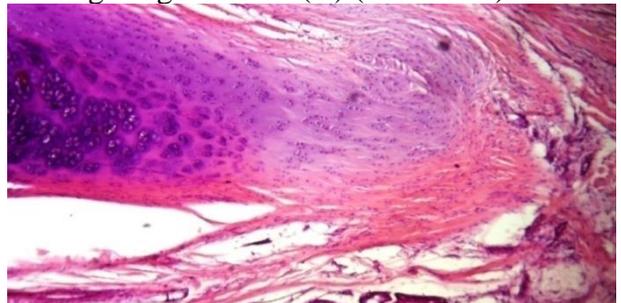
**Figure 21:** Tracheal defect at 60 postoperative day in G2 infiltration of mononuclear inflammatory cells (A) and (giant cells (B) (H&EX40).



**Figure, 22:** Tracheal defect at 15 postoperative day in G1 shows necrosis of hyaline cartilage (A) ( H&EX10).



**Figure, 23:** Tracheal defect at 15 postoperative day in G2 shows tracheal cartilage degeneration (A) ( H&EX10).



**Figure, 24:** Tracheal defect at 30 postoperative day in G2 shows new cartilage tissue formation (A) ( H&EX10).

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## دراسة مقارنة لتقنيتين لإصلاح عيوب القصبه الهوائية في الكلاب

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### الخلاصة

إن الهدف من تصميم البحث هو لتقييم إمكانية إصلاح عيوب غضروف القصبه الهوائية في الكلاب. استخدمت في هذه الدراسة 18 من الكلاب المحلية ومن كلا الجنسين. قسمت إلى مجموعتين متساويتين. تم استحداث أذى في الجزء العنقي من القصبه الهوائية على شكل نافذة وبقطر حوالي 3 سم \* 2 سم. تم غلق الأذى في المجموعة الأولى باستخدام مادة اسمنت العظم الصناعية مع شبكة البولي بروبيلين والمجموعة الثانية باستخدام شبكة البولي بروبيلين مع نخاع العظم الذاتي. تضمن تقييم الدراسة ما بعد العملية على ملاحظة العلامات السريرية، التغيرات العيانية والنسجية المرضية لكل الحيوانات. أظهرت النتائج السريرية بعد إجراء العملية حدوث نفاخ هوائي تحت الجلد في منطقة إجراء العملية الجراحية في المجموعة الثانية والتي اختفت تدريجياً بعد أيام قليلة إضافة إلى عدم حدوث أي مضاعفات مهمة في كلا المجموعتين خلال فترة الدراسة. تم ملاحظة التغيرات العيانية واخذ الخزع النسجية لكل الحيوانات خلال 15 و30 و60 يوم بعد إجراء العملية على التوالي حيث أظهرت النتائج العيانية إلى غلق الأذى المستحدث في كل الحيوانات مع وجود التصاقات قليلة مع الأنسجة المجاورة أما النتائج النسجية فقد أظهرت تكوين نسيج حبيبي جديد مع مناطق تنكس وتنخر للغضروف الزجاجي. تجدد الغضروف لوحظ فقط في المجموعة الثانية من خلال تكوين للخلايا الغضروفية الجديدة نستنتج منه يمكن استخدام كلا التقنيتين لغلق عيوب القصبه الهوائية في الكلاب ولكن الطريقة الثانية تعتبر الأفضل بسبب تحسين عملية الالتئام.

الكلمات المفتاحية: التئام أذى القصبه الهوائية، اسمنت العظم، نخاع العظم، شبكة البولي بروبيلين.