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Using polypropylene mesh or ear cartilage autograft with mini screws for the treatment of induced cleft hard palate in puppies: A comparative study

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Abstract

This experimental study aims to repair induced cleft hard palate of puppies by means of two different techniques. Eighteen puppies are divided into two equal groups. After the induction of general anesthesia by injecting a mixture of 2% xylazine at dose 2mg/kg and 10% ketamine HCL at dose 10 mg/kg., a cleft about (1x 2 cm) in diameter is established in the hard palate for each puppy. The induced cleft is closed in the first group with a polypropylene mesh, while in the second group the cleft is closed with a piece of ear cartilage autograft. In both groups, histoacryl is used. The results of the study are evaluated by monitoring clinical signs after operation. The gross and histopathological changes in both groups at the 15^{th}, 30^{th}, 45^{th} postoperative days are studied, jointly with a statistical analysis for histological scoring. No sign of aspiration pneumonia and water or milk leakage from nostrils is seen postoperatively, except some animals in group one suffered from mild, interrupted degrees of sneezing that disappeared after one week postoperatively. Grossly, the induced cleft hard palate is closed for all animals at the end of the experimental study and the implanted subjects infused and interlocking is done with tissues of the hard palate. The histopathological changes in both groups are represented by granulation tissue formation, few infiltrations of inflammatory cells and good mucosal regeneration. Finally, it is concluded that we can use these materials for closing the induced cleft in the hard palate of puppies with priority for ear cartilage.

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Introduction

Cleft palate or palatoschisis can be defined as a congenital defect that is characterized by an abnormal connection between nasal and oral cavities. It is regard one of the craniofacial defects of different animals such as dogs, cats, horse, sheep, cattle and gorillas (1-6). The development of cleft palate leads to the failure of normal feeding or process of suckling and the development of aspiration pneumonia (7,8). There are several methods to repair cleft palate, such as bone graft or palatine prosthesis where the surgical correction is still the most successful treatment, especially when the growth of maxillofacial was stopped relatively. In spite of the fact that cleft palate is the most common congenital craniofacial defect in dogs, most puppies are euthanized or die because of aspiration pneumonia (8). The corrective surgery can be performed at an age of about 4-6 months. Until the puppy is grown, nutrition should be provided to minimize the risk of aspiration pneumonia (9). The surgical correction may be failure at early stages (10) because a high degree of suture or tissue dehiscence occurrence (11). Therefore, until puppies grow older, it is challenging for surgeons and euthanasia of puppies is indicated due to the cost of intensive care requirement (12). Polypropylene mesh is a monofilament mesh that is commonly used as a biological prosthesis to treat some conditions as in hernial repair because of its ideal characteristics such as low cost, resistance to infection, strong and inert in nature with biocompatibility (13-18). Ear cartilage is used as a graft for different purposes and

locations with good results as in repairing of oronasal fistula (19) although it causes structural anomalies of the donor (20-22). Different types of adhesive tissue glue are used to close wounds and for hemostasis such as histoacryl (n-2-butyl-cyanoacrylate), which becomes solid when it contacts water and blood. It is used with successful results to repair some conditions such as in the closure of superficial lacerations, implantation of cartilage and in rhinoplasty (23-26).

The aim of this research is to evaluate the efficiency of a polypropylene mesh and an ear cartilage as autografts with mini screws instead of suturing to repair an induced cleft in the hard palate of puppies.

Materials and methods

Experimental animals

The total number of animals in this study are eighteen puppies of both sexes aging 2 -3 months old. They are divided into two equal groups. Each group consists of 9 animals. The animals are examined clinically to ensure their safety from any diseases or oral congenital defects. All animals are kept in specific cages in the animal house at College of Veterinary Medicine, University of Mosul.

Ethical approval

The research is approved by the Ethics Committee at College of Veterinary Medicine, University of Mosul. Reference no. UM. VET.2022.026.

Anesthesia

Before surgical operation, all animals are fasted from food and water. The animals are recumbent on a dorsal recumbency after induction of general anesthesia using a mixture of 10 % ketamine HCL at dose 10 mg/kg and 2% xylazine at dose 2mg/kg, given in a single syringe intramuscularly in the thigh muscles with atropine sulphate as a premedication at dose 0.05 mg/kg., subcutaneously (27).

Surgical operation

After induction of general anesthesia, an endotracheal tube was inserted into the trachea post the general anesthesia to prevent the occurrence of aspiration pneumonia due to the entrance of blood or any foreign materials to the lung during surgical operations. In all puppies, a cleft about (1x2 cm) in diameter was established in the hard palate. The nasal septum was left intact. The induced cleft in the hard palate of puppies of the first group was repaired by applying a double layer of a piece of polypropylene mesh, while for the second group, a piece of ear cartilage that is taken from the same animal was used to close the cleft. In both groups, the grafted piece of polypropylene mesh and ear cartilage was fixed to the hard palate using mini screws without the use of any suturing. In this work histoacryl (n-2-butyl-cyanoacrylate) as an adhesive tissue glue was used along the incised palatal mucosa in both groups to prevent the entrance of any material between the tissues. In addition, the glue was spread on the mesh in the first group to prevent entrance of food to the nasal cavity, postoperatively (Figures 1-3).

Figure 1: Showing induced cleft hard palate.

Figure 2: Showing fixation of mesh with screws.

Figure 3: Showing fixation of cartilage with screws.

Post-operative care

The experimental puppies received antibiotics and antipyretics for five days, postoperatively. The animals were recovered after the surgical operation with soft diet and water for about 15 days and the mini screws were removed at day 15, postoperatively.

Assessment of healing

The evaluation of the results was conducted through monitoring the clinical signs postoperatively for each animal, in addition to the study of the gross and histopathological changes of the grafted site at the 15th, 30th, 45th postoperative days. The statistical analysis of the histological scoring is also depended upon to evaluate the efficiency of each palatoplasty technique. A high dose of general anesthesia by a mixture of ketamine and xylazine was used to euthanize the puppies and to take the biopsy for histological studying.

Histological scoring

The histological sections were scored according to the following criteria (28,29), included intensity of granulation tissue, degree of reepithelization and intensity of inflammation (Table 1).

Table 1: Scoring of histological criteria

Criteria	0	1	2	3	4
Granulation tissue	Absent	Discrete	Moderate	Intense	Complete
reepithelization	Absent	Discrete	Moderate	Intense	Complete
Severity of inflammation	Severe	Moderate	Few	Few	Absent

Statistical analysis

The scores of histological data was analyzed statistically by RM ANOVA for the ranks test was used in the comparison of groups and periods with the utilized post hoc Duncan's multiple comparison in the Sigma Plot software program at $P \leq 0.05$ for statistical analysis.

Results

Clinical signs

Relatively, in all animals there are varying degree of anorexia. However, the loss of appetite started after the operation and gradually subsided during the 1th week, postoperatively. No water, soft diet or milk leakage was seen from nostrils during animals feeding postoperatively. In addition, there was no sign of respiratory distress shown in all animals after surgical repairing of the cleft except in some animals of group one, which suffered from very mild degree of sneezing that disappeared after one week, postoperatively.

Gross changes

In group one, the mucosa of hard palate showed mild degree of damage, specially near the site of some screw application. Good binding was observed between the grafted piece of mesh with mucosa and the bony structure of the hard palate at the 15th day, postoperative (Figure 4). The healing of the cleft palate improved at the 30th day, postoperative, where the damaged area of palatal mucosa disappeared with an increase in the connection between the piece of mesh and the mucosa of the hard palate through by the extension and interlocking of the granulation tissue with the mesh. At the 45th postoperative day, there was a complete closure to the cleft palate (Figure 5).

In group two, there is an excellent binding between the grafted piece of ear cartilage and the tissues of hard palate at the repaired site. Also, there is a very small area of tissue damaged at the site of screw application showed at the 15th postoperative days (Figure 6). The healing process of the induced cleft palate progresses beastly at the 30th postoperative days through increasing the insertion and binding of the grafted piece of ear cartilage with the hard palate. At the 45th postoperative days, there is excellent binding between the grafted piece and the hard palate tissues. The induced cleft is closed, where the piece of ear cartilage and mucosa of the hard palate appear as one layer (Figure 7).

Figure 4: Showing cleft palate in one of animals in G1 at the 15th postoperative days.

Figure 5: Showing cleft palate in one of animals in G1 at the 45th postoperative days.

Figure 6: Showing cleft palate in one of animals in G2 at the 15th postoperative days.

Figure 7: Showing cleft palate in one of animals in G2 at the 45th postoperative days.

Histopathological changes

In group one, the histopathological changes at the 15th postoperative days to the site of the cleft repair are characterized by new granulation tissue formation. There is an infiltration of inflammatory cells with a congestion of blood vessels and a beginning of reepithelization (Figures 8 and 9). At the 30th postoperative days, there is an increase in the maturation of the granulation tissue with more interlocking between the implanted piece of mesh and the collagen fibers. In addition, there is more degree of angiogenesis and mucosal regeneration with the infiltration of inflammatory cells. At the 45th postoperative days, there is more advancing in the healing process of the cleft palate that are represented by more maturation of the granulation tissue and the connection of the implanted mesh with the surrounding tissue and a good degree of mucosal regeneration with a reduction in the infiltration of inflammatory cells (Figure 10 and 11).

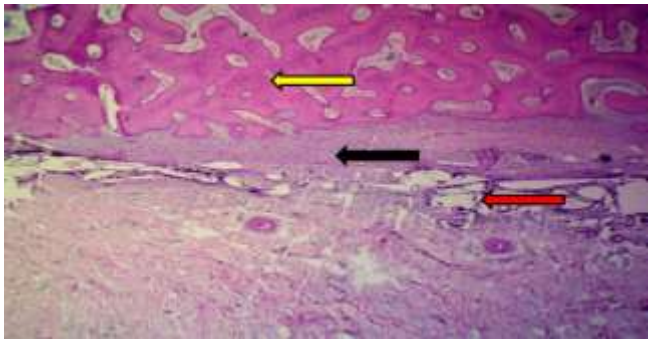

Figure 8: Micrograph at the 15th postoperative days in G1 show granulation tissue formation (black arrow) between the mesh (red arrow) and hard palate (yellow arrow). H&E, 40X.

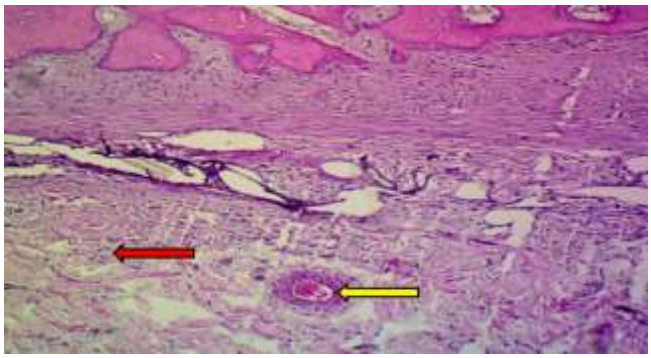

Figure 9: Micrograph at the 15th postoperative days in G1 show infiltration of inflammatory cells (red arrow) and congestion of blood vessels (yellow arrow). H&E, 100X.

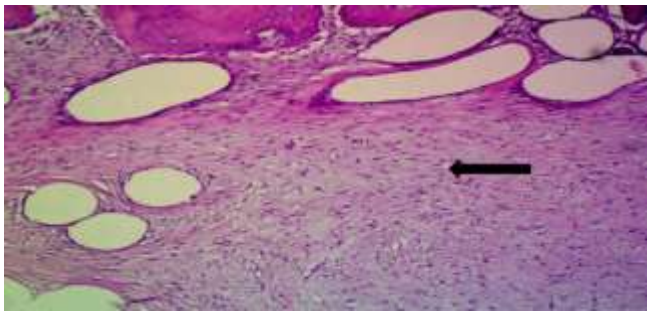

Figure 10: Micrograph at the 45th postoperative days in G1 show maturity of granulation tissue (black arrow). H&E, 100X.

For the second group, the histological changes to the site of the palatoplasty at the 15th postoperative days are characterized by a pronounced formation of granulation tissue between the grafted piece of ear cartilage with the bony structure of the hard palate and the palatal mucosa. In addition, high degree of angiogenesis with infiltration of inflammatory cells and a beginning of mucosal regeneration were seen (Figures 12 and 13). At the 30th postoperative

days, there is an excellent healing process, which is characterized by increasing in binding degree between the piece of cartilage and the surrounding tissues of the hard palate with more angiogenesis and mucosal regeneration, jointly with a decrease in the infiltrations of inflammatory cells. Cartilage hyalinization is seen, too. The healing process at the 45th postoperative was characterized by excellent changes that are represented by increasing in the degree of cartilage insertion with the hard palate tissue and the mucosal regeneration (Figures 14 and 15).

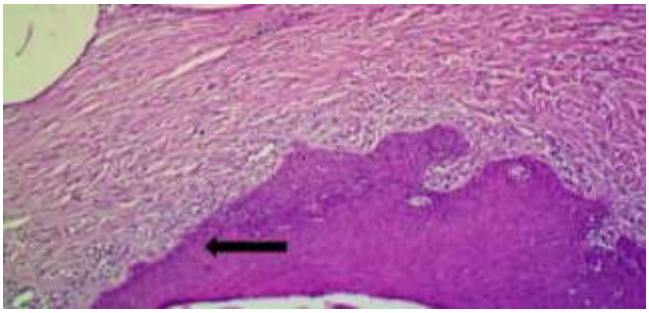

Figure 11: Micrograph at the 45th postoperative days in G1 show mucosal regeneration (black arrow). H&E, 100X.

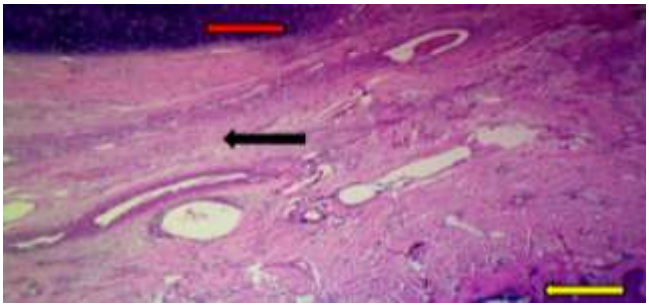

Figure 12: Micrograph at the 15th postoperative days in G2 show granulation tissue formation (black arrow) between piece of cartilage (red arrow) and mucosa (yellow arrow). H&E, 40X.

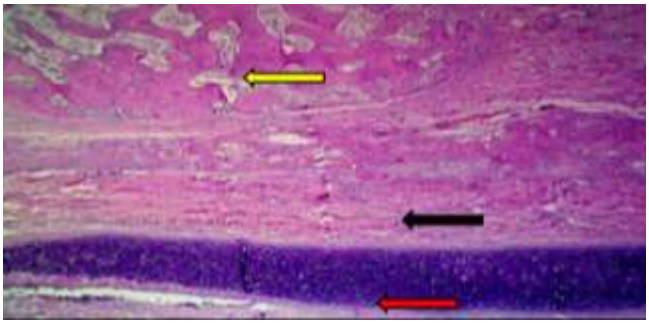

Figure 13: Micrograph at the 15th postoperative days in G2 show granulation tissue formation (black arrow) between cartilage (red arrow) and hard palate (yellow arrow). H&E, 40X.

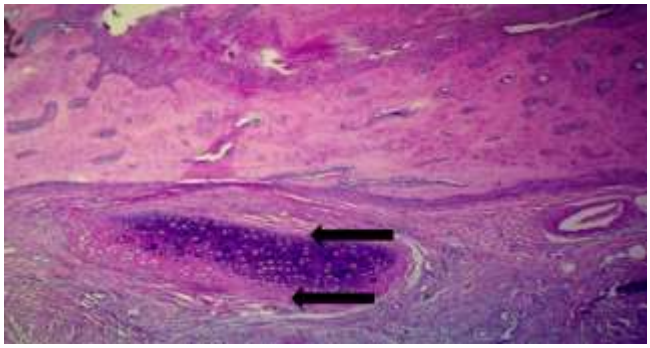

Figure 14: Micrograph at the 45th postoperative days in G2 show insertion of cartilage with hard palate tissue (black arrow). H&E, 40X.

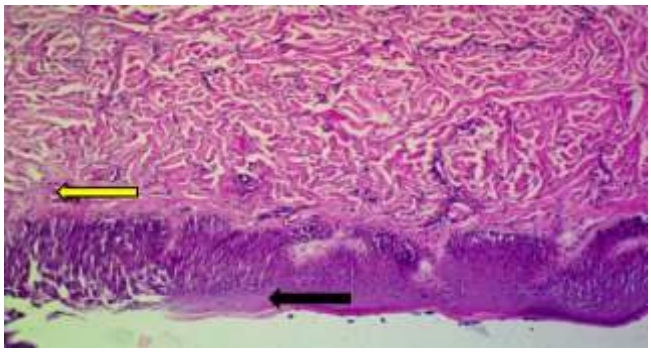

Figure 15: Micrograph at the 45th postoperative days in G2 show high mucosal regeneration (black arrow) and a few infiltrations of inflammatory cells (yellow arrow). H&E, 100X.

Histological scoring

The statistical analysis of the histological sections for both groups revealed no significant difference at P≤0.05 in the formation of granulation tissue, inflammation and reepithelization. The statistical analysis of the histological sections for all periods of the study for each group did not show any significant difference at P≤0.05 in the formation of the granulation tissue, while there is a significant difference at P≤0.05 in the reduction of inflammation and an increase in the degree of reepithelization (Tables 2-4).

Table 2: The scores of granulation tissue

Group	15th P.O.Ds.	30th P.O.Ds.	45th P.O.Ds.
G1	3.33 ± 0.33 ^{Aa}	3.66 ± 0.33 ^{Aa}	3.33 ± 0.33 ^{Aa}
G2	3.00 ± 0.00 ^{Aa}	4.00 ± 0.00 ^{Aa}	3.66 ± 0.33 ^{Aa}

Total specimens (dogs) = 3. Data expressed as Mean ± Stander error SE. The Capital letter mean there are significant differences between groups at P≤0.05. The Small letter mean there are significant differences between periods at P≤0.05.

Table 3: The scores of reepithelization

Group	15th P.O.Ds.	30th P.O.Ds.	45th P.O.Ds.
G1	1.66 ± 0.33 ^{Ab}	2.66 ± 0.33 ^{Aa}	3.33 ± 0.33 ^{Aa}
G2	1.00 ± 0.57 ^{Ab}	2.66 ± 0.33 ^{Aa}	3.66 ± 0.33 ^{Aa}

Total specimens (dogs) = 3. Data expressed as Mean ± Stander error SE. The Capital letter mean there are significant differences between groups at P≤0.05. The Small letter mean there are significant differences between periods at P≤0.05.

Table 4: The scores of inflammations

Group	15th P.O.Ds.	30th P.O.Ds.	45th P.O.Ds.
G1	1.00 ± 0.00 ^{Aa}	0.66 ± 0.33 ^{Aa}	0.00 ± 0.00 ^{Ab}
G2	1.66 ± 0.33 ^{Aa}	1.00 ± 0.00 ^{Aa}	0.33 ± 0.33 ^{Ab}

Total specimens (dogs) = 3. Data expressed as Mean ± Stander error SE. The Capital letter mean there are significant differences between groups at P≤0.05. The Small letter mean there are significant differences between periods at P≤0.05.

Discussion

The surgical treatment of the cleft palate is considered a challenge to the surgeons because there are some factors that may interfere with the healing of the cleft palate, including the growth, essential developmental deficiencies and functional deformation (30,31). In addition, the effect of surgical repair of the cleft palate on maxilla and dentition growth (32). The surgery of the cleft palate has some complications, especially the occurrence of dehiscence due to several reasons such as unwarranted tension to mucosa, infection, destruction of blood supply and rough surgical manipulation (33,34). However, in this experimental design the piece of polypropylene mesh and ear cartilage autograft was fixed to the hard palate by mini screws instead of suturing and this method yields good results and any complications due to the surgical correction specially dehiscence was not noticed. In both techniques, the grafted material acts as a barrier, which isolate the nasal cavity from oral cavity; therefore, any particle of food or water didn't enter from oral to nasal cavity. This result explains the non-occurrence of water or milk reflex from nostrils, postoperatively. The adhesive glue plays an important role to seal the pores of mesh, relatively, where Reece *et al.* , Kamer and Joseph, Detweiler *et al.* (35-37) mentioned the adhesive tissue glue use to connect wound edges until the completion of the process of wound healing and to provide a mechanical support to wound. In addition, it acts as a barrier to prevent leakage of materials. The appearance of a mild degree of sneezing in some animals of group one may be due to an incomplete closure of mesh pores by glue, which permit to enter very few drops of water or milk to nasal cavity during feeding, postoperatively or because of the entrance of little amount of glue to nasal passage, which may cause irritation and inflammation of the nasal mucosa, resulting in sneezing. However, these signs disappeared quickly without any respiratory complications where the granulation tissue that formed after the repairing replaced the adhesive glue, in addition to the granulation tissue that covered and interlocked the mesh. The palatal tissue damage that showed at the site of screw application due to the pressure produced from the tightening of the screw. This result disappeared after the removal of screws, because of the improvement of the vascular local circulation. In both groups there is a pronounced newly granulation tissue formation and reepithelization, especially in group two and this goes in agreement with Okazaki *et al.* (38), where the healing process of wounds inside the oral cavity was very faster. Moreover, the authors Zelles *et al.* (39) mention that saliva plays an important role in enhancing the healing process of intraoral wounds, because it contains an epidermal growth factor (EGF) and the ear cartilage, when implanted, has the ability to enhance and to improve the healing process of wounds (40). Infiltration of inflammatory cells occurs because the prosthetic subjects may lead to cause the infiltration of inflammatory cells (40-42), through the tissue injury, as well. The inflammatory cells are attracted to the site of injury due to releasing of cytokines (43).

Conclusion

From the findings it is concluded that both, the polypropylene mesh and the ear cartilage autograft could be used successfully to close the cleft hard palate of puppies, in addition to the use of mini screws instead of suturing during the reparation of the cleft, with priority given to the ear cartilage.

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Conflict of interest

The authors declare that there is no conflict of interest.

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استخدام شبكة البولي بروبايلين أو الترقيع الذاتي بغضروف الأذن مع المسامير الصغيرة لعلاج الشق الحنكي الصلب المستحدث في الجراء: دراسة مقارنة

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الخلاصة

تهدف هذه الدراسة التجريبية الى إصلاح الشق المستحدث في الحنك الصلب في الجراء بطريقتين مختلفتين. تقسم ثمانية عشر من الجراء الى مجموعتين متساوية. وبعد أحداث التخدير العام في هذه الدراسة، عبر حقن مزيج من الكيتامين بتركيز ١٠% وبجرعة ١٠ملغم/كغم مع الزايلازين بتركيز ٢% وبجرعة ٢ ملغم / كغم، يستحدث شق بحجم ١ - ٢ سم في الحنك الصلب لكل الحيوانات. يغلق الشق المستحدث في المجموعة الأولى من خلال استخدام قطعة من شبكة البولي بروبايلين في حين يغلق الشق في المجموعة الثانية باستخدام قطعة من غضروف الأذن مأخوذة من نفس الحيوان. كما ويستخدم الصمغ النسيجي في كلا المجموعتين. تقيم النتائج بتسجيل العلامات السريرية بعد العملية إضافة الى دراسة التغيرات العيانية والنسيجية كل ١٥، ٣٠، ٤٥ يوم بعد العملية وكذلك يعمل تحليل إحصائي للمقاطع النسيجية. لم يشاهد حدوث أي علامة لحدوث ذات الرئة الاستنشاقي بعد إجراء العملية في كل الحيوانات، ما عدا ظهور حالات بسيطة ومتقطعة من العطاس في بعض حيوانات المجموعة الأولى والتي اختفت بعد أسبوع من إجراء العملية. تبين النتائج العيانية لكلا المجموعتين اختفاء الشق الحنكي المستحدث في نهاية التجربة مع اندماج المواد المستخدمة مع نسيج الحنك الصلب. أما نسيجياً، فتظهر النتائج لكلا المجموعتين تكوين نسيج حبيبي جديد مع ارتشاح للخلايا الالتهابية مع تجدد للظهارة بشكل جيد. وأخيراً، نستنتج انه من الممكن استخدام المواد المذكورة أعلاه في إصلاح الشق الحنكي الصلب للجراء مع الأفضلية لاستخدام الترقيع الذاتي للغضروف.