



Histological Assessment of the Effects of Derma + Flex®, versus Black Silk Suture on Oral Mucosal Primary Wound Healing in Rabbits

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Abstract

Aims: The objective of this study was to compare wound healing potential between black silk suture and bioadhesive glue after induced surgical intraoral mucosal incisions in rabbits and assessed histologically. **Materials and methods:** The study was conducted on (18) male New Zealand rabbits, randomly divided into two groups (9 animals per group) according to the healing periods (1st, 3rd, and 7th) days. Two incisions were made on the buccal mucosa of each rabbit bilaterally. For the first group, the defect was sutured with black silk suture, and adhesive glue Derma + Flex for the second group applied post operatively. Three rabbits were randomly selected from each group at the (1st, 3rd, 7th) days, and biopsies were taken. The biopsy specimens were subjected to histological assessment to assess the healing parameters of the primary wound healing process. **Results:** Inflammatory cell infiltration grading in day 1 and day 3 group I was the highest and on day 7 was the lowest in both groups. Granulation tissue formation in day 1 and day 3 was the same but high in group II day 7. Re-epithelization scoring was highest at day 7 for both groups. According to the time period, there were no significant differences in group versus group (within the same day). **Conclusions:** Use of bioadhesive Derma+Flex® glue for closure of primary wounds gave a satisfied result for better healing and isolation to avoid of infection in appropriate time with less equipment's in comparison with black silk suture.

الخلاصة

الأهداف: الهدف من هذه الدراسة هو المقارنة النسيجية لانتئام الجروح بين خيوط الحرير الأسود والصمغ الحيوي بعد إحداث شقوق جراحية في الغشاء المخاطي داخل الفم. **المواد وطرائق العمل:** أجريت الدراسة على (18) أرنباً ذكراً نيوزيلندياً تم تقسيمهم عشوائياً إلى مجموعتين (9 حيوانات لكل مجموعة) حسب فترات الشفاء (1، 3، 7) أيام. تم عمل شقين على الغشاء المخاطي الشدقي لكل أرنب بكل جانب. بالنسبة للمجموعة الأولى، تم خياطة الخلل بالحرير الأسود، والغراء اللاصق Derma + Flex للمجموعة الثانية، تم اختيار ثلاثة أرانب عشوائياً من كل مجموعة في الأيام (الأول، الثالث، السابع)، وتم أخذ الخزعات. خضعت عينات الخزعة للتقييم النسيجي لتقييم معايير الشفاء لعملية التئام الجروح الأولية. **النتائج:** متوسط نتائج تسلسل الخلايا الالتهابية، حيث كان اليوم الأول والثالث للمجموعة الأولى هو الأعلى واليوم السابع هو الأقل لكلا المجموعتين وتكوين أنسجة التحبيب في اليوم الأول والثالث متماثلان ولكنهما مرتفعان في المجموعة الثانية في اليوم السابع، وأظهر تسجيل إعادة النسيج الطلائي في اليوم السابع لكلا المجموعتين. وفقاً للفترة الزمنية لم تكن هناك فروق ذات دلالة إحصائية بين المجموعة مقابل المجموعة (في نفس اليوم). لذلك هناك نشاط وثيق للغاية للطريقتين لإغلاق الجرح فيما يتعلق باستخدامهما في الجروح الأولية. **الاستنتاجات:** استخدام غراء Derma + Flex® اللاصق الحيوي لإغلاق الجروح الأولية يعطي نتيجة مرضية للشفاء والعزل بشكل أفضل للتخلص من العدوى في الوقت المناسب بمعدات أقل مقارنة بخياطة الحرير الأسود.

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INTRODUCTION

The term of oral mucosa is used to describe the wet lining of the oral cavity. The mucous epithelium of oral cavity is a remodeling barrier separating underlying tissues from adverse external environment ⁽¹⁾. Oral mucosa lines the oral cavity proper to the lip vermilion that is contiguous with the skin. The oral mucosa is located anatomically between external skin and inner gastrointestinal mucosa and shows some properties of both ⁽²⁾. The incision is the main surgical step. Proper closure of the surgical wound and optimum preservation are the most important factors influencing wound healing by initial intention and the success of surgical procedures. Approximation of wound with suture takes a long time and leads to more formation of the scar ⁽³⁾. Silk is the most widely used suture material in many surgical specialties. It is non absorbable, therefore a second date for appointment is needed to remove the stitches. Although there is absorbable type of sutures, most of them have unexpected resorption rates, as they dissolve and weaken early or stay in the wound area for too long ⁽⁴⁾. Tissue adhesives are favorable biomaterials that can be utilized for several procedures, as wound closure, hemostasis, tissue repair and implant fixation. It has many advantages when compared to sutures, which is easy to apply, faster to use, less traumatic and versatile ⁽⁵⁾. The ideal adhesive should have the criteria such

as being biocompatible, easily polymerize on site, the mechanical flexibility is adequate to accommodate complex wound contours and sizes, appropriate physical properties as tensile and shear strength and higher bonding strength with wet tissues or organs ⁽⁶⁻⁸⁾. Cyanoacrylate has strong, fast-acting adhesive properties and is widely used in medicine, industry and domestic activities. Cyanoacrylates can stick to the target surface and bond to it within 5-6 seconds of contact with base materials such as water, blood, body tissues, and moisture ^(9,10). The aims of this study are to compare wound healing potential between black silk suture and bioadhesive glue after induced surgical intraoral mucosal incisions and assessed histologically.

MATERIALS AND METHODS

This study was conducted at the department of the oral and maxillofacial surgery, faculty of dentistry, university of Mosul, Iraq, for one-week duration. All surgical procedures performed under the supervision of an experienced Veterinary Surgeon following a standardized protocol. Approval was according to the ethical approval number (UoM.Dent, A.L.7/ 21). Eighteen healthy male New Zealand rabbits, aged between four and six months, and weights between (1250 – 1500 gm). The rabbits were kept in the animal house to be prepared for study under a standard environment (temperature about 25 – 30 °

C), nutrition (fresh vegetables, lettuce and herbs) and water three times daily. All animal experiments followed the animal care protocol to prohibit any health problems and to enhance efficiency. Grouping of Animals: The animals were divided into three main groups (9 rabbits for each group) according to the periods of healing (first, third, seventh) days. Each group was subdivided into two subgroups (3 rabbits for each; first group black silk suture and the second subgroup specified for the tissue adhesive (Derma+Flex®). All rabbits were anaesthetized by an intramuscular injection of ketamine hydrochloride (40mg/kg) mixed with Xylazine base (5mg/kg) in the rabbit's thigh muscle slowly ^(11,12). After time of (10-15) minutes, reflections of rabbits were examined to make sure for anesthesia. After anesthesia was checked, the rabbit was laid down laterally and the mouth was opened to make vision to the area of surgery. The same length and depth of the incisions were made on the buccal surfaces of oral mucosa bilaterally on all rabbits of this experiment. The incision length in the oral mucosa was (0.5 cm) and the depth was (0.2cm). The surgical incision was made by using a stainless-steel scalpel no.3 with surgical blade no.15, placed in the area between mesial surface of posterior teeth and angle of mouth. For the first subgroup, the surgical wound (incision) was cleaned by gauze then sutured by single stich (simple interrupted technique) of black silk suture 3/0. For the second subgroup, after

achieving hemostasis and removing the excess blood via pressure by gauze, the wound edges were approximated by the use of tissue forceps and the wound was covered by a thin film of Derma+Flex® adhesive with maintenance of the wound edge approximation for (60-90) seconds until the adhesive has polymerized and became dry on touch. At the end of procedure, the rabbits were placed in separated cages while recovery from anesthesia, and their feeding and physical activity was monitored for (24) hour. All rabbits back to their activity and feeding usually (3-5) hours after surgery. According to the groups of experiment, biopsy of the oral mucosa was taken at the date of each group: **Group 1:** The rabbits were sacrificed after 1 day (24) hours from surgery. **Group 2:** The rabbits were sacrificed after 3 days from surgery. **Group 3:** The rabbits were sacrificed after 7 days from surgery. All these groups included two subgroups Subgroup S: meaning the control group when the suture was used in the procedure and Subgroup G: meaning the study group when the adhesive was used in the procedure. The specimen was collected directly after sacrificing the rabbit. The area of incision fully involved in the specimen during taking the biopsy.

Histological evaluation was done to assess parameters of the wound healing process depending on the following criteria:

1- Inflammatory Cells Infiltration
grading scale. Score 1: Nil No

inflammatory cells seen in the field of operation. Score 2: Mild When inflammatory cells present in few numbers, less than $\frac{1}{2}$ of the field. Score 3: Moderate Inflammatory cells could be seen in more than $\frac{1}{2}$ of the field. Score 4: Severe or abundant when Inflammatory cells present in huge numbers, more than $\frac{3}{4}$ of the field.

- 2- Granulation tissue formation grading criteria: Score 1: Absence of granulation tissue formation in the wound. Score 2: The quantity of granulation tissue formation in the wound gap is scanty. Score 3: The amount of granulation tissue formation is moderate in tissues. Score 4: The total amount of granulation tissue formation in the wound is profound.
- 3- Grading scale to evaluate Re-epithelialization. Score 0: Re-epithelialization at the edge of the wound. Score 1: Re-epithelialization covering less than half of the wound. Score 2: Re-epithelialization covering more than half of the wound. Score 3: Re-epithelialization covering the entire wound, irregular thickness. Score 4: Re-epithelialization covering the entire wound, normal thickness.⁽¹³⁾
- Statistical Analysis: The median and inter-quartile range were used to present the data, and a non-parametric test (Mann-Whitney U test) was used for statistical analysis because we have

two independent samples that do not follow a normal distribution. A P-value less than 0.05 was considered significant. Statistical analysis was done by using SPSS 19 computer software program. Two test were used to compare means. Mann – Whitney NPar Test was used to compare means between groups. Kruskal – Wallis NPar Test was used to compare means within groups.

RESULTS

Histological assessment: All rabbits were in good physical state with healthy condition post-surgery, uneventful recovery.

Day One: Inflammatory Cell Examination: For silk group, there was normal physiologic reaction to silk as a foreign body which is higher than for adhesive group which appeared minimal. Granulation Tissue Examination: For silk group, there was absence of granulation tissue formation in the wound as well as in the adhesive group. Re-epithelialization Examination: For two groups, there was no re-epithelialization at the edge of the wound.

Day Three: Inflammatory Cell Examination: In the silk group, there was still inflammatory reaction as well as in the adhesive group. Granulation Tissue Examination: In the silk group and for adhesive group a scanty amount of granulation tissue formation in the wound gap was found. Re-epithelialization Examination: In the silk group, re-

epithelialization covered more than half of the wound. while for adhesive group, re-epithelialization covered the entire wound with an irregular thickness.

One Week: Inflammatory Cell Examination: In the silk group, there was mild to moderate infiltration of inflammatory yet less in the adhesive group less. Granulation Tissue Examination: In the silk group, there was a moderate amount of granulation tissue formed, yet in the adhesive group, more amount of granulation tissue was formed. Re-epithelialization Examination: In the silk group, re-epithelialization covered the entire wound with an irregular thickness. In

the adhesive group, showed that re-epithelialization covered the entire wound with normal thickness. As a comparison of day versus day in the silk group, the results showed a non-significant difference for all parameters as shown in Table (1). Comparison of day versus day in the adhesive group, showed that there was a non-significant difference for all the parameters, as shown in Table (2). Comparison between silk group and adhesive group in (day1, day 3, day 7), showed a non-significant difference for inflammatory cell infiltration, granulation tissue formation, and re-epithelialization of oral mucosa, as shown in Table (3).

Table (1): Histological analysis results within silk group comparing daily changes (1, 3 and 7th day)

Time period	I.C.I* (p-value)	G.T.F* (p-value)	RE* (p-value)
Day 1 vs day 3	1	0.102	0.102
Day 1 vs day 7	0.414	0.083	0.102
Day 3 vs day7	0.157	0.317	0.18

* I.C. I=inflammatory cell infiltrate, G.T. F=granulation tissue formation, RE=reepithelization

Table (2): Histological analysis results within adhesive group comparing daily changes (1, 3 and 7th day)

Time period	I.C.I* (p-value)	G.T.F* (p-value)	RE* (p-value)
Day 1 vs day 3	0.317	0.18	0.102
Day 1 vs day 7	0.157	0.102	0.102
Day 3 vs day7	0.317	0.317	0.317

* I.C. I=inflammatory cell infiltrate, G.T. F=granulation tissue formation, RE=reepithelization

Table (3): Histological analysis, comparing between silk group and adhesive group in the same day in oral mucosa.

Time period	I.C.I* (p-value)	G.T.F* (p-value)	RE* (p-value)
Day 1	0.456	0.317	1
Day 3	0.114	1	0.099
Day 7	0.637	0.114	0.456

* I.C. I=inflammatory cell infiltrate, G.T. F=granulation tissue formation, RE=reepithelization

DISCUSSION

In this study, the histological findings from the two different wound closure methods of surgical incisions were compared, the high-viscosity tissue adhesive used was a mixture of n-butyl cyanoacrylate and 2-octyl cyanoacrylate. The butyl-cyanoacrylate has the following properties that is bacteriostatic, biodegradable, hemostatic compound with a long half-life and good tissue compatibility ⁽¹⁴⁾. The wounds that were closed with silk sutures showed longer periods when compared to the sides closed with cyanoacrylate. The increasing use of cyanoacrylates as an adhesive in medical field (general, oral surgery and dentistry) has raised concerns about potential tissue interaction in humans ⁽¹⁵⁾. There was no significant different between the two groups, but the silk group showed predominance of moderate inflammatory cells than the adhesive group that showed mild inflammatory cells, meaning that the body inflammatory response to suture was more than the adhesive. Kumar *et al.*, ⁽¹⁶⁾ in his clinical and histological study showed that on the 3rd and 7th postoperative day, the epithelialization was better on the sides treated with n-butyl- 2cyanoacrylate but the sites closed with black silk suture showed significant inflammation and scar formation. Ozmen *et al.*, ⁽¹⁷⁾ reported that cyanoacrylate (Histoacryl) application lead to increased adhesion formation around the anastomosis on the third day. Re-epithelialization progresses more rapidly in

oral mucosal wounds in contrast to the skin. In a mucosal wound, epithelial cells migrate directly to the moist exposed surface of the fibrin clot rather than into the dry exudates (cortex) of the dermis. When the standardized punch gingival biopsies were taken from the interdental area to histologically assess the tissue reaction of the test materials, there was no alteration in epithelialization at both the suture and cyanoacrylate sides, and the vascularity was normal to slight hyper-vascularity due to endothelial diffusion in both sites. This can be attributed to vascular proliferation as part of the normal healing process. ⁽¹⁴⁾

CONCLUSION

Utilization of the bioadhesive Derma+Flex® glue for closure of primary wounds give a pleased outcome for better primary healing and separation to get rid of bacterial infection in appropriate time with less equipment's, less time needed and less efforts in comparison with black silk suture. So we recommend to use it as a primary choice of suturing modality for wounds with primary intention.

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