



Effects of Derma + Flex®, Black Silk Suture on Cutaneous Primary Wound Healing in Rabbits, Histological Evaluation

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Abstract

Aims: The objective of this study was to compare the histological wound healing potential between black silk suture and bioadhesive glue after induced surgical skin incisions. **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 back skin of each rabbit bilaterally. In 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:** The results of inflammatory cells infiltration grading showed that in day 1 group II and day 3 group I was the highest and at day 7 was the lowest in both groups. For granulation tissue formation, the results showed that at day 1 and day 3 was the same but high in group II day 7. Re-epithelization scoring was highest in group II day 3 and day 7. According to the time period, there were no significant differences in group versus group (within the same day). A very close activity of the two methods of wound closure in relation to their use in primary wounds in skin was noticed. **Conclusions:** The use of bioadhesive Derma + Flex® glue for closure of primary wounds in skin gives a satisfying result, for better healing and isolation to avoid infection in the appropriate time with less equipment's in comparison with black silk suture.

الخلاصة

الأهداف: الهدف من هذه الدراسة هو مقارنة إمكانات التئام الجرح النسيجي بين خيوط الحرير الأسود والصمغ الحيوي بعد إحداث شقوق جراحية في البشرة الخارجية للارنب. **المواد وطرائق العمل:** أجريت الدراسة على (18) ذكور أرنباً نيوزيلندياً تم تقسيمهم عشوائياً إلى مجموعتين (9 حيوانات لكل مجموعة) حسب فترات الشفاء (1، 3، 7) أيام. تم عمل شقين على البشرة الخارجية لظهر كل أرنب بشكل ثنائي. بالنسبة للمجموعة الأولى، تم خياطة الخلل بخياطة الحرير الأسود، والغراء اللاصق Derma + Flex للمجموعة الثانية المطبقة بعد العملية، 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 تصنيف تسلسل الخلايا الالتهابية، حيث كان اليوم الأول للمجموعة الثانية واليوم الثالث للمجموعة الأولى هو الأعلى واليوم السابع هو الأقل لكلا المجموعتين وتكوين أنسجة التحبيب في اليوم الأول واليوم الثالث متماثلان ولكن مرتفعين في المجموعة الثانية في اليوم السابع وأظهر تسجيل إعادة النسيج الظهاري الأعلى في المجموعة الثانية في اليوم 3 واليوم 7، وفقاً للفترة الزمنية ولم تكن هناك فروق ذات دلالة إحصائية بين المجموعة مقابل المجموعة (في نفس اليوم). لذلك هناك نشاط وثيق للغاية لطريقتين لإغلاق الجرح فيما يتعلق باستخدامهما في الجروح الأولية في الجلد. **الاستنتاجات:** استخدام غراء Derma + Flex® اللاصق الحيوي لإغلاق الجروح الأولية في الجلد يعطي نتيجة مرضية للشفاء والعزل بشكل أفضل للتخلص من العدوى في الوقت المناسب وبأجهزة أقل مقارنة بخياطة الحرير الأسود.

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INTRODUCTION

The skin is the largest organ in the body. It covers the outer surface of the total body. It serves as a waterproof shield insulator and protects the body against environmental pressures. Any wound can or disorder of the skin can provide a gateway for bacteria and potential infections ⁽¹⁾. It has a role in temperature regulation, sensation, vitamin D synthesis, and vitamin B folate protection ⁽²⁾. Loss of integrity of large portions of skin due to injury or disease can result in significant disability or even death ⁽³⁾. 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 scar ⁽⁴⁾. Wound healing occurs by primary or first intention when the wound margins can be recombined and wound closure can be completed. The wound will be repaired by formation of granulation tissue and re-epithelialization, with little or no sign of a scar tissue. Wounds that can be completely removed and converted into a surgical wound are treated with primary closure. In cases where a wound has less damage and contamination and in cases of totally debrided wounds with a wound age of less than 8 hours, primary wound closure is recommended ^(5,6). Silk was 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 are absorbable types of sutures yet most of them have unexpected resorption rates, as they dissolve and weaken early or stay in the wound area for too long ⁽⁷⁾. 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 ⁽⁸⁾. The ideal adhesive should have criteria as being biocompatible, polymerize easily on site, mechanical flexibility to accommodate complex wound contours and sizes, appropriate physical properties as tensile and shear strength and higher bonding strength with wet tissues or organs ⁽⁹⁻¹¹⁾. 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 ^(12,13). The aims of this study were to compare the histological wound healing potential between black silk suture and bioadhesive glue after induced surgical skin incisions.

MATERIALS AND METHODS

This study was conducted at department of the oral and maxillofacial surgery, faculty of dentistry, university of Mosul, Iraq. All surgical procedures were performed under the supervision of an experienced veterinary surgeon following the standardized protocol, Approval for study 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 standard environment (temperature about 25 – 30 ° C), and all animals take the same nutrition (fresh vegetables, lettuce and herbs) and water three times daily. All animal experiments followed up the animal care protocol to prohibit any health problems and to enhance efficiency. Grouping of Animals: The animals were divided into three main groups (6 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 subgroup was the 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 dose (40mg/kg) mixed with Xylazine base dose (5mg/kg) of each rabbit's thigh muscle slowly^(14,15). After 10-15 minutes the reflections of rabbits were examined to make sure for anesthesia.

After anesthesia was checked, the rabbit was laid down laterally to make vision to the area of surgery and shaving was done using electrical hair clipper to remove hair. The same length and depth of the incisions were made on the skin of the back surfaces bilaterally for all rabbits with a length of (0.5 cm) and depth of (0.2cm). The surgical incision was made by using a stainless-steel scalpel no.3 with surgical blade no.15. For the first subgroup, the surgical wound (incision) was cleaned by gauze then sutured by a single stich (simple interrupted technique) of black silk suture 3/0. For the second subgroup, after achieving hemostasis and removing any excess of blood via pressure using gauze and ensuring that the wound edges were easily approximated using tissue forceps, the surgical wound was covered by a thin film of Derma+Flex® adhesive and the wound edge approximation was maintained for (60-90) seconds until the adhesive had polymerized and became dry to the touch. At the end of procedure, the rabbits were placed in separated cages while recovery from anesthesia, and their feeding and physical activity for (24) hour. All rabbits back to their activity and feeding usually (3-5) hours after surgery and monitored. Biopsy of the skin was taken as follows: **Group 1:** The rabbits were sacrificed after (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: the control group when the black silk suture was used in the procedure and Subgroup G: in which the adhesive was used in the procedure. Specimen were collected directly after sacrificing the rabbit, the offending area of incision involved in the specimen.

Histological analysis was done to assess the physiological 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. ⁽¹⁶⁾

Statistical Analysis: The median and inter-quartile range were used to present the data, and a non-parametric test (Mann-Whitney U test), Friedman Statistical 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.

RESULTS

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

Day One: Inflammatory Cell Examination:

In the silk group, there was normal physiologic reaction to silk as a foreign body yet less in the adhesive group.

Granulation Tissue Examination: For silk group, there was less granulation tissue formation in the wound, and more in the adhesive group. Re-epithelialization

Examination: For both groups, there was no re-epithelialization at the edge of the wound.

Day Three: Inflammatory Cell

Examination: In the silk group, a higher inflammatory reaction was evident when compared to the adhesive group.

Granulation Tissue Examination: In the silk group higher amount of granulation tissue formation was observed to compared to scanty amount found in the adhesive group.

Re-epithelialization Examination: For silk group, the re-epithelialization was less than that seen in the adhesive group, in which re-epithelialization covered the entire wound with an irregular thickness.

One Week: Inflammatory Cell Examination: In both groups mild infiltration of inflammatory cells was seen.

Granulation Tissue Examination: For silk group, there was moderate amount of granulation tissue formed. However, this was more in the adhesive group. Re-epithelialization Examination: For silk group, re-epithelialization covered the entire wound with an irregular thickness. In the adhesive group observation showed that re-epithelialization covered the entire wound with normal thickness. Comparison within each group (day versus day) showed that in the silk group, and adhesive group no significant differences were found for all

parameters as shown in Table (1). and Table (2). Comparisons between both groups in (day1, day 3, day 7), showed no-significant differences regarding inflammatory cell infiltration, granulation tissue formation, and re-epithelialization of oral mucosa, and as shown in Table (3). Comparison of day versus day in the adhesive group showed there no-significant differences for inflammatory cell infiltration, granulation tissue formation, and re-epithelialization of skin, and as shown in Table (4). Comparisons between the silk group and adhesive group (day 3) showed a significant difference for re-epithelialization of skin, and non-significant difference for inflammatory cell infiltration, granulation tissue formation of skin, as shown in Table (5). Comparisons between silk group and adhesive group at (day 1 and day 7), showed a non-significant difference for inflammatory cell infiltration, granulation tissue formation, and re-epithelialization of skin, and as shown in Table (5).

Table (1): Histological analysis results within silk group comparison within the same group (values among days).

Time period	I.C.I* (p-value)	G.T.F* (p-value)	RE* (p-value)
day1, day 3, day 7	0.097	0.082	0.061

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

Table (2): Histological analysis results within adhesive group comparison within the same group (values among days).

Time period	I.C.I*(p-value)	G.T.F* (p-value)	RE* (p-value)
day1, day 3, day 7	0.15	0.135	0.05**

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

** : Significant difference.

Table (3): 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	0.317	0.102	0.317
Day 1 vs day 7	0.157	0.157	0.102
Day 3 vs day7	0.083	0.157	0.102

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

Table (4): 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	0.18	1	0.102
Day 1 vs day 7	0.083	0.18	0.102
Day 3 vs day7	1	0.18	0.083

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

Table (5): Histological analysis, comparing between silk group and adhesive group in the same day in skin.

Time period	I.C.I* (p-value)	G.T.F* (p-value)	RE* (p-value)
Day 1	0.456	0.114	1
Day 3	0.099	0.197	0.043**
Day 7	1	0.099	0.099

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

** : Significant difference.

DISCUSSION

Healing after wound closure can be improved by proper approximation of wound edges and proper wound isolation. Superficial contamination of the wound occurs after surgery and often leads to delayed epithelialization of the wound surface and excessive granulation tissue formation. All of these factors contribute to the failure of surgery to achieve the desired result. In the current study no significant differences was noticed between the two groups, even though the adhesive group showed a predominance of moderate inflammatory cells infiltration compared to

the silk group that showed mild inflammatory cell infiltration. Jacobson and colleagues who used cyanoacrylate for vascular anastomosis reported that there was an acute inflammatory reaction that developed after 12 hours postoperatively⁽¹⁷⁾. This is similar to the reports by Ahn *et al.*,⁽¹⁸⁾ whom showed that one day after surgery, slight edema in the eyelids was observed only in the sutured group, and no secretion was observed in the cyanoacrylate group. In regard to no significant difference was observed re-epithelialization for both groups. Giray *et al.*,⁽¹⁹⁾ found in clinical evaluation after one day of operation, there

was no apparent difference between suture and adhesive (histoacryl) treated wounds concerning bleeding, epithelialization, necrosis and scar formation. In contrast, Souza *et al.*,⁽²⁰⁾ reported that at the third postoperative day, there was absence of both acute and chronic inflammatory cells in the adhesive groups compared to the suture group. This disagreed with the studies of Özyazgan and Idaci,⁽²¹⁾ whom showed that where cyanoacrylate adhesive was applied to the subcutaneous level to rabbit ears; and showed that cyanoacrylate (histoacryl) increased the inflammatory reaction and caused giant cell infiltrate to foreign bodies when it came in contact with well-vascularized soft tissues. Camacho-Alonso and Jornet,⁽²²⁾ reported that re-epithelialization was faster in (steel scalpel +N-butyl-2-cyanoacrylate) groups, because seven days after provoking the wounds, already showed re-epithelialization covering the entire wound, although of irregular thickness. Singer *et al.*,⁽²³⁾ on his comparative study of the re-epithelialization of partial thickness burns treated with either octyl cyanoacrylate or silver sulfadiazine, suggested a more rapid re-epithelialization of the octyl cyanoacrylate treated burns. At 7 days, the percent of re-epithelialization was greatest in the octyl cyanoacrylate group and lowest in the group treated with dry gauze only.

CONCLUSION

Utilization of the bioadhesive Derma+Flex® glue for closure of primary

wounds gave a pleased outcome for better primary healing and separation to avoid bacterial infection in an appropriate time with less equipment's, less time needed and less efforts in comparison with black silk suture. As such, it is recommended to be used as a primary choice of suturing modality for wounds with primary intention.

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