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Histological Assessment of the Oral Soft Tissue Regenerative Capacity of Hyaluronic Acid and Ozonated Oil (An in vivo study)

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Abstract

Aims: The objective of this study is to compare the wound healing potential between two different biomaterials after induced surgical intraoral incisions. **Materials and methods**: The study was conducted on (12) male New Zealand rabbits, randomly divided into two groups (6 animals per group) according to the healing periods (3rd, 7th, and 14th) days. Two incisions were made on the buccal mucosa of each rabbit bilaterally. For the first group, the defects were filled with (PerioKIN Hyaluronic 1%) gel, O3OHEAL-M gel for the second group, applied three times daily, two rabbits were randomly selected of each group at the (3rd, 7th, and 14th) days, and biopsies were taken. The biopsy specimens were subjected to histological evaluation to assess the physiological parameters of the wound healing process. **Results**: median results of inflammatory cells infiltration grading in which day 3 group II was the highest and day 14 is lowest and Granulation tissue formation in which day 7 is highest and re-epithelization scoring showed highest at day 14, according to the time period and there were no significant differences of among groups (within the same day). So there is a very close activity of the two materials in relation to their use in wounds with tissue loss. **Conclusions**: use of PerioKIN Hyaluronic gel 1% and O3OHEAL-M gel application three times daily for bare wounds give a satisfactory result for better healing and isolation to get rid of infection at appropriate time.

الخلاصة

الاهداف: تهدف الدراسة الى مقارنة إمكانية التئام الجروح بين مادتين حيويتين مختلفتين بعد إحداث شقوق جراحية في الاغشية المخاطية في فم الارانب. **المواد وطرائق العمل**: أجريت الدراسة على (12) أرنباً نيوزيلندياً تم تقسيمهم عشوائياً إلى مجموعتين (6 حيوانات لكل مجموعة) حسب فترات ايام الشفاء (الثالث، السابع، الرابع عشر). تم عمل شقين على الغشاء المخاطي الشدقي لكل أرنب. بالنسبة للمجموعة الأولى، تم ملء الشقوق بمادة (PerioKIN Hyaluronic gel ٪1)، و (O3OHEAL-M gel) للمجموعة الثانية، وتم وضعهما ثلاث مرات يوميًا لجميع الارانب في كل مجموعة في الايام (الثالث، السابع، الرابع عشر)، وقد تم اختيار ارنبين عشوائيا لكل توقيت زمني وتم أخذ الخزعات. خضعت عينات الخزعة للتقييم النسيجي لتقييم المعاملات الفسيولوجية لعملية التئام الجروح. **النتائج**: متوسط نتائج تصنيف تسلل الخلايا الالتهابية، حيث يكون اليوم 3 المجموعة الثانية هو الأعلى واليوم 14 هو الأدنى ويكون تكوين الأنسجة الحبيبية في اليوم السابع هو الأعلى ويظهر تسجيل إعادة التجلد أعلى في اليوم 14، وفقًا للفترة الزمنية ولم يكن هناك فروق ذات دلالة إحصائية بين المجموعة مقابل المجموعة (في نفس اليوم). لذلك هناك نشاط وثيق للغاية للمادتين فيما يتعلق باستخدامهما في الجروح مع فقدان الأنسجة. **الاستنتاجات**: استخدام PerioKIN 1 % hyaluronic gel وO3OHEAL-M gel ثلاث مرات يوميًا للجروح يعطي نتائج مرضية للشفاء والعزل بشكل أفضل للتخلص من العدوى في الوقت المناسب.

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INTRODUCTION

A wound is defined as a break in the continuity of the covering skin, and/or lining mucosa. The wound healing process is a complex mechanism involving a cascade of coordinated inflammatory and proliferative steps. [1,2] Different biomaterials might be required to improve clinical outcomes, and have a potential function in wound healing and regeneration, thereby playing an important role in tissue repair mechanisms in many oral surgical procedures [3].

Hyaluronic acid is a polysaccharide found in the extracellular matrix of connective tissue. The biological action of Hyaluronic acid comes from its potential relationship with growth factors, and serves as a lubricant for various body tissues, thus maintaining the structural integrity tissues [4,5]. The properties of Hyaluronic Acid include; biocompatibility, with viscoelastic nature make it unique for use in several applications such as facilitating scar-less healing and regeneration of incision wounds [6].

Ozone therapy is a safe bio-oxidative therapy in which a mixture of oxygen and ozone is administered to obtain therapeutic effects such as; wound healing, post-surgical pain, and many other uses in dental practice [7,8].

MATERIALS AND METHODS

Twelve healthy-looking male New Zealand rabbits, three to four months in age, and 1.2~1.4 kg in weight were included. They were kept separately in clean cages and housed in a standard environment (a temperature of 18~24°C). They were fed equal amounts of standard food (grass and fresh vegetables) and water. Their health was monitored throughout the study. The rabbits were randomly divided into two experimental groups according to the tested material used to cover the wound postoperatively, the tested agents included the following: Group I: PerioKIN Hyaluronic 1% ® gel (LABORATARIOS KIN S.A., Barcelona - Spain) contains 1% Hyaluronic acid and 0.2% Chlorhexidine DG, and excipients. And Group II O3OHEAL-M ® gel (Advanced Pharma Care, Jordan) contains Ozonated Sunflower (Seed Oil), hydrogenated vegetable oil, and propylene glycol. The surgical procedures followed a standard protocol used at the Department of Oral and Maxillofacial Surgery - College of Dentistry / Mosul University. To achieve general anesthesia, each rabbit was given 40 mg/kg ketamine intramuscular injection in the thigh muscle, mixed with Xylazine 4 mg/kg (as a muscle relaxant). After anesthesia, the surgical field in the oral cavity was disinfected by Chlorhexidine Gluconate 0.2%. Two incisions were made on the anterior buccal mucosa of each rabbit bilaterally, 0.5 cm in length, and 2 mm (0.2 cm) in depth, using blade no.15, measured by a vernier. All wounds were intentionally left to heal by secondary intention. One milliliter of each agent was applied three times daily directly in to the wound using a disposable tip with

a blunt cannula connected to a syringe, for the first group, the defects were covered by (PerioKIN Hyaluronic 1%) gel, applied three times daily. O3OHEAL-M gel was used for the second group, also applied three times daily.

Histological Analysis: Histological evaluation was done to assess the physiological parameters of the wound healing process depending on 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 ½ of the field.

Score 3: Moderate Inflammatory cells could be seen in more than ½ of the field.

Score 4: Severe or abundant when Inflammatory cells present in huge numbers, more than ¾ of the field (X10).

2- Granulation tissue formation grading criteria:

Score 1: Absent 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-epithelization.

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. [9]

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.

RESULTS

During this study, all rabbits survived and tolerated the experimental procedure and recovered without any postoperative complications such as bleeding or infection of the wound. Figures (1,2,3) show the median results of inflammatory cells infiltration grading in which day 3 group II is the highest and day 14 is lowest and granulation tissue formation in which day 7 is highest and re-epithelization scoring shows highest at day 14, according to time period all Figures (4,5,6,7,8,9) show the histological features of the two groups according to time period. Table (1) show the comparison of ICI for oral mucosa by Mann-Whitney Test the comparison appears that there are no statistically

significant differences in all days of the study and for granulation tissue formation and also re-epithelization scoring. Tables (2,3) show the comparison using Mann-Whitney Test. The comparison appears that there are no statistically significant differences in all days of the study.

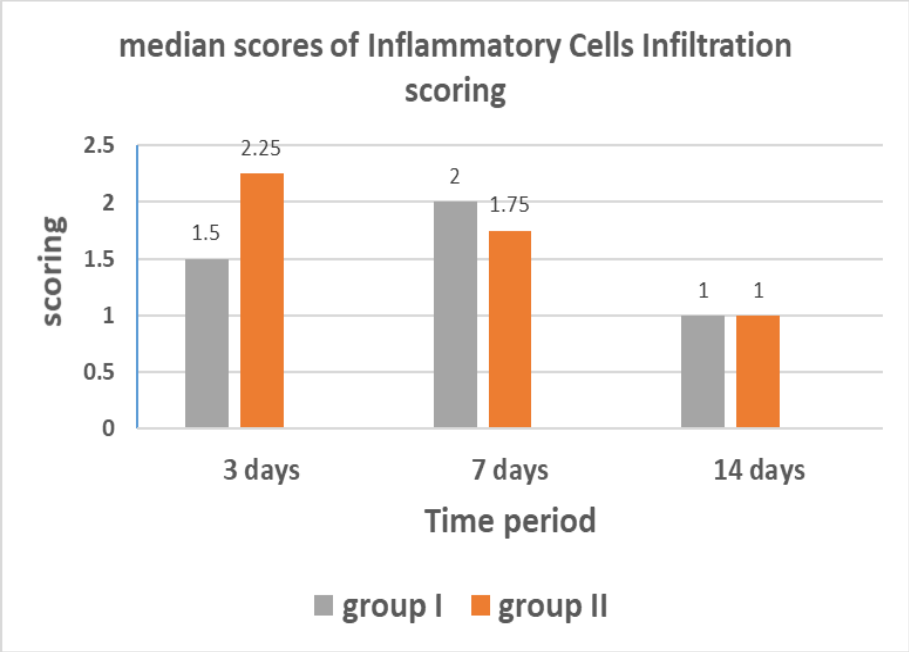

Figure 1: Inflammatory cells infiltration scoring for oral mucosa on 3rd, 7th, and 14th days after Incisions

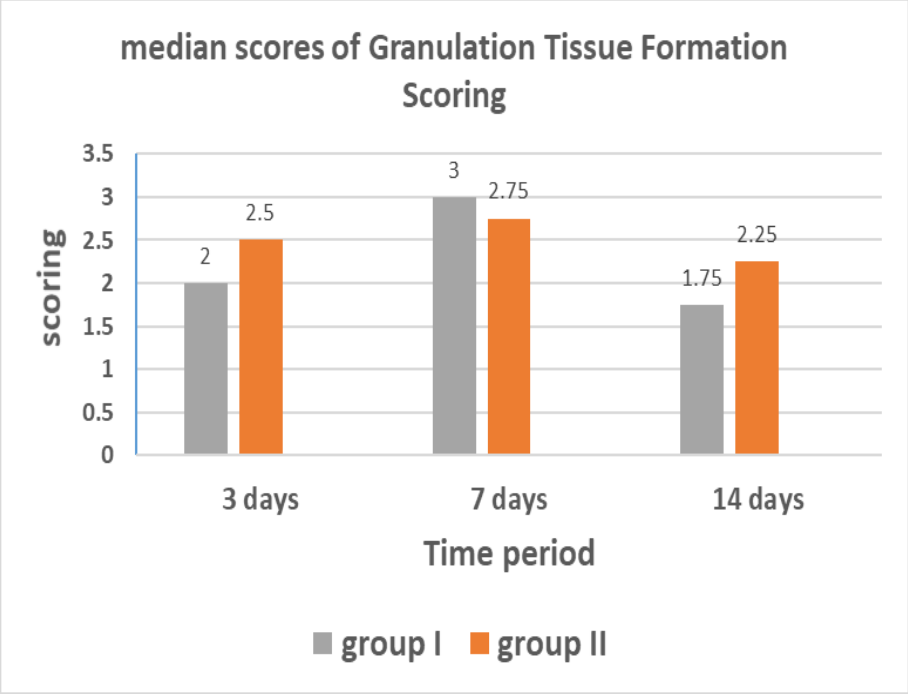

Figure 2: Granulation tissue formation scoring for oral mucosa on 3rd, 7th, and 14th days after Incisions

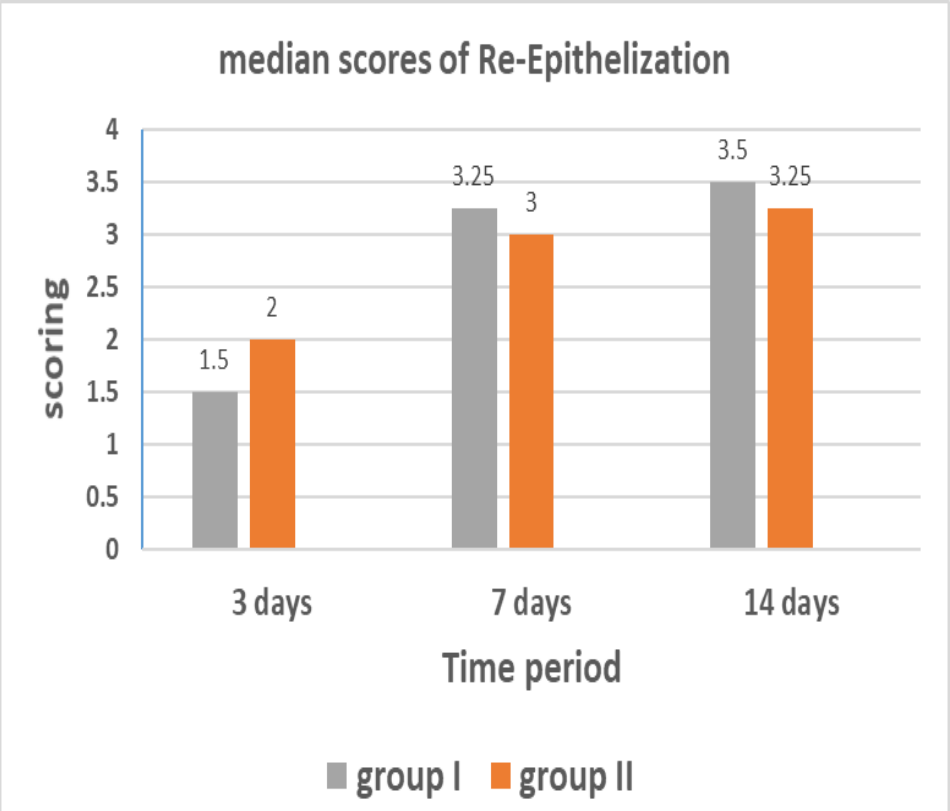

Figure 3: Re-Epithelization scoring of oral mucosa on 3rd,7th, and 14th

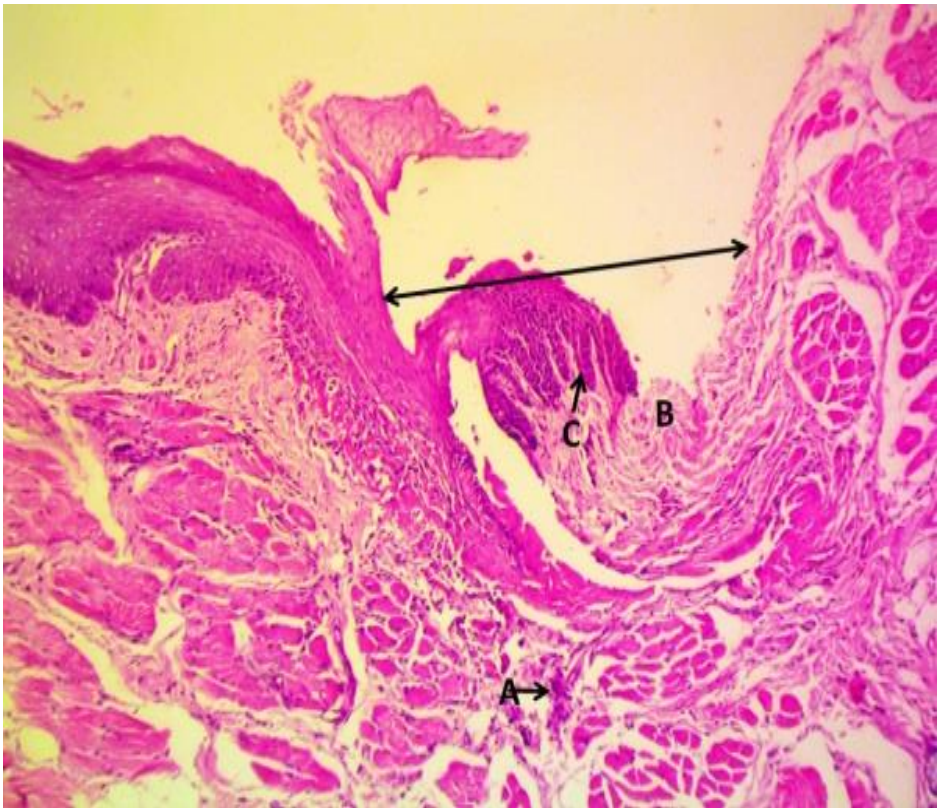

Figure 4: photomicrograph of the hyaluronic acid group (3 days) shows the site of the wound (↔) characterized by inflammatory cell infiltration (A), granulation tissue (B), and

Figure 5: photomicrograph of the ozonated group (3 days) shows the site of the wound (↔) characterized by inflammatory cell infiltration (A), granulation tissue (B), re-epithelialization (C), and angiogenesis (D). H&E stain, 100X.

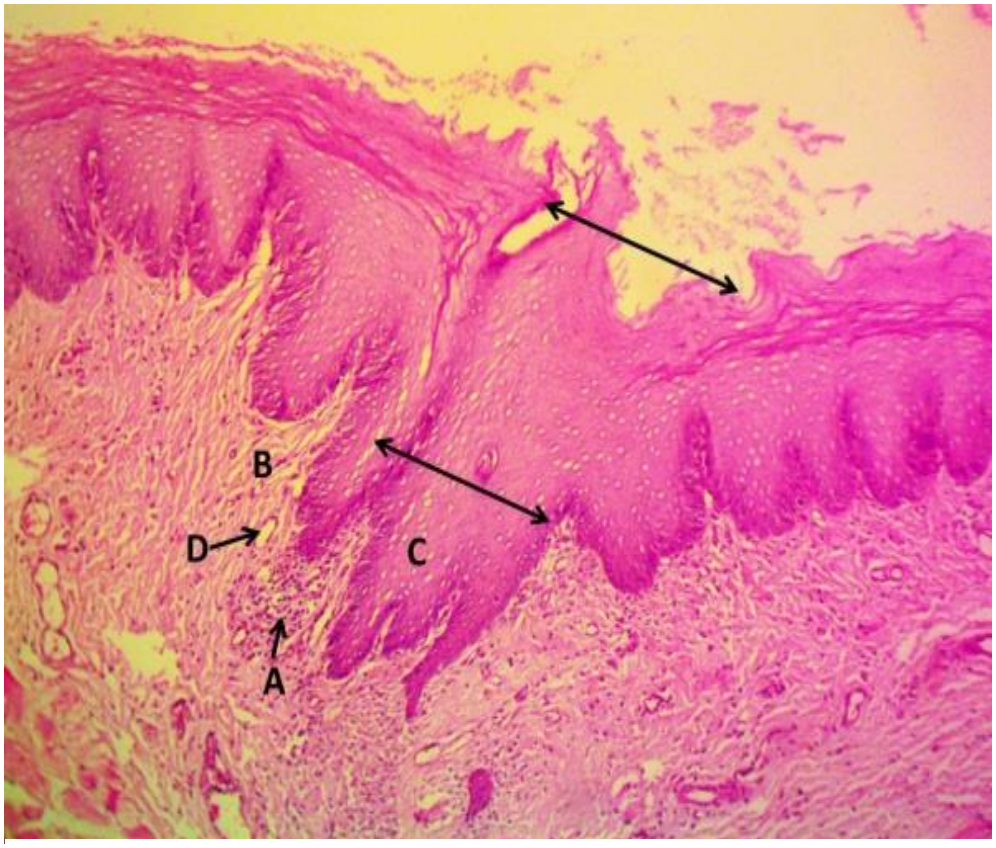

Figure 6: photomicrograph of the hyaluronic acid group (7 days) shows the site of the wound (↔) characterized by inflammatory cell infiltration (A), granulation tissue (B), re-epithelialization (C), and angiogenesis (D). H&E stain, 100X

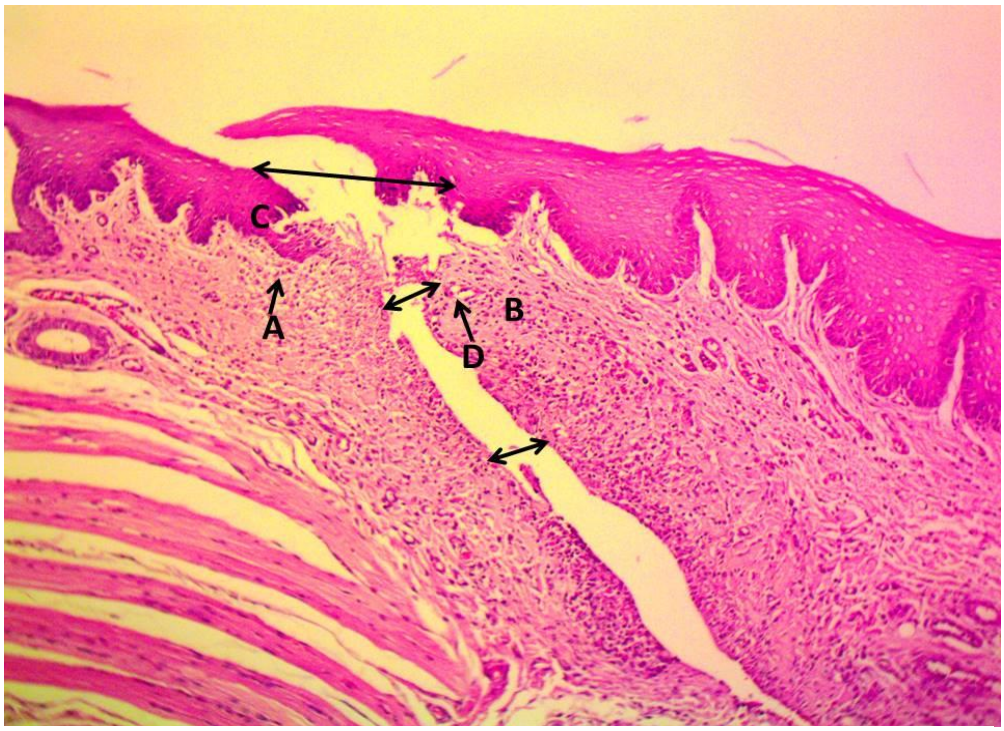

Figure 7: photomicrograph of the ozonated group (7 days) shows the site of the wound (↔) characterized by inflammatory cell infiltration (A), granulation tissue (B), re-epithelialization (C), and angiogenesis (D). H&E stain, 100X.

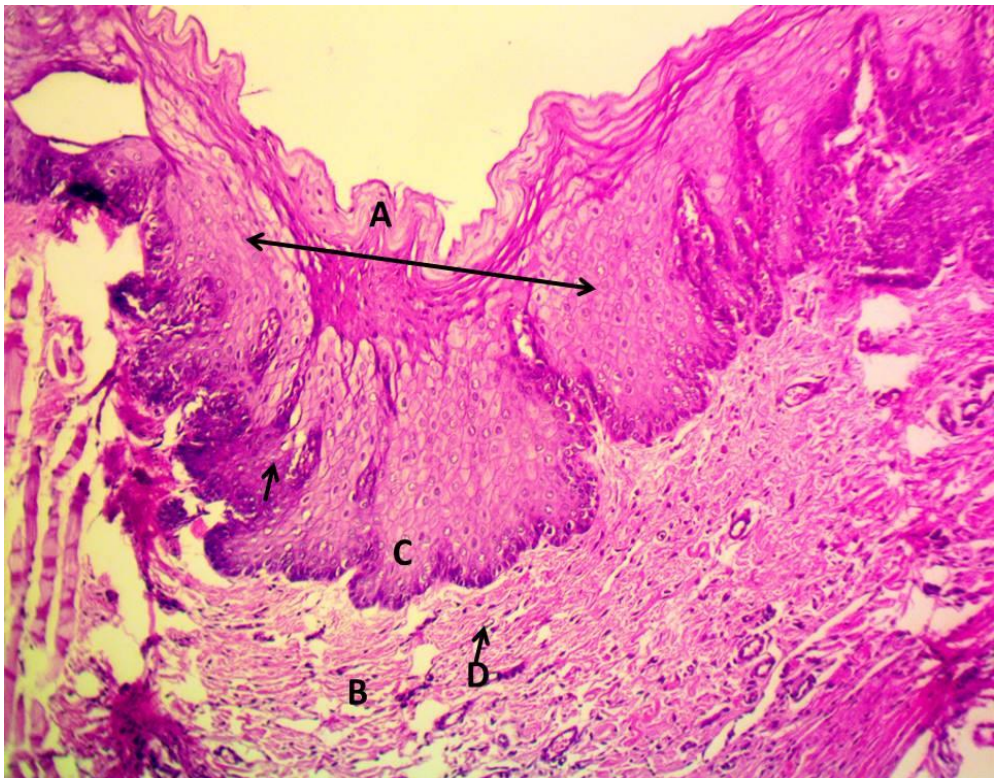

Figure. 8: photomicrograph of the hyaluronic acid group (14 days) shows the site of the wound (↔) characterized by increased keratin material (A), granulation tissue (B), re-epithelialization (C), and angiogenesis (D). H&E stain, 100X.

Figure 9: photomicrograph of the ozonated group (14 days) shows the site of the wound (↔) characterized by the presence of granulation tissue (A), re-epithelialization (B), and angiogenesis (C) without inflammatory cell infiltration

Table (1): Comparison of ICI* for oral mucosa (Mann-Whitney U Test: Comparison of Group Vs Group (within the same day).

Days	(P-value)
Group I day3 vs Group II day3	(0.222) Non-significant**
Group I day7 vs Group II day7	(0.317) Non-significant
Group I day14 vs Group II Day 14	(1) Non-significant

* ICI: Inflammatory Cells Infiltration
** P-value ≤0.05 significant

Table (2): Comparison of GTF* for oral mucosa (Mann-Whitney U Test: Comparison of Group Vs Group (within the same day).

Days	(P-value)
Group I day3 vs Group II day3	(0.127) Non-significant**
Group I day7 vs Group II day7	(0.317) Non-significant
Group I day14 vs Group II day 14	(0.186) Non-significant

*GTF: Granulation Tissue Formation
** P-value ≤0.05 significant

Table (3): Comparison of RE* for oral mucosa (Mann-Whitney U Test: Comparison of Group Vs Group (within the same day).

Days	(P-value)
Group I day3 vs Group II day3	(0.343) Non-significant**
Group I day7 vs Group II day7	(0.317) Non-significant
Group I day14 vs Group II day 14	(0.495) Non-significant

* RE: Re-epithelialization
** P-value ≤0.05 significant

DISCUSSION

The use of both PerioKIN Hyaluronic 1% ® gel and O3OHEAL-M ® gel has a positive impact on the mucosal soft tissue healing process by enhancement of the amount of granulation tissue and epithelial tissue. therefore, there is a very close activity of the two materials in relation to their use in wounds with tissue loss. the rabbit model was used because of the need for histological examination of the specimen although the rabbits were not euthanized and the area was closed by undermining of the adjacent tissue of skin and mucosa and healed by primary intention. HA has two significant functions in wound healing. It produces a short-term structure throughout the early stages of healing, and most significantly, it triggers cell proliferation and migration. [10] HA is a natural constituent of the extracellular matrix, it provides structure and hydration, and thus produces a non-immunogenic scenario that helps in renewal and healing. It affects the clinical outcomes of inflammation, supporting wound healing [11, 12]. The present study was undertaken to evaluate the therapeutic effects of topical hyaluronic gel and ozonated sunflower oil on the healing of open wound sites intraorally, with epithelial and connective tissue deficiencies that heal by secondary intention. Wound healing is a complex biological process that is commonly divided into overlapping phases: inflammation, re-epithelialization, granulation tissue formation, matrix formation, and tissue remodeling [13, 14]. The use of ozonated oil can affect the nature and quality of the inflammatory infiltrate and the granulation tissue component. [15-17]. The rate of re-epithelialization was evaluated to determine the influence of ozonated oil on the healing of lost tissue. Re-epithelialization is a major component of the wound healing process, which is achieved through a complex interplay of diverse growth factors, cytokines, and cell-cycle regulators [18]. Currently, ozone and its products are being investigated for their influence on growth factors, cytokines, and cell-cycle regulators in biological systems. Bocci *et al.* conducted a series of studies [19–21] and showed that the contact of ozone with human blood led to an increased release of TGF-*b*1; interferons-*a*, -*b*, and -c; interleukins-1, 2, 6, and 8, and tumor necrosis factor-*a* (TNF-*a*), which are important for human wound healing. Epithelial cell migration is an essential step

in wound closure, where epithelial cells migrate from the periphery of the wound towards the central part of the defect. The migration and proliferation of keratinocytes and the close relationship between the migratory cells and collagen of the newly-formed connective tissue determine the rate of re-epithelialization of the wound [(14)].

CONCLUSION

Topical use of PerioKIN Hyaluronic 1% gel and O3OHEAL-M gel three times daily for secondary intention wounds give a satisfying result of healing and isolation to get rid of the infection and ugly scar in appropriate time, so we recommend using it as an auxiliary medication for tissue loss wounds.

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