Nanotechnology Perceptions
ISSN 1660-6795
www.nano-ntp.com

Histological Study Of The Effect Of Nano Silver And Nano Zinc On The Healing Of Traumatic Ulcer In The Oral Mucosa

Dr. Yassmina Zakaria Hassan[1] , Prof. Dr. Manar Abd El Aziz Ahmed[2] , Prof. Dr. Mervat Mohamed Yousef[3] , Prof. Dr. Elham Fathy Mahmoud[4]

[1]Lecturer assistant in Oral Biology Department, Faculty of Dentistry, Suez Canal University.
[2]Professor of Oral Biology Department, Faculty of Dentistry, Suez Canal University.
[3]Professor of Oral Biology Department, Faculty of Dentistry, Suez Canal University.
[4]Professor of Oral Biology Department, Faculty of Dentistry, Suez Canal University.
[1] Yassmina_zakaria@dent.suez.edu.eg
[2]Manar3bdel3aziz@yahoo.com
[3]Dr.mervathawas@gmail.com
[4]Elham_fathy@dent.suez.edu.eg

Background: Nanoparticles have been under consideration for usage in healthcare and the field of wound healing because of their cost-effectiveness, small size, stability and safety. In wound healing, nanoparticles are evaluated based on three key criteria: their function as delivery vehicles, role in the repair process and antimicrobial properties. Because of these characteristics, metal nanoparticles like gold, silver, copper, and zinc are excellent candidates for use in the wound bed and for incorporation into dressings. Objectives: To study the healing of experimentally induced traumatic ulcers in the oral mucosa of Albino rats by using topically Nano silver and Nano zinc. Materials and methods: we used adult male albino rats which are 40 in number and were separated into four groups, 10 rats for each group: Group I: was a normal group. Group II: the ulcer was left without treatment. Group III: the ulcer was treated by twice daily topical application of Nano silver solution. Group IV: the ulcer was treated by twice daily topical application of Nano zinc solution. Each group except Group I divided into two subgroups: subgroup 1 sacrificed after 7 days and subgroup 2 sacrificed after 14 days. Results: wound healing is completed after 14 days while the usage of Nano medicine improve healing. Conclusion: Nano silver is antimicrobial agent; this prevent wound infection and that is why wound healing improved.

Keywords: Anti-microbial effect; buccal mucosa; Inflammation; Nano material and Wound healing.

Introduction

The oral cavity is commonly affected by mucosal disorders, with oral ulcers being the most frequent. Conditions involving ulcerations of the oral mucosa can significantly impact the quality of life. When the oral mucosa is broken and extends into the submucosa, breaching the lamina propria, it is defined as oral ulceration [1].

The body's protective function of wound healing prioritizes rapid recovery, while regeneration in a challenging environment is a more time-consuming process. Specifically, the oral cavity is an extraordinary environment where wound healing takes place in a warm oral fluid teeming with millions of microorganisms [2].

Nanotechnology is gaining more attention in the medical field. Nanoparticles, have small size so it has high surface area and this enhancing their effect in wound healing. [3].

Silver nanoparticles (AgNPs) have demonstrated to be anticancer, act as antioxidants and antibacterial. This quality makes them suitable for use in bandages for wound healing and in replacements for antibiotics [4].

Zinc oxide nanoparticles (ZnONPs) are compatible with living tissues, can pass through the skin layers and have shown impressive healing properties in living organisms (using rats as a model). This includes the process of re-epithelialization, the movement of keratinocytes together with the accumulation of collagen fibers and the formation of new tissue [5].

So, this investigation was designed to evaluate the healing of induced traumatic ulcer by using Nano silver and Nano zinc formulation.

Materials and methods

Materials used in this study included as follow:

- Nano silver solution: the content of metallic silver is 12 mg/mL and Polyvinylpyrrolidone (PVP) {Stabilizer}.
- Nano zinc solution: the minimal inhibitory concentration of ZnO nanoparticles was 125 μg/ml.

Preparation of nano materials:

1-Preparation of nano silver solution:

The substance of metallic silver contains 12 mg per milliliter, which is maintained through the addition of 188 mg per milliliter of Polyvinylpyrrolidone (PVP). AgNPs solutions were created using distilled and sterile water and were stored at a temperature of 4 °C in the absence of light. The dimensions of the silver nanoparticles were determined from the findings gathered through Transmission Electron Microscopy analysis, utilizing a JEOL-JEM-2010

microscope. The range of particle sizes observed was from 1 to 90 nanometers, with an average diameter of 35 nanometers [6].

2-Preparation of nano zinc solution:

The amount of ZnO nanoparticles was measured at 125 µg/ml, and particles of ZnO with dimensions between 10-30 nm were utilized. Propylene glycol solution was chosen as the diluent for the ZnO nanoparticles since they are not soluble in water [7].

Characterization of the prepared NPs by using Transmission Electron Microscopy (TEM) (figure1)

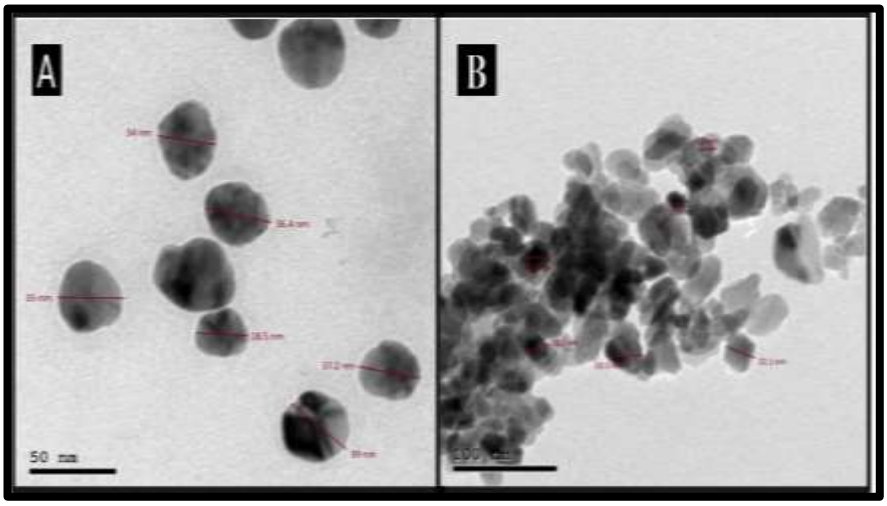

Fig. (1): **A** electron micrograph showed characterization of Ag NPs with average size 35 ± 4 nm. **B** electron micrograph showed characterization of ZnO-NP with average size 10-30 nm.

Instruments:

The instruments that used are soft tissue punch drill, mounted on low-speed surgical hand piece.

Study design:

Forty adult male albino rats with body weight of average 170 grams at the beginning of the experiment. The animals kept each five in one cage. They were fed and drank naturally. They were maintained at the ideal humidity and temperature.

Animal grouping:

Animals were classified into 4 groups, 10 rats for each group as follow:

- Group I: 10 normal rats (-ve control), no subgrouping and it will be sacrificed after 14 days.

- Group II 10 rats with induced ulceration, have no treatment (+ve control) and divided into 2.1 subgroup sacrificed at day 7 and 2.2 subgroup sacrificed at day 14.
- Group III 10 rats with induced ulceration, treated by twice daily topical application of nano zinc solution and divided into 3.1 subgroup sacrificed at day 7 and 3.2 subgroup sacrificed at day 14.
- Group IV 10 rats with induced ulceration, treated by twice daily topical application of nano silver solution and divided into 4.1 subgroup sacrificed at day 7 and 4.2 subgroup sacrificed at day 14.

Ulcer Induction:

Following the administration of anesthesia, ulcers were induced in the central region of the buccal mucosa on both the right and left sides, utilizing a 5 mm diameter punch. The resulting lesions were confined to the mucosa and did not involve the muscles [8].

Euthanization method:

Half of the animals of each group (5 animals) scarified after euthanization by overdose of ether in the 7th days, while the other half (5 animals) scarified after 14 days. After sacrificing, the buccal mucosa specimens were taken and prepared for light microscope investigations.

Histological Evaluation:

Preparation of the samples:

Tissue fixation was carried out to prevent postmortem decay by killing the tissue. For the purpose of analysis, this procedure aids in keeping biological material, such as tissue or cells, as near to their original state as feasible. Samples were immersed in 10% Neutral Buffered Formalin (NBF) [9].

After that, tissue processing involves transforming the samples into thin microscopic sections. This is achieved by embedding them in paraffin and sectioning them into 4-6 micrometer sections. The process of embedding fixed tissue in paraffin is known as tissue processing, where the samples are placed in cassettes, manually transferred to blocks, and covered with molten paraffin. Proper alignment and orientation of tissues in the paraffin block are crucial during this embedding process [10].

Staining of the samples:

In our research the stains have been used they are:

1) Hematoxylin and eosin for histological examination.

2) Masson, s Trichrome for detection of collagen fibers.

3) Immunohistochemical localization of E-cadherin to detect cellular adhesion.

Statistical Analysis:

All data was calculated, tabulated, and statistically analyzed and compared via suitable statistical tests. Statistical analysis was performed using the computer SPSS software.

Results:

Investigations under light microscope

1-By using hematoxylin and eosin stain.

Group I showed normal histological features of the buccal mucosa. Group II showed the buccal mucosa of rats in this group (positive control) with induced traumatic ulcer left without treatment showed change in the histological appearance. This group is divided into 2 sub-groups: 2.1 sub-group which left without treatment for 7 days showed complete loss of epithelium and lamina propria showed degeneration in collagen fibers, inflammatory cells infiltrations and dilation in blood vessels with engorgement of blood inside them. 2.2 sub-group which left without treatment for 14 days showed slight healing in epithelium, the epithelial ridges become longer, and irregular compared to the control group. Lamina propria showed infiltration of inflammatory cells and dilation in blood vessels and hemorrhage. Group IV showed buccal mucosal sections of rats in this group with induced traumatic ulcer treated with twice daily topical application of nano silver solution showed improvement in histological appearance than group II and group III which showed buccal mucosal section treated by nano zinc oxide solution (figure 2).

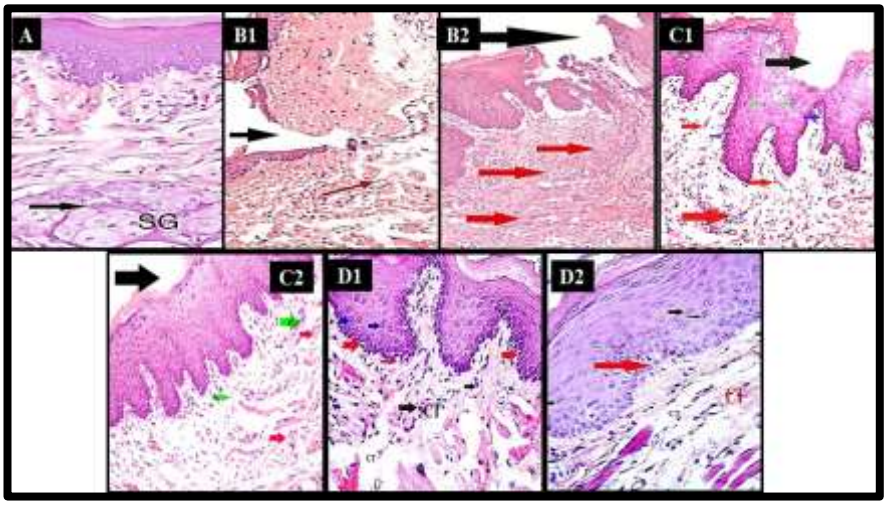

Fig. (2): **A** group I normal buccal mucosa showed normal buccal mixed salivary glands (SG), mucous acini capped with serous demilune (black arrow) and surrounded by muscular bundles. **B1** a photomicrograph of 2.1 subgroup buccal mucosa showed complete loss of epithelium (black arrow) and showed some degenerated areas in lamina propria (red arrow). **B2** a photomicrograph of 2.2 subgroup buccal mucosa showed loss in epithelium (black arrow), accumulation of inflammatory cells in lamina propria (red arrows). **C1** a photomicrograph of 3.1 subgroup buccal mucosa showed slight healing in epithelium (black arrow) with irregular

and long ridges, binucleated cells (green arrow), the prickle cell layer showed acanthosis with swelling of cells (S) and infiltration of inflammatory cells (red arrows).**C2** a photomicrograph of 3.2 subgroup buccal mucosa showed healing in epithelium (black arrow), some infiltration of inflammatory cells (green arrows) and blood vessels (red arrows). **D1** a photomicrograph of 4.1 subgroup buccal mucosa showed degeneration of the basement membrane (red arrows), binucleated cells in prickle cell layer (blue arrow), inflammatory cells infiltration (black arrows), and degenerated collagen fibers (CT). **D2** a photomicrograph of 4.2 subgroup buccal mucosa showed degeneration in basement membrane (red arrow), binucleated cells in prickle cell layers (black arrow) and degeneration in collagen fibers (CT).

(Hematoxylin & Eosin. orig. mag.200)

2-Masson'sTrichome Staining:

The histology analysis of buccal mucosa taken from rats in all groups are discussed in (figure 3).

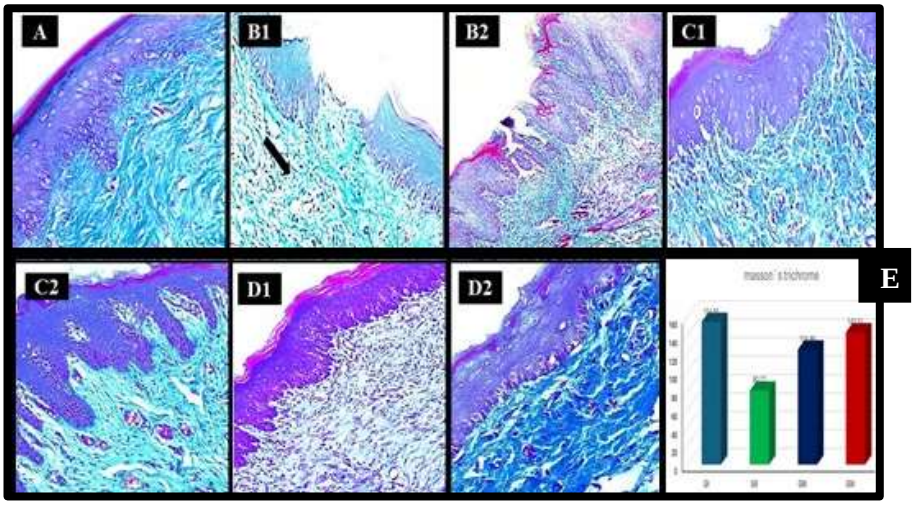

Fig. (3): **A** a photomicrograph of buccal mucosa of control rat showed strongly positive staining of the normal distributed collagen bundles in lamina propria. **B1** a photomicrograph of group 2.1 showed weakly positive staining of collagen bundles in the lamina propria (arrow). **B2** a photomicrograph of sub-group 2.2 showed weak to moderately positive staining of collagen bundles in the lamina propria. **C1** a photomicrograph of group 3.1 showed weak to moderately positive staining collagen bundles in the lamina propria. **C2** a photomicrograph of sub-group 3.2 showed moderately positive staining of collagen bundles in the lamina propria. **D1** a photomicrograph of sub-group 4.1 showed moderately positive staining of collagen bundles in the lamina propria. **D2** a photomicrograph of group 4.2 showed strongly positive staining of collagen bundles in the lamina propria. **E** histogram showed the mean of Masson's trichrome staining intensity among the studied groups.

(Masson trichrome orig. mag. 200)

Statistical Analysis for Masson's trichrome staining intensity:

Mean and Standard deviation (SD), minimum and maximum values for masson`s trichrome for different groups were presented in table (1).

The results showed that there are clearly significant differences between groups for masson`s trichrome Using one-way ANOVAs (F=306.969, P<0.001) at a significant level P< 0.05. The pairwise comparison showed a significant difference between each group to others. The high mean value was recorded in group I (154.91±5.41) followed by group IV (143.51±3.94) and group III (124.88±7.32) while the GII group was the lowest (80.27±6.48).

Table 1, Masson`s trichrome

Groups	Mean	SD	Min.	Max.	F test	P value
GI	154.91[a]	5.41	142.33	161.19	306.969	<0.001**
GII	80.27[d]	6.48	72.05	92.46		
GIII	124.88[c]	7.32	106.57	130.67		
GIV	143.51[b]	3.94	136.34	148.82		

** and different superscript letters mean significant difference at P<0.05

3-Immunohistochemical localization of E-Cadherin

The histology analysis of buccal mucosa taken from rats in all groups are discussed in (figure 4).

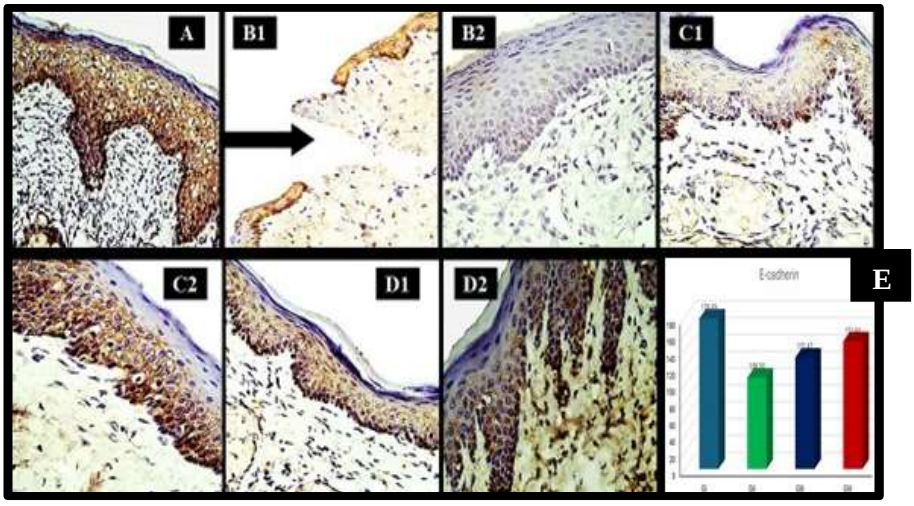

Fig. (4): **A** The buccal mucosa of control group incubated with mouse monoclonal antibody of E-cadherin clone 36B5 showed strongly positive staining reactivity of epithelial cells. **B1** The buccal mucosa of group 2 showed loss of epithelium (arrow). **B2** a photomicrograph of buccal mucosa of group 2.2 showed negative to weakly positive staining reactivity of epithelial cells. **C1** a photomicrograph of buccal mucosa of group 3.1 showed moderate to strongly positive staining reactivity of epithelial cells. **C2** a photomicrograph of buccal mucosa of group 3.2 showed strongly positive staining reactivity of epithelial cells. **D1** a photomicrograph of buccal mucosa of group 4.1 showed moderate to strongly positive staining reactivity of epithelial cells. **D2** a photomicrograph of buccal mucosa of group 4.2 showed strongly positive staining reactivity of epithelial cells. **E** a statistical chart illustrates the staining reactivity of buccal mucosa of different groups to E-Cadherin

(orig. mag. 200)

Statistical analysis of E-cadherin expression:

Mean and Standard deviation (SD), minimum and maximum values for E-cadherin for different groups were presented in table (2)

The results showed that there are clearly significant differences between groups for E-cadherin Using one-way ANOVAs ($F=175.614$, $P<0.001$) at a significant level $P< 0.05$. The pairwise comparison showed a significant difference between each group to others. The high mean value was recorded in group I (178.89±2.22) followed by group IV (151.84±5.15) and group III (132.47±7.31) while the GII group was the lowest (108.50±9.65).

Table, 2. E-cadherin

Groups	Mean	SD	Min.	Max.	F test	P value
GI	178.89^{a}	2.22	175.50	183.00	175.614	<0.001**
GII	108.50^{d}	9.65	95.91	120.11		
GIII	132.47^{c}	7.31	119.63	140.25		
GIV	151.84^{b}	5.15	137.41	160.28		

** and different superscript letters mean significant difference at P<0.05

Discussion:

Ulcer healing is a biological process that involves the planned migration and proliferation of cells, as well as the angiogenesis, remodeling, and extracellular matrix deposition. An oral ulcer is a common oral condition which is extremely hurtful and can be infected and lead to local tissue necrosis and inflammation, which can lengthen the disease's course and make the patient's suffering worse [11].

Since traumatic ulcers are the most prevalent inflammatory ulcerative conditions in the oral cavity and the cheek mucosa is the most traumatized site, traumatic ulcers are induced in the buccal mucosa in this study. These injuries can result from habits, poorly fitting prostheses, occlusal disharmony, broken restorations, tooth crowns, or even unintentional bites during chewing [12]. Inflammation, proliferation, and remodeling are some of the critical processes that wound healing goes through to replace lost tissue layers and cellular structures [13].

Because topical application of antimicrobial agents allows the drug to enter the targeted site directly without causing systemic adverse effects, it was chosen for this study instead of systemic administration.

A silver-based compound's antimicrobial activity is dependent on its morphology, or size and shape, in addition to its type (silver nitrate, silver-sulfadiazine, etc.) and released species (Ag+, Ag0, etc.). The compound's surface area grows as its size decreases. Increased antimicrobial activity is the consequence of a high silver species efflux rate caused by a large surface area. For most bacteria, the antimicrobial activity of the silver nanorods was greater than that of bulk silver. [14].

In our histological results, clear cells have been found among epithelial cells of normal buccal mucosa. It was explained that clear cells are defined as cells that have an empty appearance due to a clear halo surrounding their nuclei, which may be caused by an abundance of glycogen or another material not stained by hematoxylin or eosin. One could consider clear cells to be the defining characteristic of the cellular remnants of the stomodaeum, the ancestral oral cavity. Thus, it is discovered that these cells have a significant amount of PAS positive diastases labile material, which is suggestive of glycogen. [15].

In this study, it was recognized an increase in clear cells in the epithelium of the induced traumatic ulcer and that could be explained by the increase of Langerhans cells may have a role in wound healing. Our opinion was confirmed by the study reported that the Langerhans cells (LCs) represent one of the first cells of immunological barrier and play an important role during the inflammatory phase of acute wound healing [16].

By examination of group II rats' buccal mucosa where the traumatic ulcer was induced and left without treatment, after 7 post-surgical days; the histological results revealed complete loss of epithelium while after 14 days showed slight healing in epithelium, the epithelial-connective tissue interface showed degeneration of the basement membrane at certain areas. The prickle cell layer showed edema. Lamina propria showed infiltration of inflammatory cells and dilation in blood vessels and hemorrhage.

These findings were consistent with Saetta's [17] research, which demonstrated that the control group experienced significant inflammatory changes at 3–5 days after a bronchial ulcer caused by high-dose brachytherapy (a procedure in which radioactive material is inserted into the body). These changes included an increase in the number of inflammatory cells in the connective tissue, extensive epithelial desquamation, and a loss of the celery appearance of the bronchial epithelium.

The histological results of buccal specimens of group III rats with induced traumatic ulcer treated with twice daily topical application of nano zinc solution showed slight improvement in histological appearance than ulcer that left without treatment in group II.

The binucleated cells that observed in prickle cells during healing in group III was explained by Losick [18] who noted that puncture wounds heal rapidly by forming a scab as it helps in plugging the wound and holding its edges together to aid in wound healing. Although the resealing of the epithelium beneath the scab was seen, the cellular mechanism responsible for this process has only recently been identified. Reentering S phase, the surrounding epithelial cells heal a puncture wound by becoming polyploid instead of dividing. So, the epithelial cells repair as a very large polyploid cell.

Masson's trichrome staining is advantageous and provide further understanding in histopathological study of wound healing [19]

The buccal mucosa obtained from group II with induced traumatic ulcer left without treatment days revealed that the collagen fibers of the lamina propria showed weakly positive staining with Masson's trichrome stain and this supported by Suvik and Effendywhen [20] eighteen clinically healthy Sprague Dawley's female rats were obtained for wound healing study. The gross observation showed complete epithelization at day 17, the collagen was still less deposited at the wound area even at day 21 post-wounding, a sign of the formation of fine collagen fiber.

In group III showed improvement than group II as fibroblasts start to invade the wound site two or three days after the wound is made, signaling the start of the tissue proliferative phase. Collagen and extra cellular matrix deposition, which acts as a scaffold for repair, is linked to fibroblast infiltration. Microvasculature, keratinocytes, and epithelium can migrate because collagen acts as a bed for their migration. TGFβ/SMAD (Mothers against decapentaplegic homolog protein) signaling is one of the main modulators of ECM deposition and fibrosis. Because zinc is an essential cofactor for SMAD signaling, it is crucial for the development of granulation tissue [21].

Collagenases and plasminogen activators worked together to break down fibrin clots, and zinc-dependent matrix metalloproteinase broke down dermal basal membranes and extracellular matrix (ECM), making space for angiogenesis, migration, and cell growth [22].

In group IV there is improvement of collagen arrangement during wound healing. It was reported that wound treated by silver enhance the fibroblasts' rate of migration. Additionally, fibroblasts treated with Nano silver exhibited increased expression of α-SMA, a marker of myofibroblasts, indicating that these fibroblasts could develop into myofibroblasts. Since myofibroblasts are highly mobile and contractile, Nano silver may be able to help speed up wound healing and dermal regeneration by changing fibroblasts into myofibroblasts (which are then involved in strengthening the wound by depositing extracellular collagen fibers in the wound tissue) [23]

The molecules known as cadherins are involved in the formation of adherent junctions, which allow cells to adhere to one another. The E-cadherin antibody recognises the E-cadherin protein, which is encoded by the CDH1 gene. E-cadherin is a calcium-dependent cell adhesion protein that is necessary for cell migration and proliferation. This antibody is used to analyze E-cadherin protein expression throughout the epithelial mesenchymal transition. [24].

In group II showed a week staining reaction of the epithelium. Our results are confirmed by Masamitsu [25] who prepared a round full thickness excisional wounds (6 mm in diameter) and full-thickness incisional wounds dorsally in mice. On various days after the operation, E-cadherin expression was examined by immunohistochemical staining using a monoclonal antibody specific for E-cadherin. According to the findings, epithelial cells express less E-cadherin. The rate of migration and mitosis may be a factor in this decline. Furthermore, the alteration in incisional and excisional wounds was comparable.

In group III with induced traumatic ulcer treated with nano zinc for 14 days revealed better results than previous group.

According to Asti Meizarini's research [26], which examined the effectiveness of a dressing made of zinc oxide and turmeric extract in promoting the reepithelization phase of rat wound healing, E-cadherin expression in the control rats' basal layer dramatically increased on day 7 and continued to rise steadily until day 14. On the other hand, in the treatment groups, E-cadherin expression increased earlier on day 5 and peaked on day 7. This suggests that the dressing containing zinc oxide and turmeric extract may hasten the formation of adherent junctions. Hence, cadherin-dependent regulation of suprabasal junctional EGFR (epidermal growth factor) is essential for the establishment of epithelial barrier function, for regeneration of the barrier and restoration of homeostasis on barrier disruption [26].

In group IV showed a massive improvement than group II. As mentioned before silver has an antimicrobial effect which improve wound healing and Nano silver better than silver nitrate and this increase the activity of migration and mitosis of cells and this confirmed by Rajendran [27] who claimed that AgNPs caused neutrophils to undergo apoptosis, which had an anti-inflammatory effect. Consequently, lowering the concentration of pro-inflammatory cytokines which enhancing the rate of wound healing as well as lowering the hypertrophic scarring.

Conclusion:

From the results of the present investigation, the following conclusion could be reached: Infections may delay and deteriorate wound healing so antimicrobial agents should be used. Nanomaterial has the best effect due to its size improve penetration to wound.

Acknowledgement:

I would like to express my appreciation to my professors for their help and support and I would to express my deep thanks for all colleagues in the oral biology department who helped me, encourage me and participated in way or another to accomplish this work.

References:

1. Johnston L., Warrilow L., Fullwood I., Tanday A. Fifteen-minute consultation: oral ulceration in children. Arch Dis Child Educ Pract Ed. 2022; 107:257–264.
2. Wong, V. W., Gurtner, G. C., and Longaker, M. T. wound healing: a paradigm for regeneration. Mayo Clin. Proc. 2013; 88, 1022–1031.
3. Mordorski B., Rosen J. and Friedman J. Nanotechnology as an innovative approach for accelerating wound healing in diabetes. Diabetes Manag. 2015; 5(5): 329–332.
4. Majeed, S., Danish, M., Mohamad Ibrahim, M. S., Sekeri, S. H., Ansari, M. T., Nanda, A. Bacteria mediated synthesis of iron oxide nanoparticles and their antibacterial, antioxidant, cytocompatibility properties. J. Cluster Sci. 2021; 32, 1083–1094.
5. Yadid, M.; Feiner, R.; Dvir, T. Gold Nanoparticle-Integrated Scaffolds for Tissue Engineering and Regenerative Medicine. Nano Lett. 2019; 19, 2198–2206.
6. Ashokkumar S, Ravi S, Kathiravan V, Velmurugan S. Synthesis of silver nanoparticles using A. indicum leaf extract and their antibacterial activity. Spectrochim Acta A Mol Biomol Spectrosc. 2015; 134:34–39.
7. Jansen J, Karges W, Rink L. Zinc and diabetes—clinical links and molecular mechanisms. J Nutr Biochem. 2009; 20:399–417
8. Duarte C, Quirino M, Patrocínio M and Anbinder A. `Effects of Cha- Effects of Chamomilla recutita (L.) on oral wound healing in rats`, Medicina Oral Patologia Oral y Cirugia Bucal. 2011 Sep.; 1; 16 (6): e716-21.
9. Eltoum I, Frdenburgh J, Grizzle WE. Advanced concepts in fixation. 1. Effects of fixation on immunohistochemistry, reversibility of fixation and recovery of proteins, nucleic acids, and other molecules from fixed and processed tissues. 2. Developmental methods of fixation. Journal of Histotechnology. 2001; 24:201-210.
10. Eltoum I, Frdenburgh J, Myers RB, Grizzle WE. Introduction to the theory and practice of fixation of tissues. Journal of Histotechnology. 2001; 24:173-190.
11. Miao, M., Peng, M., Xing, Z., and Liu, D. "Effect of Shuangjinlian mixture on oral ulcer model in rat". Saudi Journal of Biological Sciences. 2009; 26(4), 790–794.
12. Compilato D, Cirillo N, Termine N, Kerr AR, Paderni C, Ciavarella D, Campisi G. Long-standing oral ulcers: proposal for a new “S-C-Dclassification system”. J Oral Pathol Med. 2009; 38:241-53.
13. Li H., Fu L., Ouyang Y., Cai C., Wang J., and Sun Z. Adult bone marrow-derived mesenchymal stem cells contribute to wound healing of skin appendages. Journal of Dermatol Sci. 2007; 14:325–335
14. Warriner R., and Burrell R. Infection and the chronic wound: a focus on silver. Journal of Advanced Skin Wound Care.2005; 18:2–12.
15. Vahabi S, Salman BN, Rezazadeh F, Namdari M. Effects of cyclosporine and phenytoin on biomarker expressions in gingival fibroblasts of children and adults: An in vitro study. J Basic Clin Physiol Pharmacol. 2014; 25:167-73
16. Gallo PM, Gallucci S. the dendritic cell response to classic, emerging, and homeostatic danger signals. Implications for autoimmunity. Frontiers in immunology. 2013; 4:138.
17. Saetta M., Stefano A., and Maestrelli P. Activated T-lymphocytes and macrophages in bronchial mucosa of subjects with chronic bronchitis. Am journal of Rev Repir Dis. 1992; 47:301– 306.
18. Losick VP, Fox DT, Spradling AC. Polyploidization and cell fusion contribute to wound healing in the adult Drosophila epithelium. Curr Biol. 2013; 23:2224–2232.
19. Elbischger P.J., Bischof H., Regitnig P. and Holzapfel G.A. Automat ic analysis of collagen fiber orientation in the outermost layer of human arteries. Patter n Analytical Application. 2004; 7:268-284.

20. Suvik A. and Effendy A.W.M the use of modified masson's trichrome staining in collagen evaluation in wound healing study. 2012; Volume 3 No. 1; 39-47.
21. Maywald M., Meurer S.K., Weiskirchen R., Rink L. Zinc supplementation augments TGF-beta1-dependent regulatory T cell induction. Mol. Nutr. Food Res. 2017; 61:3.
22. Arwert E.N., Hoste E., Watt F.M. Epithelial stem cells, wound healing and cancer. Nat. Rev. Cancer. 2012; 12:170–180.
23. Chuangang You, Qiong Li, Xingang Wang, Pan Wu, Jon Kee Ho, Ronghua Jin, Liping Zhang, Huawei Shao. Silver nanoparticle loaded collagen/chitosan scaffolds promote wound healing via regulating fibroblast migration and macrophage activation. 2017; 7: 10489.
24. Padmanaban V, Krol I, Suhail Y, Szczerba BM, Aceto N, Bader JS. Ecadherin is required for metastasis in multiple models of breast cancer. Nature. 2019; 573:439.
25. Masamitsu Kuwahara, WeMitsuo Hatoko, Hideyuki Tada and Aya Tanaka. E-cadherin expression in wound healing ofmouse skin. 2001; 28: 191–199.
26. Asti Meizarini, Aryati Aryati, Devi Rianti, Wibi Riawan and Astari Puteri. Effectivity of zinc oxide-turmeric extract dressing in stimulating the reepithelization phase of wound healing. 2020; 13; 2221-2225.
27. Rajendran N., Kumar S., Houreld N. A review on nanoparticle-based treatment for wound healing. Journal of Drug Delivery Science and Technology. 2018; 44:421-430.