

Study the Effect of Alum Activated by Cold Plasma on Mice Wounds Using Textural Analysis

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Abstract

The objective of this research is to concentrate on the gray-tone spatial-dependence matrices to extract the texture features of the wounded skin treated with an alum solution activated with plasma. This method depends on the manual selection of the region of interest, which results in the dependence of parameter values on the extracted region. Potassium alum is used to clean and treat wounds, as well as to prevent bacteria from spreading, where the aqueous solution has aluminum hydroxide which forms a white precipitate on the wounds that works to coagulate the blood and stop bleeding, its efficiency increases when activated by plasma. A group of mice were used; their ages ranged from 2–3 months and their weight ranged from 200–250 g. Wounds were created in mice, and the experiment included using alum in the form of powder, an aqueous solution, and an aqueous solution activated with plasma for a duration of 5 minutes. The textural analysis investigated the effect of alum powder and alum solution on wounds and skin. According to the results of the textural analysis, the histopathology image and gray-tone spatial-dependence matrices, the skin regained its external appearance (near normal), and the wounds healed quickly as a result of employing the plasma-activated aqueous solution. The results were investigated from the histopathology images.

Keywords: Gray tone spatial-dependence matrices, Plasma, Potassium alum, Textural analysis, XRF analysis.

Introduction

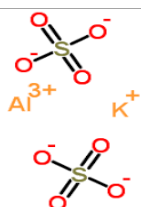
A wound is any disruption of the skin's, mucous membranes, or organ tissue's integrity. Simple wounds that are confined to the skin are characterized by complicated wounds that are deeper and entail harm to muscles, nerves, and arteries. Mechanical, thermal, chemical, and radiogenic damage can all result in wounds. Injuries to the skin or other body tissues are known as wounds. Cuts, scrapes, scratches, and perforated skin are among them¹. Aluminum compounds have been utilized as adjuvants in veterinary and human vaccinations for

about 90 years². Alum tends to help hosts develop effective and long-lasting protective immunity by generating antibody responses. Despite significant advancements in human vaccine manufacturing in recent years, alum remains the most routinely used adjuvant³. Glenny et al. revealed the adjuvant effect of alum for the first time when they discovered that processing an Immunogenic toxoid, diphtheria toxoid, by precipitation with potash (crude $KAl(SO_4)_2 \cdot 12H_2O$), resulted in a greatly enhanced immune response against the toxoid⁴. They later

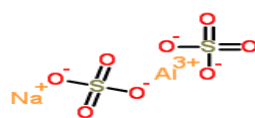
discovered that protein preparations precipitated with alum were highly heterogeneous, depending on which anions were present during precipitation, for example sulfate, bicarbonate, or phosphate. Wen Yand Shi Y. Alum employed aluminum gels to refine and optimize the protein–alum preparation, which represents a huge step forward in standardizing the use of alum as a common adjuvant. In contrast to the alum-precipitated vaccines of the past, vaccine preparations based on this technology are known as aluminum-adsorbed vaccines⁵. Asmaa Ali Hussein, study of the effect of Alum concentration on bleeding levels in this clinical trial showed that there are significant reductions in bleeding and the reduction increase with the increase of treatment concentration and have a significant correlation with the date the bleeding reduced to zero⁶, Oryan A. et al used the potash alum to treated the burn wounds⁷, Mariyam Roqaiya and Wajeeha Begum make a review on medical application of alum in medicine application⁸. The research aims to find the best locally available method for treating wounds that occur in our daily lives through the use of potash alum activated by plasma for the purpose of complete wound healing.

Alum is a generic double sulphate containing aluminum. Alum (potassium alum) is considered a double aqueous salt because it contains 12 water molecules in its crystalline structure. Alum is known in general as an aqueous double salt consisting of two types of positive ions, one of which is mono-charged and the other is triple-charged. A water molecule is composed of 12 (water of crystallization), which

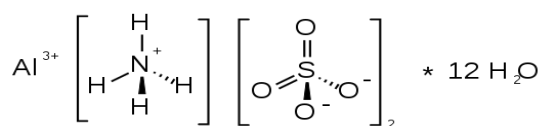
plays an important role in this type of salt, and two molecules of negative sulfate ions⁹. It is a mineral-derived medication. Swollen mucous membranes and excessive secretions are reduced when potassium alum is present. The coagulation cascade will be activated, which will stop the bleeding. In pharmaceuticals, potassium alum is used as an astringent, antiseptic, and adjuvant. The production of alum ions causes the blood to clot by neutralizing the charges on plasma proteins. Disinfectants have a similar effect because these ions react with the free organic acid and thiol groups of proteins on bacteria and free proteins, causing protein precipitation. The tissue will constrict, and secretions will dry up as a result of this action. Because alum has antibacterial properties, it helps prevent infection. Alum's Lekhan characteristic (chemical debriding effect) aids in the removal of dead, necrosed tissue from burn wounds, allowing healthy granulation tissue to develop. Alum and clove crude extracts were evaluated for antibacterial activity against *S. aureus*, *S. epidermidis*, *E. coli*, and *Klebsiella pneumoniae*. It demonstrated antibacterial and growth inhibitory activity against gram-positive and gram-negative bacteria isolated from diverse infection sites when compared to regular cefotaxime. Alum and clove crude extracts were evaluated for antibacterial activity against *S. aureus*, *S. epidermidis*, *E. coli*, and *Klebsiella pneumoniae*. It demonstrated antibacterial and growth inhibitory activity against gram-positive and gram-negative bacteria isolated from diverse infection sites when compared to regular cefotaxime^{10,11}. The most important types of alum are shown in Fig 1



a-Potassium alum, $KAl(SO_4)_2 \cdot 12 H_2O$, also called "potash alum" or simply "alum"¹².



b-alum, $NaAl(SO_4)_2 \cdot 12 H_2O$, also called "soda alum" or "SAS"¹².



c-Ammonium alum, $NH_4Al(SO_4)_2 \cdot 12 H_2O$ ¹²

Figure 1. The Chemical formal for different types of Alum: (a) Potassium alum, $KAl(SO_4)_2 \cdot 12 H_2O$, (b) Sodium alum, $NaAl(SO_4)_2 \cdot 12 H_2O$, and (c) Ammonium alum, $NH_4Al(SO_4)_2 \cdot 12 H_2O$.

It's an inorganic salt called potassium aluminum sulphate. It has a sweetish, astringent taste and is a colorless, transparent, and odorless crystalline mass or granular powder. When heated, it melts and loses its water of crystallization at around 200°C, resulting in the creation of anhydrous salt. Aluminum ammonium sulphate is soluble in 7.5 parts water and 1 part boiling water¹³. It operates via astringent protein precipitation at the cell surface and superficial interstitial spaces¹³. As a result, capillary permeability decreases, intercellular space contraction occurs, vasoconstriction occurs, capillary endothelium hardens, edema, and inflammation occur, and exudates diminish. Alum is beneficial for gingivitis, mucositis, and mouth ulcers. By contacting alum crystals, either as a solid or as a solution, alum can be utilized as a hemostatic for superficial abrasions, lesions, and ulcers on the lips¹⁴.

Pharmacodynamics

When potassium alum is present, it reduces swollen mucous membranes produced by inflammation of the nasal, urinary, and gastrointestinal pathways, as well as excessive secretions. The coagulation cascade will be triggered, resulting in the cessation of bleeding¹⁵. Potassium alum is used as an antiseptic, astringent, and adjuvant in pharmaceuticals. The astringent effect is achieved by

Materials and Methods

Preparation of Aqueous Solution

To make the aqueous solution, 40 g of alum powder is placed with 200 ml of distilled water in an Erlenmeyer flask, and the components are stirred for one hour with a magnetic stirrer device²⁰. The

To make the aqueous solution, 40 g of alum powder is placed with 200 ml of distilled water in an Erlenmeyer flask, and the components are stirred for one hour with a magnetic stirrer device²⁰. The resulting solution is divided into two parts, part is left as it is and the other is exposed to plasma²¹. The aqueous solution of alum is placed in a container and exposed to plasma for five minutes as shown in Fig 2.

Plasma Activation

The microwave-induced plasma is composed of

inducing coagulation in the superficial tissue layers until a crust is created. The production of alum ions causes the blood to clot by neutralizing the charges on plasma proteins. Disinfectants have a similar action, where these ions react with free organic acids on bacteria and free proteins, causing protein precipitation. The tissue will contract, and secretions will dry up as a result of this process. Its adjuvant qualities are mostly used in the manufacture of vaccines, where this chemical boosts the immune response^{16,17}.

Alum Advantage

Stop bleeding

Alum promotes rapid wound healing, reduces swelling, and restores the skin's natural texture. Its astringent and therapeutic effects account for this. Because of its hemostatic qualities, alum also works on the wound by reducing bleeding. Alum and honey are used to treat whooping cough by reducing mucus buildup in the lungs. Because of its drying properties, alum treats dysentery and diarrhea. Due to its powerful healing effect, topical administration of alum has been reported to be beneficial for mouth ulcers¹⁸. Because of its astringent properties, alum helps to control vomiting by reducing mucus in the lungs. Alum is used for the treatment of nasal hemorrhage. It allows nosebleeds to stop within seconds¹⁹.

five essential parts²²⁻²³

- 1- Microwave source (magnetron).
- 2- Tapered rectangular waveguide.
- 3- Plasma discharge tube.
- 4- Ignition system.
- 5- Gas supply and flow controller

Fig 2 shows the system of microwave plasma that used to active alum²⁴



Figure 2. The system of microwave plasma

To generate the torch for the active alum subjected to plasma for 5 minutes, a microwave-induced plasma system of non-thermal atmospheric pressure operated at frequency 2.45 GHz was employed using argon gas and a microwave power of 800 watts with voltage 175v and the gas flow at 2liter /min.

Gray Tone Spatial-Dependence Matrices

It assumes that texture information is adequately specified by a set of gray tone spatial-dependence matrices, which are computed for various angular relationships and distances between neighboring resolution cell pairs on the image, all textural features are derived from these angular nearest neighbor gray-tone spatial dependence matrix²¹.

Results and Discussion

Physical properties of Alum

XRF Analysis: The alum sample was collected from a Al-basher scientific office which sells scientific material and herbs in Baghdad with CAS number 7784-24-9. The chemical synthesis of the alum sample has been obtained via the technique of XRF (X-ray fluorescence). The results of the XRF examination technique are shown in Table 1, together with the element's concentration. The table only lists the elements with high concentration values; those with low concentrations are irrelevant to the outcome and are not listed. Because they encouraged the skin to mend itself, the Na₂O and MgO have made a significant contribution to the healing of the wounds²⁴.

Table 1. The Elements Concentration for the Alum

Symbols	Concentration
Na ₂ O	8.365
MgO	1.018
Al ₂ O ₃	15.75
SO ₃	45.47

The Test Groups

Four groups are used in the experiment which are the control positive, the group of Alum powder, the group of Alum solution and Alum solution exposed to plasma.

1- The (F1) moment of inertia ^{25,26}

$$F1 = \sum_{i=0}^{Ng-1} \sum_{j=0}^{Ng-1} (i-j)^2 M(i,j) \dots \dots \dots 1$$

2- Inverse difference moment (F2) that

$$F2 = \sum_{i=0}^{Ng-1} \sum_{j=0}^{Ng-1} \frac{M(i,j)}{1+(i-j)^2} \dots \dots \dots 2$$

3- Angular second moment

$$F3 = \sum_{i=0}^{Ng-1} \sum_{j=0}^{Ng-1} (M(i,j))^2 \dots \dots \dots 3$$

4-Entropy

$$F4 = \sum_{i=0}^{Ng-1} \sum_{j=0}^{Ng-1} (M(i,j)) \log(M(i,j)) \dots \dots \dots 4$$

In which M(i,j) is the gray level matrix and Ng is the number of gray level ^{27,28}.

1- Group of Control Positive

In comparison with sections of the control group Fig 3 and Fig 4. This group showed a very thick layer of necrotic tissue infiltrated with inflammatory cells and fibrin covered the site of injury. Underneath the dermal layer showed extensive granulation tissue, fibroplasia and infiltrated leukocytes (control positive) Fig 5. and Fig 6.

2- Group of Alum Experiment

Sub group of Alum powder

In comparison to sections of control groups (Fig 3 and Fig 4). Sections of this group were similar to those in control negative, revealing normal epidermal epithelium and dermal collagen bundles, hair follicles and cytoarchetecture (Fig 7 and Fig 8).

Sub Group of Alum Solution

The sections of the skin showed severe dermatitis which was characterized by the replacement of the epidermis by very thick layer of fibrin deposited followed by a layer of necrotic tissue and zone of inflammatory cells. Dermis was revealed severe cellulitis which is characterized by edema, degeneration and necrosis of collagen bundles associated with massive infiltration of mono and multinuclear leukocytes (neutrophils, lymphocytes and histocytes) (Fig 9, Fig 10 and Fig 11). Fig 12

and Fig 13 shows the section of skin (solution alum) in which the solution is exposed to plasma.

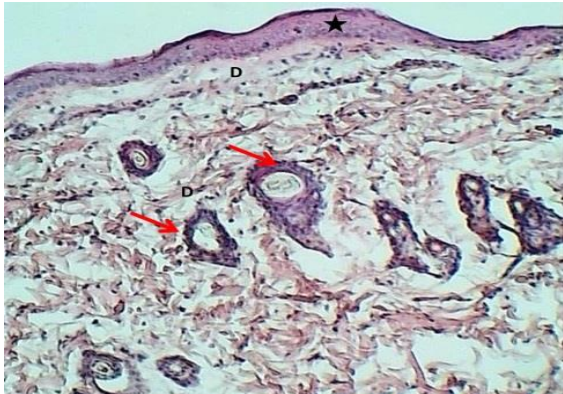


Figure 3. Section of skin (C-) (control negative) shows: normal appearance of epidermis (Asterisk), dermis (D) with hair follicles (Arrows). H&E stain. 100x.

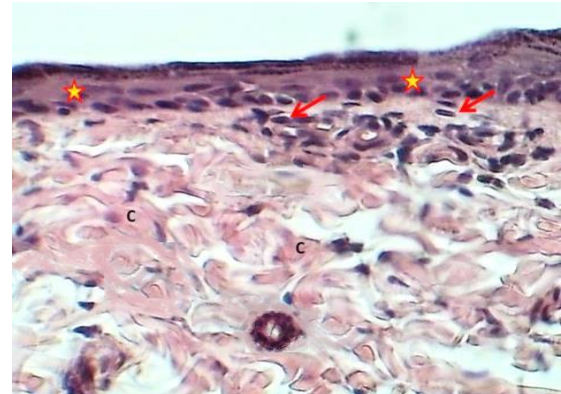


Figure 4. Section of skin (C-) (control negative) shows: normal appearance of epidermal epithelium (Asterisk), dermal collagen bundles (C) with normal content of fibroblasts (Arrows). H&E stain. 400x

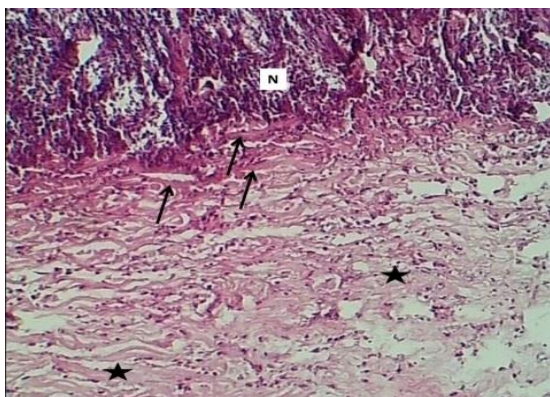


Figure 5. Section of skin (control positive) shows necrotic tissue (N), fibrin network (Arrows) & granulation tissue (Asterisks). H&E stain. 100x.

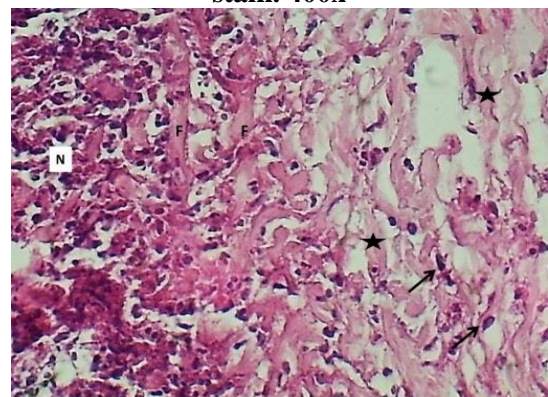


Figure 6. Section of skin (control positive) shows: necrosis tissue (N) collagen bundles (Asterisk), and fibroblasts (Arrows). H&E stain. 400x.

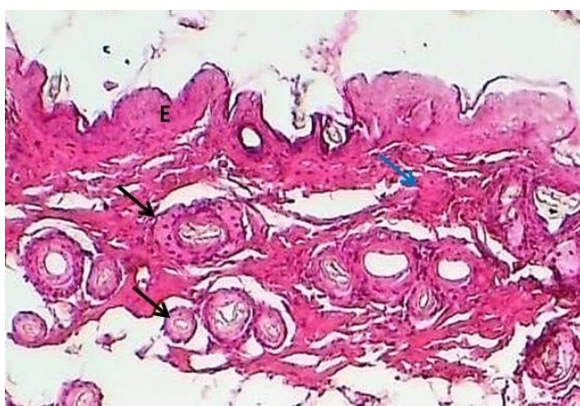


Figure 7. Section of skin (alum powder) shows: normal epidermis (E), normal dermis fibrous tissue (Blue arrows), & hair follicles (Black arrow) . H&E stain.100x.

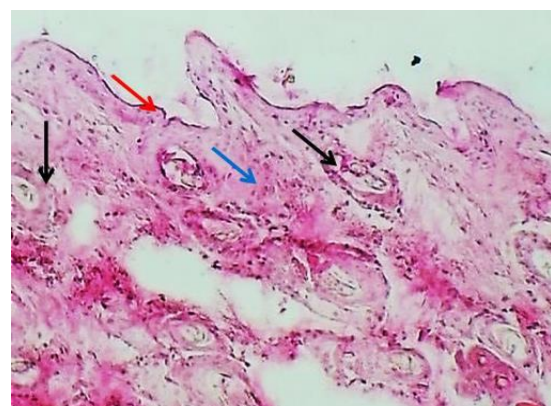


Figure 8. Section of skin (alum powder) shows: very thin epidermis (Red arrow), with normal dermis fibrous tissue (Blue arrows), & hair follicles (Black arrow). H&E stain.100x.

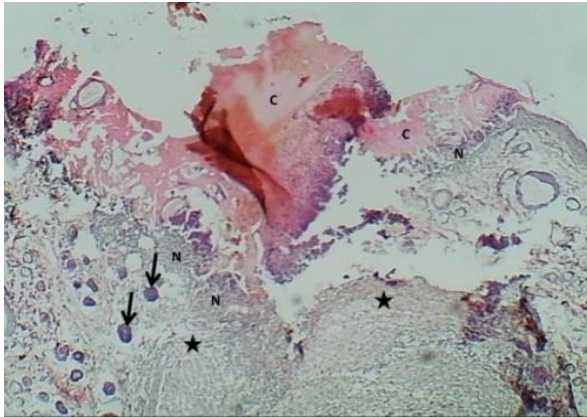


Figure 9. Section of skin (Alum solution) shows: thick fibrin clot (C), necrotic tissue (N), inflammatory zone (N), normal dermal tissue necrosis (Asterisks) & degenerated hair follicles (arrows).H&E stain. 40x

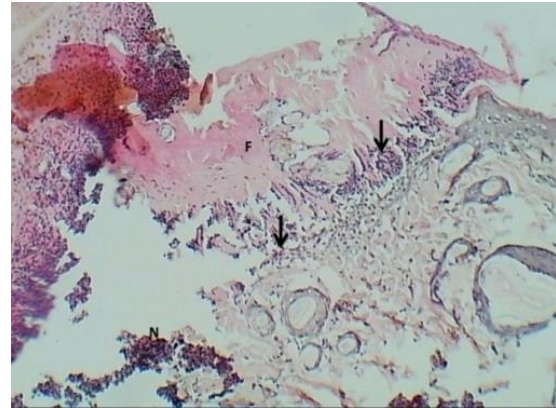


Figure 10. Section of skin (Alum solution) shows: thick fibrin clot (F), necrotic tissue (N), inflammatory zone (Arrows). H&E stain. 100x.

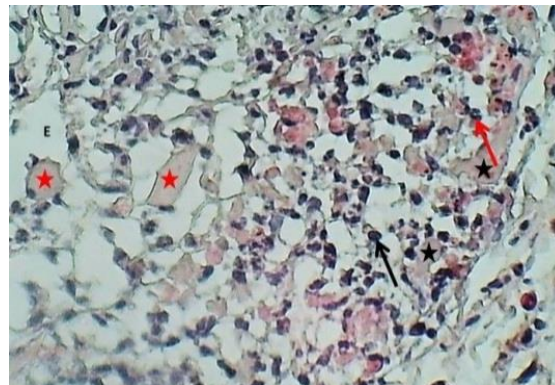


Figure 11. Section of deep part of dermis skin (Alum solution) shows: degenerated collagen bundles (Red arrows), necrotic collagen bundles (Black asterisks), mononuclear leukocytes (Red arrows) & multi nuclear leukocytes (Black arrows). H&E stain. 400x.

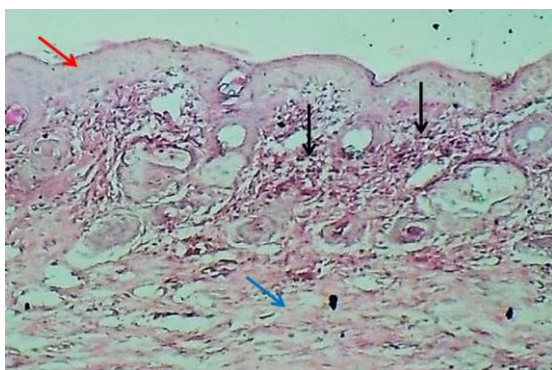


Figure 12. Section of skin (Alum solution) shows: normal epidermis (Red arrow) & mild dermatitis revealed mild degeneration of collagen fibers with infiltration of inflammatory cells (Black arrows), normal fibrous tissue of deep dermis layer (Blue arrows) & inflammatory cells (Black arrows). H&E stain. 100x.

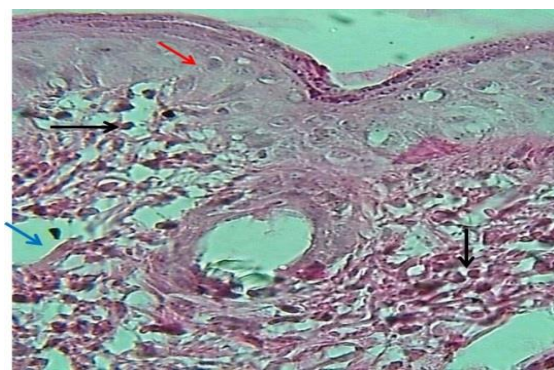


Figure 13. Section of skin (Alum solution) shows: normal epidermis (Red arrow) mild degeneration of collagen fibers (Blue arrow) with infiltration of inflammatory cells (Black arrows). H&E stain. 400x

Textural Analysis

The textural features can be obtained after the images transformed to a gray level in order to depict what would happen to the skin during a wound and healing. Table 2, represents the features for Control negative and positive, Table 3 the features for the Alum powder and Table 4 represents the textural features of the Alum Solution and Alum Solution exposed to plasma. Table 5 displays the average textural features for all cases and Fig 14 represents the textural features distribution. From the Figures and tables, the result shows that for the control negative the value of moment of inertia and entropy is small since the skin is normal, there is no wound defecting the skin, the entropy gives information about the increase in the number of gray level in the texture image, when its value is small there are low level of gray levels number and this means there are no defect in the skin, in which the skin shows the normal appearance of epidermal epithelium, dermal collagen bundles with normal content of fibroblasts. The moment of inertia for the control negative is small because it represents the natural measure of the degree of spread value for the control positive the value increase because of the wound which causes a defect in the skin. The inverse difference moment and the angular second moment have high values and

approach one when the skin is normal, giving an indication of the local homogeneity of the skin. The moment of inertia value gives information about the differences between adjacent points on the skin because the differences are small, this means that the skin has the same gray level value. So that the skin in Fig 5 which represents the control positive shows necrotic tissue, fibrin network and granulation tissue while the skin in Fig 6 shows the necrosis tissue, collagen bundles and fibroblasts it's an infected skin (control positive), as result, entropy and moment of inertia values rise while inverse difference moment and angular second moment values decrease. In the groups that had therapy with alum in its three forms: powder, solution, and solution treated with plasma their skin returned to its normal case since, the energy increased alum's solubility, and this aiding in healing. The solution treated with plasma received special attention since it produced more energy than the other treatments. The skin treated with (Alum solution exposed to plasma) shows normal epidermis and mild dermatitis revealing mild degeneration of collagen fibers with infiltration of inflammatory cells, normal fibrous tissue of the deep dermis layer and inflammatory cells.

Table 2. Textural features for the Control negative and positive.

Textural features	Control Negative1	Control Negative2	Control Positive1	Control Positive2
Moment of inertia	0.213	0.341	0.872	0.978
Inverse difference Moment	0.981	0.911	0.652	0.713
Angular second Moment	0.976	0.982	0.467	0.679
Entropy	0.225	0.213	0.851	0.721

Table 3. Textural features for the Alum Powder

Textural features	Alum Powder No.1	Alum Powder No.2
Moment of inertia	0.471	0.469
Inverse difference moment	0.903	0.891
Angular second moment	0.956	0.962
Entropy	0.352	0.378

Table 4. Textural features for the Alum Solution

Textural Features	Alum Solution1	Alum Solution2	Alum Solution3	Alum Solution Exposed to Plasma1	Alum Solution Exposed to Plasma2
Moment of Inertia	0.321	0.362	0.302	0.206	0.221
Inverse Difference Moment	0.970	0.954	0.932	0.985	0.918
Angular Second Moment	0.963	0.951	0.974	0.987	0.982
Entropy	0.326	0.312	0.254	0.239	0.212

Table 5. Represented the Average Textural Features for all Cases

Textural features	Control Negative	Control Positive	Alum Powder	Alum Solution	Alum Solution Exposed to Plasma
Moment of Inertia	0.282	0.925	0.47	0.328333	0.2135
Inverse Difference Moment	0.946	0.6825	0.897	0.952	0.9515
Angular Second Moment	0.979	0.573	0.959	0.962667	0.9845
Entropy	0.219	0.786	0.365	0.297333	0.2255

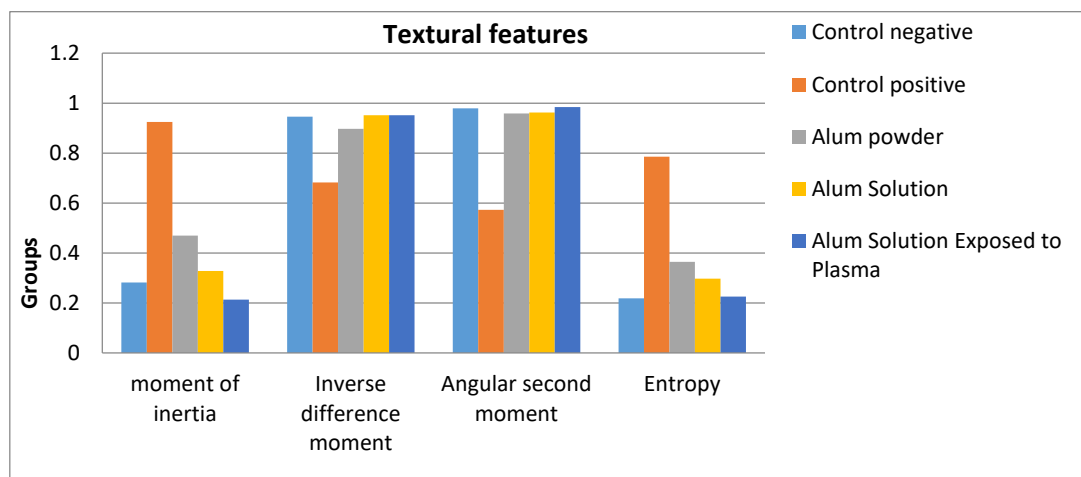


Figure 14. Represented the Textural Features Distribution.

Conclusion

Examinations of XRF showed that among the components of alum there are magnesium oxide and sodium oxide, which have a major role in stimulating the skin to restore and contribute to the healing of wounds. The results showed the best method among the three methods in treating wound is the solution treated with plasma, that the aqueous solution has a better ability to heal than the powder, and that the

aqueous solution exposed to plasma is better than the aqueous solution without plasma because the plasma increases the temperature of the solution, which helps increase the efficiency of potassium because the solubility of potassium alum increases significantly with increasing temperature. This result was explained by the texture analysis features of the skin and the histopathology images.

Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- The author has signed an animal welfare statement
- Ethical Clearance: The project was approved by the local ethical committee at University of Baghdad.
- The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors' Contribution Statement

N. F. M., A. H. Ali, S N. M., R. S. A. Contributed to the design and implementation of the research to the

analysis of the results and to the writing of the manuscript.

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دراسة تأثير الشب المنشط بالبلازما الباردة على الجروح في الفئران باستخدام التحليل النسيجي

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الخلاصة

يهدف البحث الى استخدام مصفوفة الاعتماد المكاني ذات اللون الرمادي لاستخراج السمات النسيجية للجلد المصاب بالجروح و المعالج بمحلول الشب المفعّل بالبلازما ، وتعتمد هذه الطريقة على الاختيار الذاتي لمنطقة الدراسة والذي ينتج عنه اعتماد قيم المعلمات على المنطقة المستخرجة. يستخدم شب البوتاسيوم لتنظيف الجروح وعلاجها وكذلك لمنع انتشار البكتيريا حيث يحتوي المحلول المائي على هيدوكسيد الالمنيوم الذي يكون راسب ابيض على الجروح والتي تعمل على تخثير الدم وتوقف النزف وتزداد كفاءته عند تفعيله بالبلازما. لقد تم استخدام مجموعة من الفئران ؛ تراوحت أعمارهم بين 2 - 3 أشهر ووزنها يتراوح بين 200-250 جم ، تم إنشاء جروح في الفئران ، وتضمنت التجربة استخدام الشب على شكل مسحوق ومحلول مائي ومحلول مائي مفعّل بالبلازما وكانت مدة التعريض خمس دقائق. تمت دراسة التحليل النسيجي للجلد المصاب بالجروح من حيث تأثير مسحوق الشب ومحلول الشب ومحلول الشب المفعّل بالبلازما على الجروح والجلد. وفقاً لنتائج التحليل النسيجي وصورة التشريح النسيجي ومصفوفة الاعتماد المكاني ، استعاد الجلد مظهره الخارجي (شبه الطبيعي) ، والتئمت الجروح بسرعة نتيجة استخدام المحلول المائي المنشط بالبلازما.

الكلمات المفتاحية: مصفوفات الاعتماد المكاني ذات اللون الرمادي، البلازما، شب البوتاس ، التحليل النسيجي، تحليل XRF