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Efficacy of Hyaluronic Acid and Mupirocin Mixture Therapy on Skin Burn Healing in a Rat Model

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Abstract: Infection within avascular necrotic tissue impedes the healing process of burn wounds. Hyaluronic acid (HA), a common component of the extracellular matrix (ECM), is a promising biomaterial for use in dermatology. Its biocompatibility, coupled with its hydrophilic characteristics that assist in moisture regulation and its bacteriostatic attributes, makes it particularly advantageous for managing bioburden and promoting burn healing. **The objective of the study** is to evaluate the efficacy of the combined treatment of mupirocin and a hyaluronic acid (MUP+HA) gel in a rat model of third-degree skin burn. **Methods:** Thirty male rats were divided into five groups: healthy, negative control, mupirocin gel, hyaluronic acid gel, and a combination of mupirocin and hyaluronic acid gel. A topical gel was applied twice daily to a 10-mm circular burn, and the rats were euthanized after 21 days. We assessed the burn contraction % (BCA%) and performed histological and immunohistochemical analyses on skin samples. **Results:** The new MUP+HA gel significantly increased BCA%, showing an 80±9.23% rise (P≤ 0.001) compared to the negative control group. Histology showed a marked decline in inflammatory cells (P ≤ 0.004), along with notable increases in collagen deposition (P ≤ 0.001), re-epithelialization of skin tissue (P ≤ 0.01), and new blood vessel formation (angiogenesis) (P ≤ 0.05) compared with the negative control group. Immunohistochemistry (IHC) demonstrated substantial reductions in TNF-α (P≤0.05) relative to the negative control group, with IL-10 and VEGF levels returning to baseline after full healing. **Conclusion:** The MUP+HA gel demonstrated superior efficacy to either the MUP or HA gel alone in promoting burn healing in a rat model, as evidenced by accelerated wound closure and improved histological and immunohistochemical outcomes.

Keywords: Burn healing, Hyaluronic acid, Mupirocin, Combination therapy, Rat model, Topical gel.

Introduction

The skin, the body's largest external organ, covers about 1.5 to 2 square meters [1]. Wounds, whether they occur suddenly or develop over time, can result from medical errors, underlying health problems, or exposure to chemical, thermal, or other physical factors, and they can damage the epidermis. Healing burn wounds is a complex process. It involves removing foreign materials, the inflammatory response, the formation of granulation tissue, scar formation, and many other complex biological processes [2].

The repair of a burn wound is a complex process that requires the cooperation of several body systems, including hemostasis, inflammation, tissue growth, and remodeling [3]. Immediately after the injury, platelets begin to clump together, and blood vessels constrict to reduce blood loss. Following this, the burn-healing process enters the inflammatory phase, during which immune cells, such as neutrophils and monocytes, migrate to the injury site [4].

Most skin problems usually heal on their own within one to two weeks. However, serious injuries or burns, especially full-thickness burns, can hinder the skin's natural healing process. This is mainly because of bacterial infections that can occur during wound healing. These infections can cause significant tissue damage, worsen inflammation, and slow down the healing process [4].

Burn wound infection is characterized by the presence of microorganisms, biofilm formation, and the potential for spread. Burns, by their very nature, breach the skin's protective barrier, thereby inviting bacterial growth. Moreover, the burn eschar creates a nutrient-rich environment that promotes bacterial growth and hinders healing. When microbes attach to a burn, they can form biofilms, which are communities of bacteria protected by a matrix. This matrix protects the bacteria from the immune system and antibiotics, making treatment more difficult. Moreover, biofilms help microbes survive in difficult situations, such as when nutrients are scarce or the pH changes [5].

Within the first 5 to 7 days following a burn injury, wounds are susceptible to colonization by a range of microorganisms, including Gram-negative bacteria, fungi, and viruses. Pseudomonas aeruginosa, a Gram-negative bacterium, frequently causes burn wound infections in intensive care units, largely due to its multidrug resistance and an array of virulence factors [5].

HA is a linear glycosaminoglycan and a polymer that is naturally found in the extracellular matrix of vertebrate tissues, such as connective, epithelial, and neural tissues [6]. It participates in several biological functions, including embryonic development, wound healing, cancer progression, angiogenesis, inflammation, and bone regeneration [7].

HA is essential for wound healing, particularly during the inflammatory and angiogenic phases. Various HA-based biomaterials, either alone or in combination with other biopolymers, have been developed and evaluated to support these phases. These comprise sponges, films, hydrogels, and electrospun membranes [8].

Applying HA directly to wounds helps the body hold onto water. This creates a more favorable environment for collagen

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and elastin synthesis and supports cell growth and specialization. This method accelerates the healing process and improves tissue properties, such as elasticity and the density of small blood vessels. Moreover, HA's anti-inflammatory effects help regulate tissue responses, thereby reducing the risk of keloids and hypertrophic scars [9].

Studies in vivo, including both animal models and human participants, provide strong evidence that HA significantly speeds up wound healing and shortens recovery time. HA serves a dual purpose. At first, it provides a temporary structure during the early stages of tissue repair, which helps deliver essential nutrients and remove metabolic waste. Subsequently, it supports the proliferation and migration of keratinocytes, which are critical for effective re-epithelialization [9].

Mupirocin (MUP), a topical antibiotic often used, is effective in treating skin infections. Its broad antibacterial and antibiofilm properties make it useful against infections caused by both Gram-positive and Gram-negative bacteria, including nasal MRSA. MUP (2%) is available in various forms, such as creams and ointments, including Bactroban and Bactoderm [10]. The main way MUP works is by competing with the bacterial enzyme isoleucyl-tRNA synthetase (IleRS). Because the epoxy side chain is similar to isoleucine, the natural substrate, it binds strongly to the enzyme's active site. As a result, this binding prevents the enzyme from attaching isoleucine to tRNA. As a result, the stopping of protein synthesis leads to the death of the bacterial cells [10] [11].

Its unique mechanism of action ensures there is no cross-resistance to other antibiotics. Conversely, the efficacy of MUP is constrained by its short half-life (less than 30 minutes) and significant protein binding. Moreover, standard formulations can elicit adverse reactions, including burning, dryness, pruritus, rashes, erythema, nausea, discomfort, stinging, edema, and soreness [12].

Notwithstanding, HA established roles in bacteriostasis and wound healing [13]. and MUP has potent bactericidal capabilities [14]. There is a conspicuous paucity of research concerning their combined application within a therapeutic context. This synergistic approach is expected to produce an additive effect, integrating the regenerative and anti-inflammatory properties of HA with the direct antibacterial action of MUP. Consequently, this combination could potentially enhance infection management and accelerate tissue regeneration more effectively than current therapeutic options. Objective of the study. This research sought to measure the wound-healing capabilities of the newly developed MUP+HA gel formulation by measuring burn contraction percentage and histological parameters, including inflammatory response (macrophages and lymphocytes), collagen deposition, angiogenesis, and re-epithelialization. Additionally, immunohistochemical (IHC) analysis was performed using markers including vascular endothelial growth factor (VEGF), interleukin-10 (IL-10), and tumor necrosis factor-alpha (TNF-α).

Materials and Methods

MUP pure powder was procured from Baoji Guokang Bio-Technology Co., Ltd., located in Baoji, China. Hyaluronic acid was procured from Bide Pharm in China and Mallocell in the Netherlands. Hydroxypropyl methylcellulose (HPMC) grade E10M was obtained from Mallocell in Randstad, Netherlands. Ketamine hydrochloride at 100 mg/mL and Xylazine hydrochloride at 20 mg/mL were obtained from Bayer in Leverkusen, Germany. The antitumor necrosis factor (TNF-α) antibody and the two-step plus poly-HRP anti-mouse/rabbit IgG detection system kit, which includes DAB solution, were provided by Elabscience in China. Elabscience in China supplied polyclonal interleukin-10 (IL-10) antibodies and monoclonal antibodies against vascular endothelial growth factor (VEGF).

Preparation of Hydroxypropyl Methylcellulose (HPMC)

A hot plate magnetic stirrer was employed to slowly dissolve 5 g of HPMC polymer with 50 mL of boiling water that had been deionised, after which the mixture was topped up to 100 mL with deionised water to create a uniform gel [15].

Preparation of HA Gel 1%

Using the vortex, 50 mg of HA powder was combined with 3 ml of cold deionized water. Stirring gently for 40 minutes, then refrigerated overnight at 4°C before carefully mixing with 2 ml of HPMC gel base to create a 1% (W/v) gel [15, 16].

Preparation of MUP Gel 2%

A MUP 2% gel was prepared by mixing 100 mg of MUP powder with 3 drops of ethanol in a glass screw-cap tube, then stirring continuously for 15 minutes until the mixture was completely homogeneous. Then, allow the ethanol to evaporate, and add 3 ml of deionized water. The previously prepared HPMC gel, which contains 2 ml, was combined with the MUP solution to create a homogeneous 5 ml gel suitable for topical skin application [15, 17].

Preparation of Combination MUP+HA Gel

MUP 4% gel was prepared by mixing 200 mg of MUP powder with 3 drops of ethanol in a glass screw cap tube, then stirring continuously for 15 minutes until the mixture was completely homogeneous. Then, allow the ethanol to evaporate, and add 3 ml of deionized water. The previously prepared HPMC gel (2 ml) was combined with the MUP solution to form a homogeneous 5 ml MUP gel. At the same time, using the vortex mixer, 100 mg of 2% HA powder was combined with 3 ml of cold deionized water. Stir gently for 40 minutes, then refrigerate overnight at 4 °C. Carefully mix with 2 ml of HPMC gel base to obtain a 5 ml 2% HA gel.

Blending the MUP 4% and HA 2% gels after preparation yields a 10 ml single composite gel MUP 2% and HA 1% suitable for skin application.

Measurement of PH

The pH of the gel specimens was assessed with a pH meter, which was calibrated prior to each use with buffer solutions at pH 4, 7, and 9. The gel was examined directly on the electrode. The pH of the formulation was assessed thrice at 25°C, and the mean value was computed [18].

Sterilization of Gel

All preparations were performed under strictly aseptic conditions inside a laminar airflow hood. All glassware, instruments, and materials were sterilised by autoclaving before use to maintain sterility throughout the experiment.

Experimental Animal Design

Thirty male Wistar albino rats, approximately two months old and with a body weight between 180 and 200 grams upon arrival,

participated in the experiment. They were preserved in a climate-controlled environment, with humidity and temperature carefully regulated and a 12-hour light-dark cycle. The rats could go anywhere they wanted, drinking potable water and eating a regular pellet-based diet during the experiment. All rats received topical antiparasitic medication and were acclimated to the experimental conditions for 14 days prior to the commencement of the study.
The rats were randomly distributed to five experimental groups, each including six animals, as shown in Table 1 below. Treatments were applied twice daily at the burn site. The particulars of the therapies and group information are delineated below.

Table 1: Experimental Groups and Treatment for Burn Wound Healing in Rats.

groups	Explanation
Healthy group	Rats have no burn injuries and no treatment for specific comparisons (e.g., inflammatory response, collagen deposition, angiogenesis, and re-epithelization) to assess baseline healing.
Negative control group	Rats have burn injuries treated with HPMC gel.
Burn + Mupirocin (MUP) alone group	Topical application of MUP gel (2%).
Burn + Hyaluronic acid (HA) alone group	Topical application of HA gel (1%).
Burn + MUP + HA group	Combined therapy (same doses as individual treatment).

Model of the Burn Induction and Specimen Collection

The rats received anesthesia by an intraperitoneal (IP) injection of ketamine (100 mg/kg) and xylazine (20 mg/kg). The hair on their dorsal area was clipped using an electric shaver, followed by cleaning the skin using sterile saline and disinfection with 70% ethanol [19]. After drying, the skin surface was lifted and peeled away from the underlying organs, leaving a flat surface. A 10 mm diameter circular full-thickness burn was inflicted at the dorsal upper region of the back using a stainless-steel rod (1 cm²). The rod was dipped in boiling water (100 °C) and pressed firmly onto the rat's skin for 15 seconds to induce a third-degree burn (see Figure 1). The burns were then rinsed with normal saline, left unstitched, and exposed for treatment [20]. To reduce biting and fighting among rats and to maintain skin burn therapy, each group of three rats was kept in their own plastic cage. Treatments were applied to the burn twice daily and monitored for effectiveness through imaging of the rat's dorsum at a normal height on different days of treatment [20].

On day 21 post-burn, skin tissue samples from all groups were collected and fixed in 10% formalin. These samples underwent histopathological and immunohistochemical (IHC) analysis. The evaluation was semi-quantitative, scored as absent (0), mild (1), moderate (2), and marked (3) [21, 22].

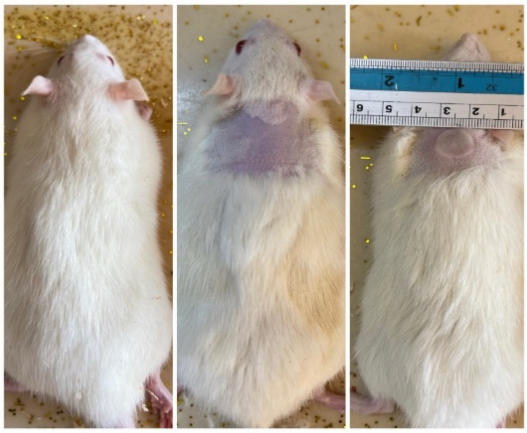

Figure 1: Experimental burn wound model in rats showing the procedure: anesthesia was administered, the dorsal area was shaved, and the burn wound was induced.

Measurement of Burn Diameter and Contraction Percentage

The diameter of each rat's burn was evaluated in millimeters on days 0, 3, 7, 10, 14, 18, and 21 after burning using an electronic vernier caliper. The burns were also photographed. The percentage of burn contraction was calculated using the following equation [23, 24].

Percentage of Burn Contraction = (Original Burn Area – Burn Area at imaging time) / Original Burn Area × 100%.

Histopathological Analysis

Collected skin tissue specimens were fixed using 10% formalin, followed by dehydration via graded ethanol from 50% to 100%, and then rinsed with xylene. They were then penetrated and encapsulated in paraffin wax at 60 °C. Slices of the designated controlled thickness were excised employing a microtome and placed on glass microscope slides. It underwent deparaffinization, rehydration, and hematoxylin and eosin staining. Ultimately, they were affixed with Canada balsam and examined under a light microscope [25].

A blinded histopathologist examined each slide for changes, including inflammatory response (lymphocytes and macrophages), collagen deposition, angiogenesis, and re-epithelialization. The scale of histological sections was evaluated semi-quantitatively, as described by (Absent; 0), (Mild; 1), (Moderate; 2), and (Strong; 3) [21, 22].

Immunohistochemistry (IHC) Analysis

The IHC reactions utilized specific antibodies and were standardized at the IHC special laboratory. The manifestation of a brown precipitate in tissue sections is a positive staining signal. Quantification of tumor necrosis factor-α (TNF-α), interleukin-10 (IL-10), and vascular endothelial growth factor (VEGF) inflammatory mediators was performed using a digital light microscope at 10X and 40X magnifications and assessed by an expert pathologist. The scale was used to calculate the intensity of the positive signal (0, 1, 2, and 3). When there is no detectable signal, the scale is 0; when the signal intensity is mild, moderate, or marked, the scales are 1, 2, and 3, respectively [26]. Measurements are in the cytoplasmic fraction for TNF-alpha and IL-10 and in the endothelial cell-specific fraction for VEGF.

Results

HPMC Gel

The HPMC gel formulation was successfully prepared. All gels were clear and transparent, with complete dissolution of the powder and no visible drug precipitation.

HA 1% Gel

The HA gel had a pH of 5.4±0.05, indicating a slightly acidic nature suitable for skin application. No visible particulate matter or instability was observed.

MUP 2% Gel

The MUP gel exhibited pH 5.5±0.05, and the gel remained clear without any drug precipitation, confirming its stability for topical use.

Combination MUP+HA Gel

The MUP+HA gel combination was clear and transparent, with complete dissolution of both powders. The gel exhibited a pH of 4.8 ± 0.05, which is appropriate for topical application on skin. The combination did not result in any visible precipitation or phase separation, indicating physical stability.

Morphological Burn Healing

Figure 2 illustrates the measurement of the Burn Contraction Area Percentage (BCA%) across all treatment groups. The MUP+HA gel-treated group exhibits a significant increase in BCA% on day 3 compared to the negative control (P≤0.05). After 7 days, compared with the negative control groups, the increase in burn contraction percentage in the MUP group is statistically significant (P ≤ 0.05). On the other hand, the HA- and MUP+HA-treated groups exhibit a highly statistically significant difference (P≤0.001) in wound contraction percentage compared to the negative control group. On day 10, no significant differences were observed among the groups. Furthermore, by day 14, compared with all negative control groups, the MUP group shows no statistically significant change. Compared with HA, a statistically significant change is observed (P≤0.05). Ultimately, compared with the MUP+HA group, the MUP+HA group shows a very statistically significant difference (P≤0.01). On day 18, the MUP-treated group showed no significant difference compared with the negative control group. While both HA and MUP+HA gel treatment groups showed a significant and highly significant difference (P≤0.05) and (P≤0.001), respectively, relative to the negative control group. There was a significant increase in the contraction percentage with MUP+HA combination therapy compared to the HA-treated group (P≤0.05) and the MUP-treated group (P≤0.01), as indicated by the connecting line between the bars. Finally, on day 21, compared with the negative control group, the MUP-gel-treated group shows a statistically significant difference (P ≤ 0.05), and compared with the HA group, it also shows a statistically significant difference (P ≤ 0.05), indicated by the connecting line between the bars. The MUP+HA and HA-treated gel groups were highly significant compared with the negative control group (P ≤ 0.001 for both). Ultimately, the combination therapy in the MUP+HA gel group shows a statistically significant difference compared to the MUP-treated group (P≤0.001), indicated by the connecting line between the bars. All differences are illustrated in Figure 3.

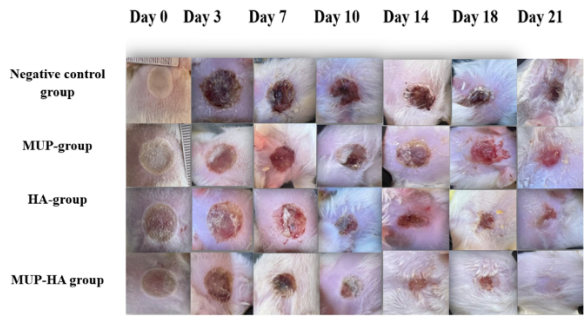

Figure 2: Morphological representations of burns in rat treatment groups on different days. MUP: mupirocin, HA: hyaluronic acid, MUP+HA: mupirocin-hyaluronic acid.

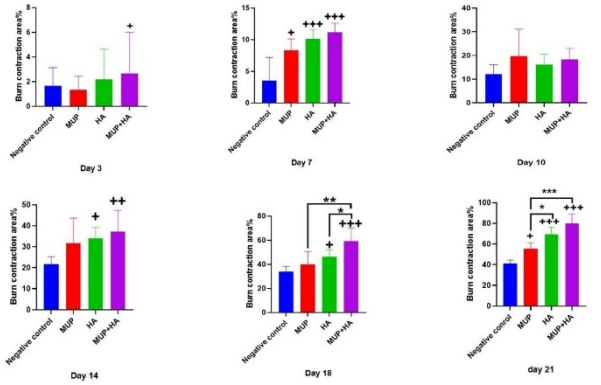

Figure 3: Burn contraction area percent (BCA%) on different days. ***P ≤ 0.001, **P ≤ 0.01, *P ≤ 0.05, **+**: significant, **++**: very significant, **+++**: highly significant changes compared with the negative control group. The lines connecting the bars indicate comparisons between the groups they connect. MUP, mupirocin; HA, hyaluronic acid; MUP+HA, mupirocin + hyaluronic acid.

Histopathological Findings

Figure 4 illustrates statistical differences among groups across the histological parameters. All four treatment groups (negative control, MUP, HA, and MUP+HA gels) showed a highly significant increase in inflammatory cell scores (macrophages and lymphocytes) on day 21 post-burn compared to the healthy group (P ≤ 0.001). Both the MUP- and MUP+HA-gel-treated groups demonstrated a very significant reduction in macrophage and lymphocyte scores relative to the negative control (P ≤ 0.01 for both). The HA-treated-gel group also showed a significant difference from the negative control (P ≤ 0.05).

The treated groups (MUP, HA, and MUP+HA gels) showed no significant differences in collagen deposition after burn injury compared with the healthy group. The negative control group experienced a notable decrease compared to the healthy group (P ≤ 0.001). Rat groups treated with gel HA and MUP+HA demonstrated a statistically significant increase in collagen deposition scores relative to the negative control group (P ≤ 0.001 for both). MUP alone showed no statistically significant difference when compared to the negative control group. A highly significant difference was found between the MUP+HA groups and the MUP group (P ≤ 0.001), as indicated by the connecting line between the bars, while a notable disparity was detected between the HA- and MUP-treated groups (P ≤ 0.05), indicated by the connecting line between the bars.

The angiogenesis scores for the treatment groups (HA, MUP, and MUP+HA gels) showed a highly significant increase at 21 days post-burn compared with the healthy group (P ≤ 0.001, ≤ 0.01, and 0.01, respectively). The negative control group showed no significant difference compared to the healthy group. Rats treated with HA and MUP+HA exhibited a highly significant increase in angiogenesis scores relative to the negative control group (P ≤ 0.01, 0.05, respectively). MUP showed no significant change compared to the negative control group.

The negative control group showed a statistically significant decrease in skin tissue re-epithelialization after burn injury compared with the healthy group (P < 0.001). Rats treated with MUP, HA, and MUP+HA showed notable and highly significant improvements in skin tissue re-epithelialization scores relative to the negative control group (P ≤ 0.05, 0.01, and 0.01, respectively). All histological changes are explained in Figure 5.

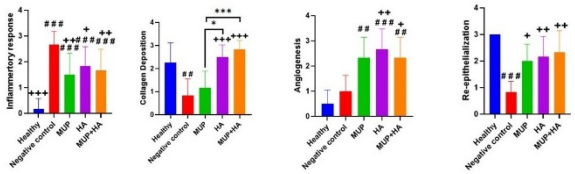

Figure 4: illustrates the impact of different treatments on histological parameters such as inflammatory response, collagen deposition, angiogenesis, and re-epithelization at day 21 post-burn. The significance levels are indicated as ***P ≤ 0.001, **P ≤ 0.01, and *P ≤ 0.05. The **+:** marks a significant, **++**: very significant, and **+++**: highly significant difference compared to the negative control group, while **#:** denotes significant, **# #**: very significant, **and ###**: highly significant differences relative to the healthy group. The lines connecting the bars indicate comparisons between the groups. MUP: mupirocin, HA: hyaluronic acid, and MUP+HA: a combination of mupirocin and hyaluronic acid.

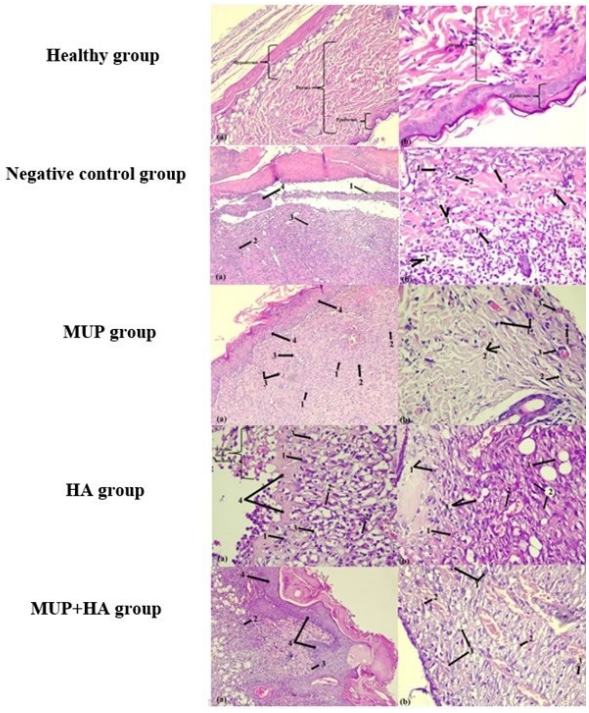

Figure 5: Histopathological changes in skin tissues of all treated and healthy groups on day 21 post-burn. H&E stain (10× and 40×). Data expressed as mean ± SD. 1. The presence of inflammatory cells (macrophages and lymphocytes) (healthy group: 0.17±0.40, negative control group: 2.67±0.51, MUP: 1.5±0.54, HA: 1.83±0.75, MUP+HA: 1.67±0.81). 2: collagen deposition (healthy group: 2.16±0.98, negative control group: 0.83±0.75, MUP: 1.16±0.75, HA: 2.5±0.54, MUP+HA: 2.83±0.40). 3: angiogenesis (healthy group: 0.5±0.54, negative control group: 1±0.63, MUP: 2.33±0.81, HA: 2.66±0.81, MUP+HA: 2.33±0.81). and 4: re-epithelialization (healthy group: 3±0, negative control group: 0.83±0.4; scoring system: 0: absence, 1: mild, 2: moderate, 3: marked, MUP: 2±0.63, HA: 2.17±0.75, 2.33±0.81). MUP: mupirocin, HA: hyaluronic acid, MUP+HA: mupirocin + hyaluronic acid.

Immunohistochemical Finding

Figure 6 shows statistical differences between groups for each parameter. On day 21, the negative control group showed a significant rise in tumor necrosis factor (TNF-α) levels compared to the supposedly healthy group (P≤0.01). Animals treated with MUP, HA, and MUP+HA gels had significantly lower TNF-α levels than the negative control group (P ≤ 0.05 for all).

Interleukin-10 (IL-10) levels were higher in the MUP and HA gel-treated groups than in the healthy group (P≤0.001 for both). Animals receiving MUP and HA gel treatments showed a highly significant increase in IL-10 compared with the negative control (P ≤ 0.05 and 0.01, respectively). The MUP+HA gel treatment group showed a notable reduction in IL-10 levels compared with the MUP and HA groups (P ≤ 0.05 for both), indicated by the connecting line between the bars. The combined MUP+HA gel demonstrated no significant difference in IL-10 levels compared to the healthy group.

Regarding Vascular Endothelial Growth Factor (VEGF), on day 21, the MUP- and HA-gel-treated groups showed a highly significant increase in VEGF levels compared with the healthy group (P ≤ 0.001 for both groups). Both groups also demonstrated a significant increase relative to the negative control group (P ≤ 0.05 for both). There was a significant difference between the HA group and the MUP+HA group (P≤0.05), indicated by the connecting line between the bars. However, no significant difference was found between the MUP+HA group and the healthy group. All IHC changes are shown in Figure 7.

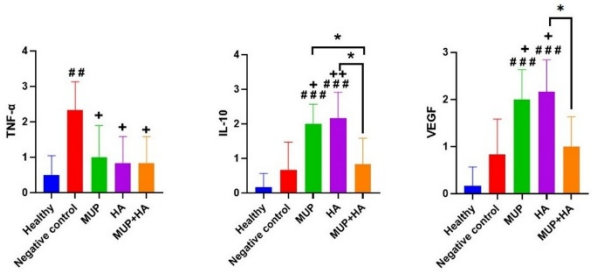

Figure 6: Effect of treatments on immunohistochemical parameters: TNF-α, IL-10, VEGF. ***P ≤ 0.001, **P ≤ 0.01, and *P ≤ 0.05 group. The **+:** marks a significant, **++**: very significant, and **+++**: highly significant difference compared to the negative control group, while **#:** denotes significant **# #**: very significant**, # # #**: highly significant differences relative to the healthy group. The lines connecting the bars indicate comparisons between the groups. MUP refers to mupirocin, HA is hyaluronic acid, and

MUP+HA indicates a combination of mupirocin and hyaluronic acid.

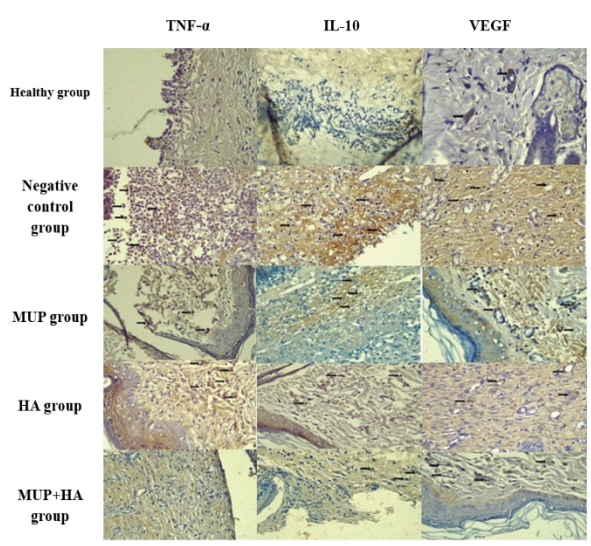

Figure 7: Skin immunohistochemical changes in the healthy and treatment groups on day 21 post-burning were examined under a digital light microscope at different magnifications (10X and 40X). date expressed as mean ± SD for TNF-α (healthy group: 0.5±0.54; negative control group: 2.5±0.54; MUP: 1±0.89; HA: 0.83±0.75; MUP+HA: 0.83±0.75). IL-10 (healthy: 0.16±0.4; negative control group: 0.66±0.81; MUP: 2±0.57; HA: 2.1±0.75; MUP+HA: 0.83±0.75. VEGF (healthy group: 0.16±0.4, negative control 0.83±0.75; MUP: 2±0.63; HA: 2.1±0.68; MUP+HA: 1±0.63), scoring system 0: absence, 1: mild, 2: moderate, 3: marked, MUP: mupirocin, HA: hyaluronic acid, and MUP+HA: mupirocin+ hyaluronic acid.

Discussion

The World Health Organization recognizes burns as a major global health concern. These injuries are notoriously painful and can be devastating, sometimes resulting in long-term disability or even death. Every year, countless individuals around the globe suffer burns. Many of these cases require hospitalization, and about 200,000 deaths are recorded each year [27].

HA is a compound that occurs naturally in the outermost layer of cells of the skin, as well as in cartilage, mammalian bone marrow, synovial fluid, vitreous humor, and other tissues. The extensive application of this substance in dermatological formulations in recent years has garnered significant interest [28].

The combination gel, which contains MUP and HA, offers a multifaceted approach to treating burn injuries, addressing two key aspects: preventing infection and promoting tissue repair. HA, a biocompatible material, helps to keep and distribute MUP in the affected area, which then improves its antibacterial effectiveness [29]. MUP provides a strong defense against bacterial infections, creating a favorable environment for HA to support healing. This combined effect helps wounds heal, reduces inflammation, and lowers the chance of further infections, highlighting the importance of this combination in treating burn wounds [30].

Using an animal model for burn healing helps replicate human physiology and forecast treatment results. Rats frequently serve as subjects in wound healing studies, and for good reason: they're readily available, compact, and their healing processes are rapid. Also, it's practical to test many of them at once. Among various methods, heated devices are among the most frequently used to induce burn injuries. The methods used in this study followed established protocols. The burn wounds were consistent in size and depth, and a 15-second burn resulted in a full-thickness, third-degree injury [31].

The MUP+HA gel has an acidic pH, aligning with the pH standards for topical skin treatments. If the burn area becomes alkaline, healing slows, harmful bacteria can grow, and antibiotics may become less effective. Treatment could be adjusted accordingly [32]. The antibacterial efficacy of MUP is pH-dependent; at acidic pH, MUP enhanced antibacterial activity in vitro, establishing an optimal milieu for efficacious therapy [33].

The findings showed that on day 21, the HA- and MUP-HA-gel-treated groups experienced rapid contraction, indicating significantly faster healing than the other groups. The most notable changes in BCA% were 69.33±6.9 and 80±9.2, respectively, a pattern not observed in the other groups. HA gel accelerates wound healing by stimulating the regeneration of both dermal and epidermal cellular elements [34].

While inflammation is necessary to form granulation tissue, stabilizing the resulting tissue matrix is crucial to reducing inflammation and promoting effective tissue repair. Moreover, HA's capacity to neutralize free radicals can beneficially modulate inflammatory responses, thereby protecting cells and the extracellular matrix from oxidative stress and proteolytic damage [35].
HA molecules are indispensable for the normal functioning of the epidermis, where they are present in high concentrations within the basal layer. HA, with its three-dimensional structure in the extracellular space, plays a key role in hydration and the movement of nutrients [35, 36].

The MUP gel-treated group showed a 55.66% increase in BCA% compared with the negative control group. It demonstrated a dual mechanism involving the modulation and reduction of inflammation, indirectly by decreasing bacterial load, thereby preventing a prolonged inflammatory response and creating a favorable environment for healing. Simultaneously, MUP promotes keratinocyte proliferation and enhances the production of key growth factors such as erythropoietin, hepatocyte growth factor, macrophage colony-stimulating factor, and platelet-derived growth factor-AA in the early inflammation phase of healing, which accelerates re-epithelialization and burn closure [37].

MUP, when combined with HA, showed a notable elevation in BCA% compared to MUP alone and HA alone. This effect is primarily due to HA's capacity to enhance wound contraction by increasing fibroblast activity, accelerating granulation tissue development, and modulating the inflammatory response [38] and the effect of MUP, which enhances the burn environment by reducing bacterial load [37].

Integrated assessment of histopathological changes and IHC staining expression is essential for elucidating the mechanistic contribution of MUP+HA gel in burn healing. The faster healing observed with MUP+HA gel is likely due to its influence on the inflammatory phase. The group treated with MUP+HA showed significant improvements in collagen production and re-epithelialization, along with decreased TNF-α

levels, a return to baseline IL-10 levels, and support for the critical stages of angiogenesis [39].

Following skin damage, the body quickly releases TNF-α, which then starts the inflammatory response in the burned tissues. Additionally, during the proliferative phase of healing, it is secreted by macrophages and promotes extracellular matrix formation [40].

The findings demonstrated that HA gel, when used alone, significantly lowers TNF-α levels. This indicated that HA may modestly reduce the inflammatory response. The effect of HA has been found to modulate the function of immune cells, including macrophages and lymphocytes, which are central to regulating inflammation. This has been shown to promote a shift toward an anti-inflammatory profile and to reduce the severity of inflammation [41]. HA can regulate the activity of CD44 and Toll-like receptors (TLRs) on macrophage surfaces by binding to them. Additionally, HA exerts anti-inflammatory effects by altering macrophage polarisation. Research demonstrates that HA facilitates the activation of M2-type macrophages (anti-inflammatory) while leaving M1-type macrophages (pro-inflammatory) unaffected, thereby diminishing the release of pro-inflammatory cytokines [42]. The MUP gel reduced TNF-α levels, consistent with many previous studies on the role of MUP as an IHC biomarker during excisional wound healing. For example, topical MUP gel treatment decreased the levels of the pro-inflammatory cytokine TNF-α [43].

Complete healing in the MUP+HA-gel-treated group after burns correlates with a significant reduction in TNF-α levels, bringing them back to levels observed in healthy skin. An IHC analysis revealed that rapidly healing burns had less TNF-α labeling in inflammatory cells. As healing progressed and the lesion exhibited complete re-epithelialization, TNF-α immunostaining, which had become weak or undetectable, resembled that of normal, undamaged skin. This decrease indicates the return of a balanced inflammatory response, marked by less consistent activation of pathways like NF-κB [44].

In the absence of burn treatment, the negative control group exhibited impaired healing. The persistent inflammation observed in the untreated rats impedes the transition to the subsequent phase of healing, thereby slowing the process and prolonging burn closure. Consequently, macrophages and neutrophils remain at the injury site in the untreated burn cases. Chronic inflammation affects keratinocyte cohesiveness, delaying re-epithelialization and healing [45]. High levels of inflammation allow chemokines and inflammatory cells to communicate, leading to persistent inflammation and hindering wound tissue growth. Re-epithelialization in the untreated rat group takes longer because chemical mechanisms impede regenerative changes needed for wound healing [45].

IL-10, a powerful anti-inflammatory cytokine, reduces the activity of monocytes and macrophages. At the same time, it reduces the production of pro-inflammatory cytokines, such as IL-1β, IL-6, and TNF-α, in activated macrophages. T cells, monocytes, and macrophages are the main sources of this cytokine [46].

A decrease in IL-10 levels leads to a stronger inflammatory response, as evidenced by an increased number of monocytes in the burn area, similar to that observed in the negative control group (47). In contrast, increased IL-10 production in the MUP and HA gel groups reduces the inflammatory response after injury. The experimental results showed a decrease in both macrophages and lymphocytes in the burn area, which matched the histopathological findings. In contrast, skin samples from healthy rats did not show any IL-10 [43, 47]. No IL-10 was detected in skin biopsies of healthy rats [47].

The complete recovery in the MUP+HA-gel-treated group is associated with a reduction in IL-10 to levels similar to those in healthy tissue, which then return to baseline after healing concludes. The return of IL-10 to normal levels during the healing process suggests that the inflammatory response is fully controlled, indicating that recovery is complete. Therefore, the healing process depends on the careful regulation of these specific molecules. If their levels are unbalanced, it could lead to problems like scarring or long-lasting inflammation [46].

The VEGF family is a key modulator of angiogenesis. VEGFs promote the growth of small blood vessels and also help metastasis by activating the receptor's cytoplasmic tyrosine kinase domain. Restoring blood flow to burn tissue increases nutrient and oxygen delivery, promoting cell growth and burn healing [48].

In this study, the rats treated with HA gel showed a significant increase in new blood vessel formation compared to the other groups. This finding supports earlier research on hyaluronic acid's role in healing burns, particularly its ability to increase vascular endothelial growth factor in burned areas [49]. HA is crucial in regulating angiogenesis through its dynamic interplay with the extracellular matrix and endothelial cells. HA enhances angiogenesis by diminishing cell-to-cell and cell-to-matrix adhesions. Consequently, it promotes the migration and sprouting of endothelial cells from existing arteries. Additionally, HA interacts with cell-surface receptors, particularly CD44, triggering intracellular signaling pathways that promote endothelial cell proliferation, survival, and differentiation [50].

The present finding is that MUP treatment significantly enhances angiogenesis, but unfortunately, this increase was not statistically significant in new blood vessel formation compared with the negative control group. This finding aligns with a previous study that confirms that promoting angiogenesis is not a primary mechanism of action for this antibiotic in wound healing [50]. Simultaneously, the evaluation of VEGF expression showed that MUP treatment significantly increased its levels. This finding corroborates prior research suggesting that MUP can upregulate VEGF. It's essential to recognize that this VEGFA elevation was assessed in the context of a comprehensive investigation of wound healing, given that VEGF is a critical signaling molecule. Specifically, it facilitates angiogenesis, the process of new blood vessel formation, thereby aiding in the delivery of oxygen and essential nutrients to injured tissues. The current study's findings suggest that MUP's ability to increase VEGF could contribute to its therapeutic effect beyond simply preventing bacterial infection. The use of MUP helped promote granulation tissue formation by gradually shifting the wound from the inflammatory to the proliferative phase of healing. This aligns with VEGF's role in tissue repair [51].

In MUP+HA gel-treated groups, showing a low level of VEGF after complete healing, which means the return of VEGF expression to near-baseline levels after complete healing, reflects appropriate downregulation of angiogenic activity and indicates a successful transition from active tissue repair to tissue maturation and remodeling [52].

The interaction between IL-10 and VEGF is crucial for skin wound healing. After the initial inflammatory phase, IL-10 starts

the Janus kinase/signal transducer and activator of transcription 3 (JAK/STAT3) signaling pathway. As a result, this process promotes VEGF production by both skin fibroblasts and nearby immune cells. VEGF is crucial for increasing blood flow to tissues and promoting granulation tissue formation. This is because VEGF stimulates endothelial cell growth, migration, and the formation of new capillaries [53]. Moreover, IL-10 helps stabilize HIF-1α in hypoxic regions of wounds. As a result, this stability promotes VEGF production and activates pathways that promote the growth of new blood vessels. IL-10 protects endothelial cells by reducing pro-inflammatory cytokines and oxidative stress. Moreover, IL-10 promotes endothelial progenitor cell migration and survival via VEGF. This, in turn, increases capillary density and the amount of oxygen available to the healing tissue [53, 54].

Dermal collagens are fibrillar proteins with high tensile strength and play a key role in wound healing. Collagen deposition lays the groundwork for angiogenesis and tissue remodeling [51].

The groups treated with HA and MUP+HA-gel showed a significant increase in collagen in their tissues, a finding that supports previous research. HA is known for its ability to speed up wound healing. This occurs because it stimulates collagen production and tissue renewal. This process boosts fibroblast activity, which in turn increases collagen production. Initially, this involves the creation of type III collagen, followed by the production of type I collagen. HA is crucial for both wound healing and the maintenance of skin elasticity [55]. It regulates the activity of matrix metalloproteinases (MMPs), thereby modulating the equilibrium between collagen synthesis and degradation; this process helps prevent the formation of disorganized scar tissue and promotes the development of organized collagen fibers. Moreover, HA stimulates TGF-β1 synthesis, which then interacts with CD44 on fibroblasts, thereby accelerating collagen deposition and tissue remodeling. As a result, HA reduces scarring and promotes collagen organization, thereby expediting wound healing [56].

In contrast to the negative control group, the MUP gel treatment did not significantly increase collagen deposition. These findings suggest that the effect of topical MUP on wound healing isn't due to a direct stimulation of collagen synthesis. Similar to the previous study, there was no significant increase in collagen deposition compared to the control group [57].

Re-epithelialization is a critical step in the healing process to restore the skin's protective barrier and mitigate inflammation [58].

The degree of re-epithelialization varied across the experimental cohorts; notably, the HA- and MUP+HA-gel-treated groups showed higher re-epithelialization percentages, as indicated by burn closure ratios. This observation could be attributed to their ability to maintain a moist wound environment, thereby preventing tissue dehydration and cellular necrosis and fostering favorable conditions for epithelial cell migration and proliferation. Furthermore, HA has demonstrated the capacity to expedite angiogenesis and facilitate the degradation of necrotic tissue, both of which are critical processes in wound healing. The topical application of HA has been observed to positively affect re-epithelialization; it absorbs burn fluid and exudate, thereby maintaining a dry wound environment and a distinct fibrous layer, which supports the hypothesis that HA promotes keratinocyte migration and proliferation [59].

The group treated with MUP gel exhibited improved skin re-epithelialization, with results significantly superior to those of the negative control group. This finding supports previous research suggesting that MUP indirectly aids re-epithelialization by reducing the bacterial presence in the wound. This reduction then lessens excessive inflammation, which speeds up the inflammatory phase. As a result, the wound environment becomes more favorable for epithelial cells to migrate and proliferate from the edges, thereby promoting epithelialization [60].

In summary, the MUP+HA gel exhibits superior performance compared to either MUP or HA gel alone in a rat model, accelerating wound closure, enhancing epidermal regeneration, stimulating collagen production and new blood vessel formation, and reducing inflammatory cell infiltration relative to negative controls. IHC analysis further supports these findings, with lower TNF-α levels and IL-10 and VEGF levels returning to normal after complete healing.

Conclusion

The MUP+HA gel exhibits superior performance compared to either MUP or HA gel alone in a rat model, accelerating wound closure, enhancing epidermal regeneration, stimulating collagen production and new blood vessel formation, and reducing inflammatory cell infiltration relative to negative controls. IHC analysis further supports these findings, with lower TNF-α levels and IL-10 and VEGF levels returning to normal after complete healing.

Disclosure Statements

Ethics approval and consent to participate

Animal handling and experimental procedures adhered to the ethical standards outlined by the Institutional Review Board (IRB) for the use of laboratory animals. The research protocol was reviewed and approved by the Animal Ethics Committee at the College of Pharmacy, University of Basrah, under approval code EC87, issued on October 7, 2025.

Consent for publication

Not an application.

Availability of data and materials

Contact the corresponding author to request access to the data; they will provide it upon reasonable request.

Author's contribution

Maysaa Banay Zubairi participated in the study's design. Atyaf Ali Faisal Al Ameri handled material preparation, conducted experiments, analyzed, and collected data. They also drafted the initial manuscript, with two authors contributing and providing feedback on the final version. All authors reviewed and approved the final manuscript.

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Conflicts of interest

The authors declare that there are no conflicts of interest related to the publication of this article.

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