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Copper Ions Ameliorated Thermal Burn-Induced Damage in ex vivo Human Skin Organ Culture

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Keywords
Burn · Stasis area · Ex vivo model · Copper ions · Cytokines

Abstract
Introduction: The zone of stasis is formed around the coagulation zone following skin burning and is characterized by its unique potential for salvation. The cells in this zone may die or survive depending on the severity of the burn and therefore are target for the local treatments of burns. Their low survival rate is consistent with decreased tissue perfusion, hypotension, infection, and/or edema, resulting in a significant increase in the wound size following burning. Copper is an essential trace mineral needed for the normal function of almost all body tissues, including the skin. ***Objective:*** The aim of the work was to study the effect copper ions have on skin burn pathophysiology. ***Methods:*** Skin obtained from healthy patients undergoing abdominoplasty surgery was cut into 8 × 8 mm squares, and round 0.8-mm diameter burn wounds were inflicted on the skin explants. The burned and control intact skin samples were cultured up to 27 days after wounding. Immediately following injury and then again every 48 h, saline only or containing 0.02 or 1 μM copper ions was added onto the skin explant burn wounds. ***Results:*** We found that exposing the wounded sites immediately after burn infliction to 0.02 or 1 μM copper ions reduced the deterioration of the zone of stasis and the increase in wound size. The presence of the copper ions prevented the dramatic increase of pro-inflammatory cytokines (interleukin (IL)-6 and IL-8) and transforming growth factor beta-1 that followed skin burning. We also detected re-epithelialization of the skin tissue and a greater amount of collagen fibers upon copper treatment. ***Conclusion:*** The deterioration of the zone of stasis and the increase in wound size following burning may be prevented or reduced by using copper ion-based therapeutic interventions.

Introduction

Exposure to thermal hazard may cause injuries that constitute a significant burden, ranging from mild skin burn to life-threatening conditions that are accompanied with long-term morbidity and disability complications. In some cases, major fire disasters may strike large number of casualties at once both in civilian and military situations [1]. Thermal burns are inflicted by exposure of the

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skin to high temperature often by hot liquids, steam, or flames [2]. The severity of burn injury is determined by temperature, time of exposure, and exposure area. Severe burns cause extreme physiological burden on the patient for a prolonged period of time. The local endogenous skin response to thermal burn generally results in 3 zones, depending on the severity of the burn and the cause [3]. The point of maximum irreversible damage is referred to as the zone of coagulation since there occurs tissue loss due to immediate coagulation and denaturation of tissue proteins. The zone around the coagulation zone, denoted the zone of stasis, is characterized by decreased tissue perfusion, and depending on the severity of the burn, hypotension, infection, and/or edema may lead this zone into an area of complete tissue loss or recovery. Around the zone of stasis, a zone of hyperemia is characterized by increased perfusion that will invariably recover unless there is severe sepsis or prolonged hypoperfusion [4]. Some cells in the middle zone of stasis, while initially viable, following the reduced perfusion, typically die, and the initial size of the wound deepens and widens [4]. The role of local and systemic treatment is to minimize the extension of the zone of injury thus saving as much viable tissue as possible.

Following severe skin injury, release of cytokines and other inflammatory mediators at the site of injury may lead to several systemic responses that can be detrimental. These include (a) significant increase in capillary permeability, leading to loss of intravascular proteins and fluids into the interstitial compartment, peripheral and splanchnic vasoconstriction, and myocardial contractility decrease. These cardiovascular changes, coupled with fluid loss from the burn wound, may result in systemic hypotension and end-organ hypoperfusion; (b) bronchoconstriction and potentially respiratory distress syndrome; (c) increase of the basal metabolic rate by up to 3 times its original rate; and (d) nonspecific cell-mediated and humoral immune modulation [4].

The zone of stasis is deemed a therapeutically critical section of the burn surface that can be salvaged [5]. Prevention of the progression and expansion of the wound injury into larger and deeper areas may have important local and systemic consequences that could significantly decrease complications and morbidity. Thus, local therapy that can halt the further deterioration of the zone of stasis following wound burn injury is an important modality, and the ideal local therapy is yet to be revealed.

Copper is an essential trace element involved in plenty of cellular, metabolic, and physiological pathways in the majority of body tissues [6]. In the skin, copper stimulates dermal fibroblast proliferation [7]; enhances production and secretion of various collagen and elastin types by fibroblasts in vitro [8] and ex vivo [9]; stabilizes the skin extracellular matrix (ECM) once formed [10, 11]; serves as a cofactor of superoxide dismutase, an antioxidant enzyme present in the skin, important for protection against free radicals [12, 13]; serves as a cofactor of lysyl oxidase, an enzyme that catalyzes lysine-derived crosslinks in ECM [14]; is vital for the catalytic activity of tyrosinase, essential for melanin synthesis in melanocytes [15]; and inhibits cellular oxidative effects, such as membrane damage and lipid peroxidation [8]. Recently, the capacity of copper oxide-impregnated wound dressings to enhance wound healing of chronic wounds was reported [16]. The goal of this study was to examine the therapeutic effect of local copper ions treatment on the skin burn model pathophysiology, using ex vivo skin explants [17].

Materials and Methods

Ex vivo Model

Skin was obtained from 35- to 65-year-old healthy patients undergoing abdominoplasty surgery. The patients under general anesthesia went standard or circumferential abdominoplasty. After excision of the excess skin, the subcutaneous fat was removed using a sterile technique. The defatted full-thickness skin was submerged in culture medium and transferred to the laboratory. The skin was cut into 8 × 8 mm squares by using a custom-made apparatus. Round 0.8-mm diameter burn wounds were inflicted on the skin explants by exposure to a soldering iron (200°C, 10 s). The injured and control intact skin samples were placed dermis down and kept at the air-liquid interface at 37°C with 5% CO_2 in Dulbecco's Modified Eagle's Medium supplemented with 100 IU/mL penicillin and 100 mg/mL streptomycin and 10% serum (Biological Industries, Beit Ha'emek, Israel). Culture medium was refreshed every 48 h. The explants were cultured up to 27 days after wounding. Each individual experiment was performed with skin explants obtained from the same donor. At least 3 independent experiments from 3 different donors were performed for each parameter studied. A sketch of the model is presented in Figure 1.

Copper Source and Application on the Skin Explants

As a source of copper ions, 2 items were used: woven fabric impregnated with 1% copper iodide microparticles (Cupron Inc., USA) and sterile wound dressings impregnated with 1.2% wt/wt cuprous oxide microparticles (MedCu Technologies Ltd., Israel). Prior to use, the copper iodide-impregnated fabric was sterilized using UV light. 0.84 g of the copper iodide-impregnated fabrics and 3.6 g of the cuprous oxide-impregnated wound dressings were immersed each separately in 25 mL of 0.9% saline overnight at 37°C for each experiment. The resulting concentrations of copper ions in the saline were determined using Aquachek™ copper ions test strips (Hach Company, USA), and the solutions served as copper ions stock solutions. From the stock solutions, working concentrations of 0.02 or 1 μM of copper ions were diluted in saline. A

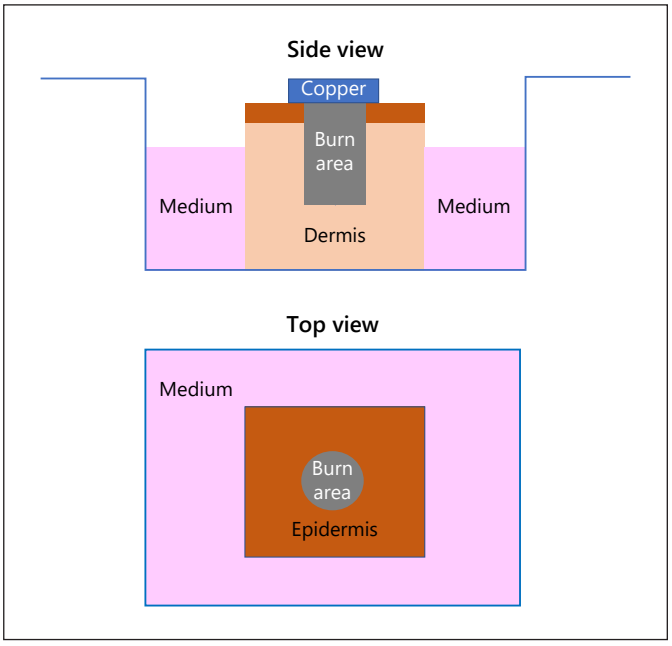

Fig. 1. Side and top views of the ex vivo explant model. 8 × 8 mm squares of skin explants were cut, placed dermis down in an air-liquid interface, and left for recovery for 24 hours. Round 0.8-mm diameter burn wounds were inflicted to the cut explants by exposing the epidermis to a 200°C hot soldering iron for 10 s. The injured and control intact skin cut explants were placed in 24-well plates. Culture media were added to the bottom of the well, without reaching the epidermal layer, which was exposed to the air. Three microliters of saline only or containing different concentrations of copper was carefully added on top of the burned area and around it, making sure it does not reach the culture media in the bottom of the well. Each experiment was done in triplicates using the skin explants from the same donor.

droplet of 3 μL of saline or copper solution (0.02 or 1 μM copper) was added onto the skin explant burn wound immediately following injury (day 0) and then again every 48 h. The solutions were mounted on top of the burn area and the epidermis around the burn area making sure that they do not reach the medium in the chamber but stay on the air interface on top of the skin. Each control and treatment was performed in at least 3 replicate explants.

Measuring Wound Area

Pictures of burns were captured at designated time points using a Moticam 5+ camera. Wound area was measured using ImageJ software. The results are presented as means ± SEM.

Measurement of Viability

To assess the cell viability throughout the culture period, a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed 1, 3, 5, 10, 14, 21, and 27 days following wounding, as follows: skin explants were incubated for 60 s in prewarmed PBS (56°C). Then, the epidermis layer of each replicate was separated from the dermis by grabbing the tissue edge with tweezers and peeling the epidermis off using a scalpel. Epidermal pieces were transferred to a 96-well plate, and each well contains 150 μL MTT (Sigma, 0.5 mg/mL) and incubated for 1 h at 37°C and reduced light conditions. Then, the epidermis pieces were transferred to a new 96-well plate containing 150 μL of isopropanol. Plates were placed for 5 min at 200 rpm in a plate shaker (MRC, Beijing, China). Following incubation, epidermis sections were removed, and absorbance was measured at 570 nm in a Tecan plate reader (Tecan Group Ltd., Switzerland).

ELISA (IL-6, IL-8, and TGF-β1)

During harvesting time points, the spent medium from all test groups was collected and centrifuged at 1,500 *g* for 5 min to remove particulates. Media were kept at −80°C until use. The secretion levels of interleukin (IL)-6, IL-8, and transforming growth factor beta 1 (TGF-β1) were measured by commercial enzyme-linked immunosorbent assay kits according to manufacturer instructions (Biolegend, San Diego, CA). In brief, plates were coated for 24 h with a specific anti-human capture antibody. Then, the coated plates were incubated with samples, followed by washes for unbound molecules. Then, detection (anti-IL-6, IL-8, or TGF-β1) antibody was added and detected by Avidin-HRP solution. Finally, wells were incubated with a substrate solution, while absorbance was measured at 570 nm.

Histology and Immunohistochemistry

At indicated harvesting time points, skin explants were fixed with 4% formaldehyde for 1 h at room temperature. Then, the samples were washed twice with PBS and kept in 70% ethanol at 2–8°C until use. Following dehydration in gradual increasing concentrations of ethanol and embedment, paraffin sections (8 μM) were prepared, pasted on slides, and either stained with hematoxylin & eosin (H&E) or Masson trichrome stain, according to the manufacturer specification (Sigma-Aldrich) followed by mounting with DPX mountant (Sigma, Israel). Immunostaining with Rabbit-anti Ki67 (Abcam, UK) was performed following rehydration, blocking in 2% BSA in PBS for 20 min, for 2 h, followed by exposure to goat anti-rabbit IgG H&L (HRP) for 1 h and detection by DAB substrate (Abcam, UK). Washes were performed in 0.025% Tween/PBS. Counterstain was performed with Hematoxylin (Sigma, Israel). Images were captured using a Zeiss PrimoVert microscope connected to a Moticam 5+ camera.

Statistical Analysis

Statistical analysis was performed using SigmaPlot 12.0 software. Values are presented as the average of 3 replicates, and standard errors of the mean are provided. Significant differences between values were analyzed using the unpaired *t* test, while significant results are for $p < 0.05$.

Results

Epidermal Viability

The exposure of healthy, nonburned, skin explants to 0.02 or 1 μM of copper ions obtained from copper oxide- or copper iodide-impregnated fabrics did not significantly affect epidermal viability compared to vehicle (saline)-treated controls during ex vivo explants culture and were

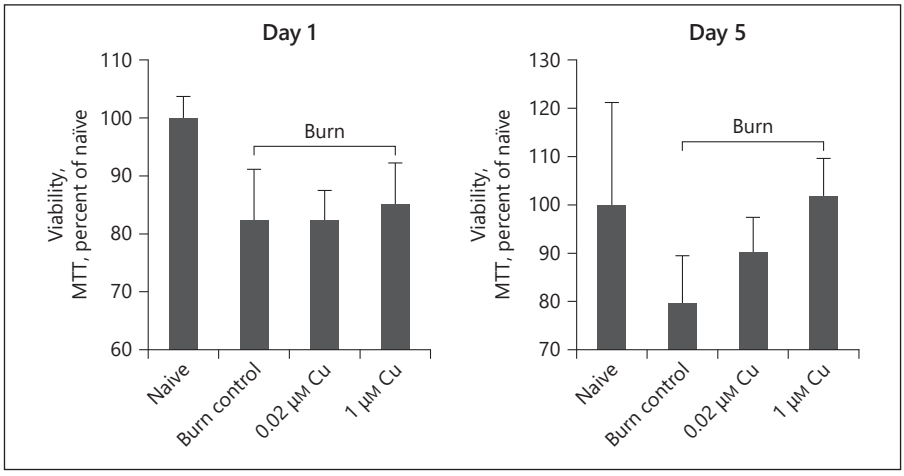

Fig. 2. Epidermal viability. The viability of the epidermis after 1 and 5 days following burn infliction was determined by MTT. The results are presented as means ± standard error, of 3 replicates in a representative experiment performed with skin explants obtained from the same donor. MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

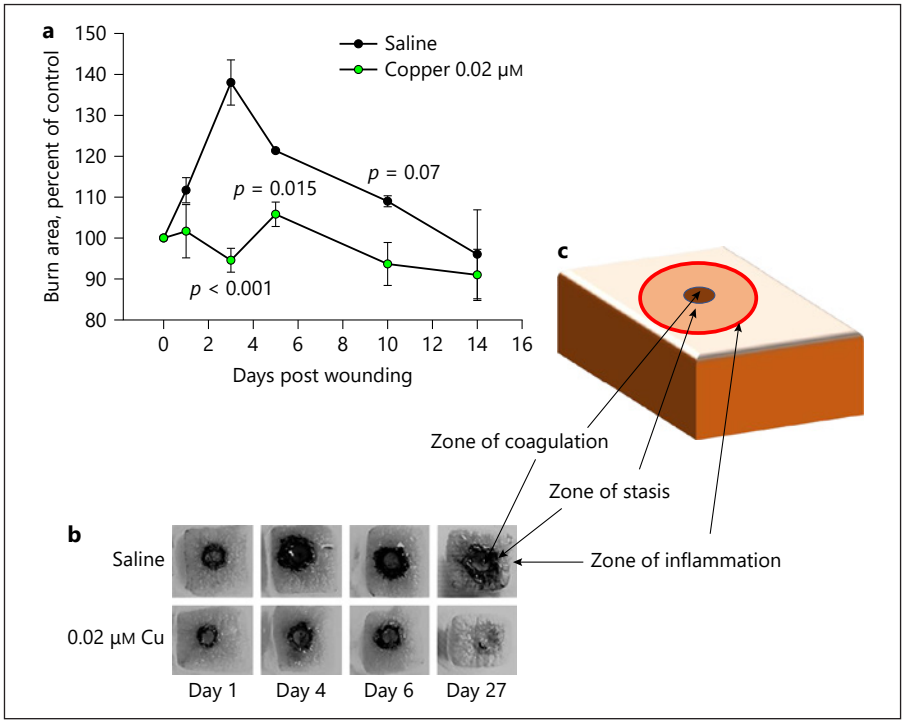

Fig. 3. Burn wound size. **a** Quantitation of burn size (area) at different days following burn infliction. The *p* values between the treatment groups and the untreated burn control groups per a given day are shown. **b** Representative pictures of the explants treated with saline only (control) or 0.02 μM copper ions, showing clear differences in the size of the wounds between the treatments, especially in the zone of stasis as illustrated in **c**.

found to be safe for use in the ex vivo experimental system (data not shown). When examining viability of the epidermal layer upon burn inflictions following 1 and 5 days, the viability of the epidermis was reduced in an average of 20% compared to naïve, nonburned pieces, based on the average of 3 independent experiments, from 3 different donors. When burned skin explants were treated with 0.02 or 1 μM of copper ions, the viability of the treated pieces remained similar to the untreated pieces after 1 day. However, following 5 days since the burn was inflicted, a trend of increased viability was observed, depending on the copper concentration (Fig. 2), though the differences were not statistically significant.

Wound Area Measurement

In order to evaluate the size of burns, pictures of the skin explants were taken and burn size was measured. Three independent experiments from 3 different donors were performed. As can be seen in Figure 3a, which depicts a representative experiment, the size of the burn wounds treated by saline only increased significantly following wounding, becoming almost 40% larger than the

initial size after 3 days of wounding; this is attributed to the zone of stasis. Following aggravation, the size of burn started to decrease gradually reaching the initial wound size 14 days following wounding. In contrast, the size of the wounds treated with 0.02 μM copper ions obtained from either copper iodide or cuprous oxide particles did not change significantly following wounding. It remained statistically smaller than the wounds treated with saline only at 3 days following wounding, and similarly smaller wounds were measured in the copper-treated explants at days 5 and 10 following wounding. As depicted in Figure 3a and as shown in the representative pictures in Figure 3b of a different experiment, the main zone, which did not increase following burning in the copper-treated explants, was the zone of stasis. The significant effect of the copper ions, on the prevention in the increase of the wound size following the burning of the skin explants, was observed in all 3 experiments performed.

Measurement of Cytokines IL-6, IL-8, and TGF-β1

In order to further understand how addition of copper ions affected the burned skin, the amounts of IL-6, IL-8, and TGF-β1, found in the growth media obtained from the various treated explants, were determined. Figure 4 presents a representative experiment of 3 independent experiments. As depicted in Figure 4a, in the burn controls, there was a clear increase in IL-6 secretion on the 1st and 4th days following burning as compared to the naïve controls. Measurements were normalized to amount of cytokine secreted from normal skin and are presented as percent of naïve control. Addition of 0.02 or 1 μM of copper ions from both sources of copper to the burned explants abolished the increase in IL-6 secretion by the burned explants on day 1 and actually resulted in reduced IL-6 secretion even compared to the naïve controls (Fig. 4a, right panel). On day 4 and forward, there were no significant differences in IL-6 levels between burn control samples and copper-treated samples (data not shown). On day 1, the amount of IL-8 secreted by the samples treated with copper-containing saline did not differ significantly than the amounts secreted by the burn control samples, which were all statistically significantly lower than the IL-8 secretions by naïve controls. In contrast, IL-8 levels, which were elevated in the burn controls as compared to the naïve controls (Fig. 4b) at day 4, were attenuated by the addition of the copper ions, being similar to the IL-8 levels secreted by the naïve controls (Fig. 4b, right panel). Similarly, the levels of TGF-β, which increased gradually during the first days after burning reached a peak on day 6 (Fig. 4c), were significantly inhibited by the presence of the copper ions in the solution on day 4 (Fig. 4c, right panel). On the other examined days, there were no statistically significant differences between the TGF-β secretion of the burn control samples and the copper-treated samples.

Histological Examination

Examination of tissue formation was performed on days 12 and 27. Sections of normal and burned skin, treated or not with copper ions, were stained with H&E and Masson's trichrome staining, respectively. Figure 5 presents full-length images of sections stained with H&E. In the upper panel, upon burn, the damage of the burn is observed in the center of the tissue, where the epidermal layer is absent and old, and dead epidermis is detached above (dark purple arrows). Additionally, newer separation of the epidermis from the dermis is observed further from the burn area (orange arrow) as a continuous damage. When the burns were treated with 0.02 or 1 μM copper ions, there was less detachment of the epidermal tissue from the dermis (Fig. 5, middle and lower panels). Higher magnification (inset) enables detecting newly formed epithelia (leading epithelial tongue, gray arrows) at the edges of the epidermal detachment. To strengthen these results, sections were immunostained against Ki67 to detect proliferating cells at the site of re-epithelialization. When burned skin was treated with 0.02 μM of copper ions, a significantly higher amount of Ki67-stained cells compared to control was observed (Fig. 6). Burned skin explants were harvested after 27 days, sectioned, and stained with Masson's trichrome staining which enables to detect both epidermal and dermal changes by staining cells in red and collagen fibers in blue. In normal tissue, healthy epidermal layer was attached to the dermis, whereas immediately after burning, the epidermis was detached, and the damaged tissue is indicated by the gray arrow (Fig. 7a). Following 27 days, re-epithelialization is detected upon copper treatment but not in the control samples. In addition, greater collagen stain is observed in the copper ion-treated samples (Fig. 7b).

Discussion

The impact of thermal burns on the skin tissue has been extensively investigated due to its high relevance to public health. In this study, we show evidence that supports the beneficial role of local copper administration for the treatment of thermal burns with emphasis on the reduction of the burn area at early periods of the healing

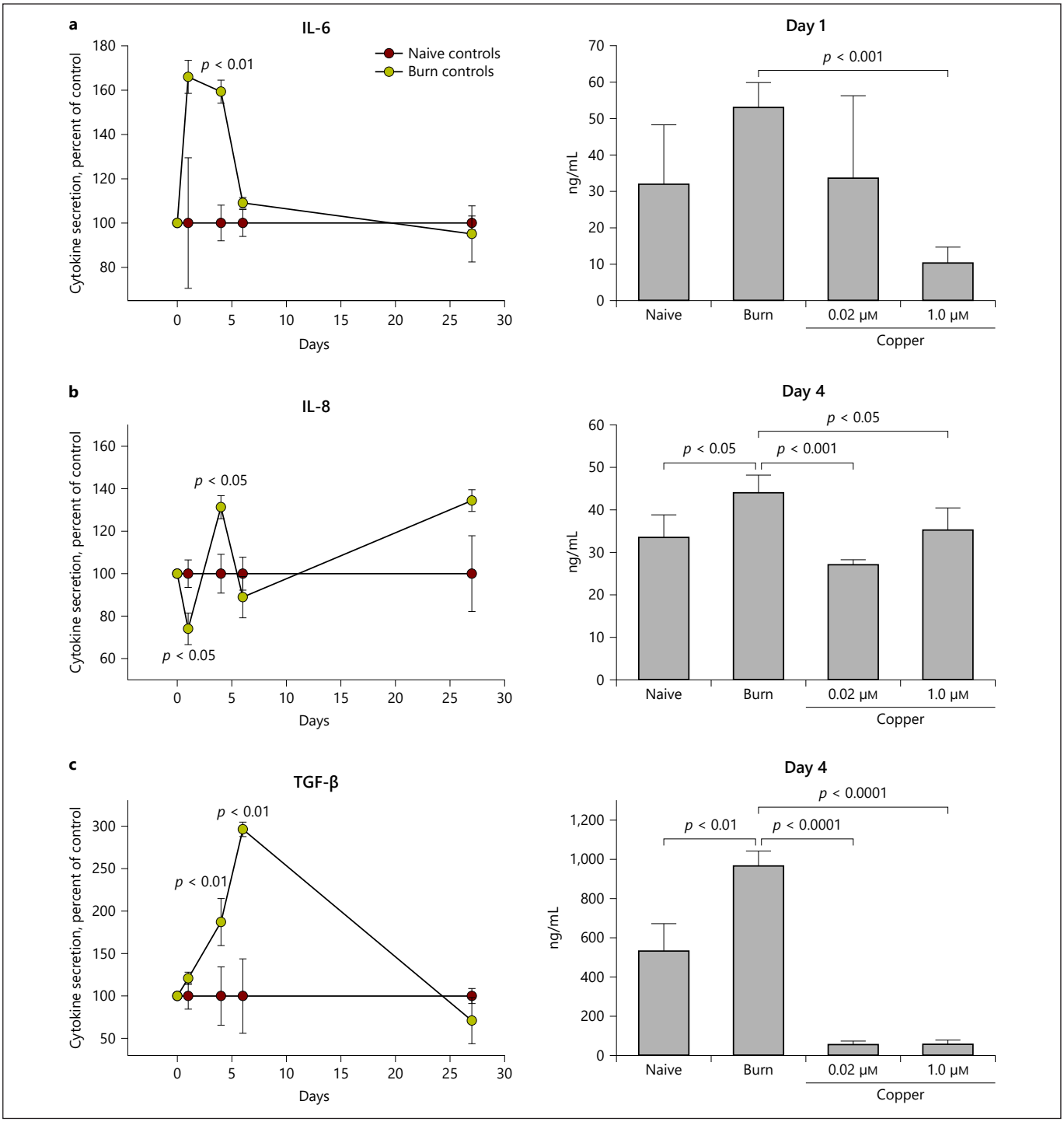

Fig. 4. Cytokine expression. **a–c** Left panels: comparison between IL-6, IL-8, and TGF-β1 kinetic secretion by the untreated skin explants (naïve controls) and the wounded skin explants (burn controls) following wounding. Results are presented as percent of controls and normalized each day to naïve control. The *p* values of a *t* test between the cytokine expressions per a particular day following wounding are shown. **a–c** Right panels: effect of addition of 0.02 or 1 μM of copper ions on IL-6, IL-8, and TGF-β1 secretion, respectively, as compared to the naïve controls and the burn controls on day 1 or 4 after wounding. The *p* values of a *t* test between the cytokine secretions of the tested groups that reached a *p* value of <0.05 are shown. The data shown are a comparison of the cytokine secretions from a single explant donor. Similar results were obtained by 2 additional independent explants. IL, interleukin; TGF, transforming growth factor.

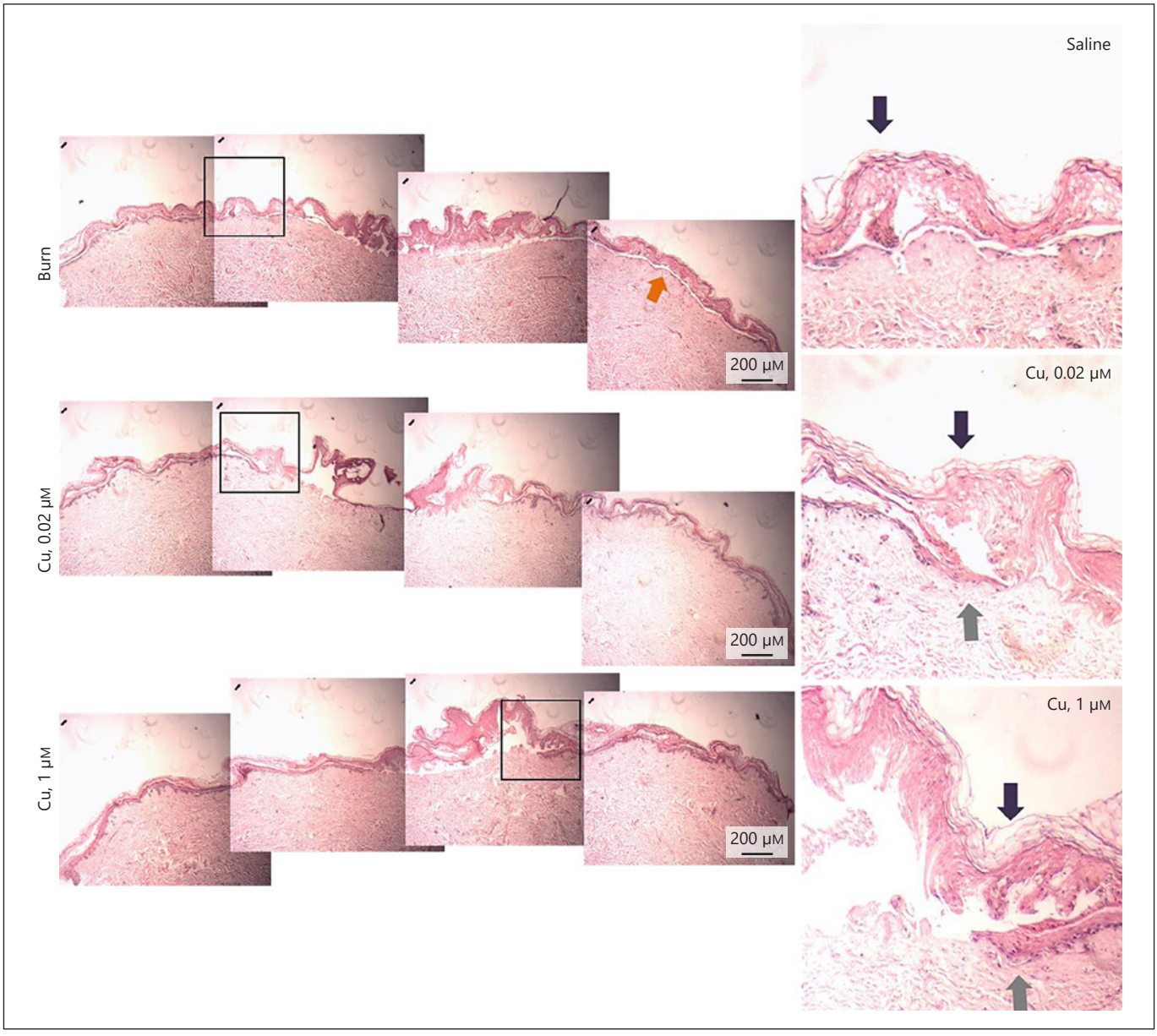

Fig. 5. Re-epithelialization following burn is catalyzed by copper ions. Burned skin treated or not with copper ions (0.02 and 1 μM) was harvested after 12 days, sectioned, and stained with HE. Orange arrow indicates separation of the epidermis from the dermis as a secondary damage in burned untreated skin. **Inset** Focus on the site of re-epithelialization. Dark purple arrow indicates detached dead epidermis. Beginning of epithelial regeneration (gray arrows) is seen upon treatments with copper ions, but not in the burn control section.

process and the accompanying inflammatory process. Collectively, our study lays the ground for clinical implications of copper-impregnated fabrics for the treatment of thermal burns.

The ex vivo human skin organ culture is a valuable tool in dermatological research, with increasing numbers of disease-oriented screening models [18–20]. Several studies have shown the relevance of ex vivo human skin models for studying thermal and chemical burns as useful tools for both basic and applied research. Liu et al. [21] defined various criteria, including heat temperature, that affect the severity and depth of wounds upon thermal burns caused by soldering iron, to create recurring conditions. They also demonstrated re-epithelialization in this

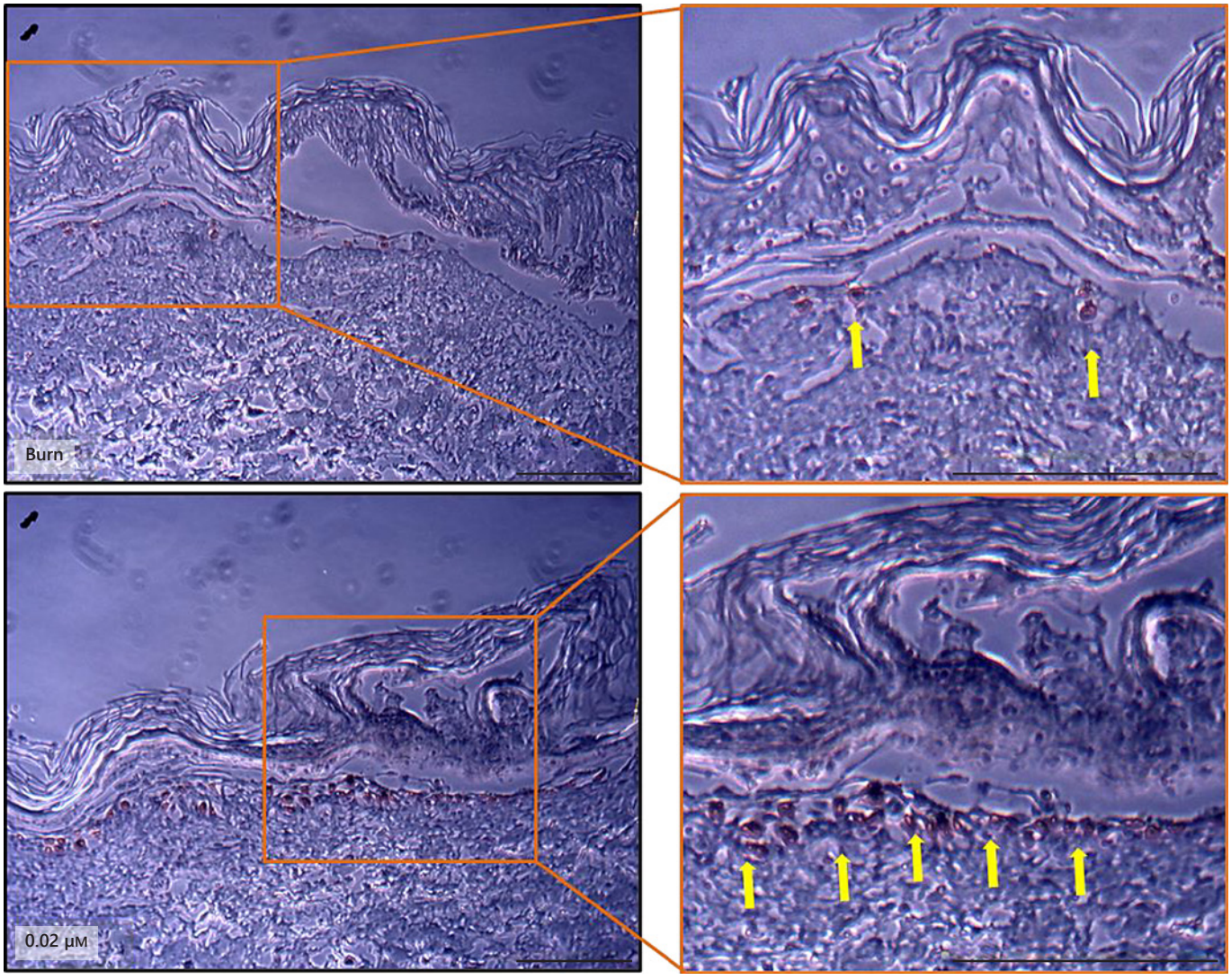

Fig. 6. Keratinocytes proliferation at the re-epithelialization area. Skin pieces fixed after 12 days were labeled with anti-Ki67 and secondary HRP-conjugated antibody. Counterstain was performed with hematoxylin. Upon burn, newly proliferating cells, stained with Ki67 (brown nuclei), indicated by yellow arrows, are significantly more common in the copper-treated explants.

model [21]. In their study, Coolen et al. [17] challenged a similar model, by adding lipopolysaccharide to mimic bacterial infections, which resulted in decreased re-epithelialization. Burgher et al. [22] demonstrated that the majority of damage caused by hydrofluoric acid burn in skin explants occurs within minutes. Therefore, extremely rapid decontamination should be applied to minimize the severity of the damage. Furthermore, a recent study focuses on detecting early-stage responses in the microenvironment of thermal burns ex vivo [23]. Here, we explored the healing process of thermal burns in an ex vivo human skin model upon treatment with copper ions, from 2 different sources, by using various healing markers by means of histology and immunohistochemistry methods, as well as cytokine secretion to the growth media and ECM remodeling.

After burning, monitoring of the skin pieces was performed kinetically. The findings are consistent with known phases of burn healing [24]. During the early days following burn infliction, at the inflammatory stage, we detected an elevated secretion of the pro-inflammatory cytokines IL-6 and IL-8. Although in this experimental system the skin pieces are detached from an organism and the vascular system and, therefore, cannot simulate an

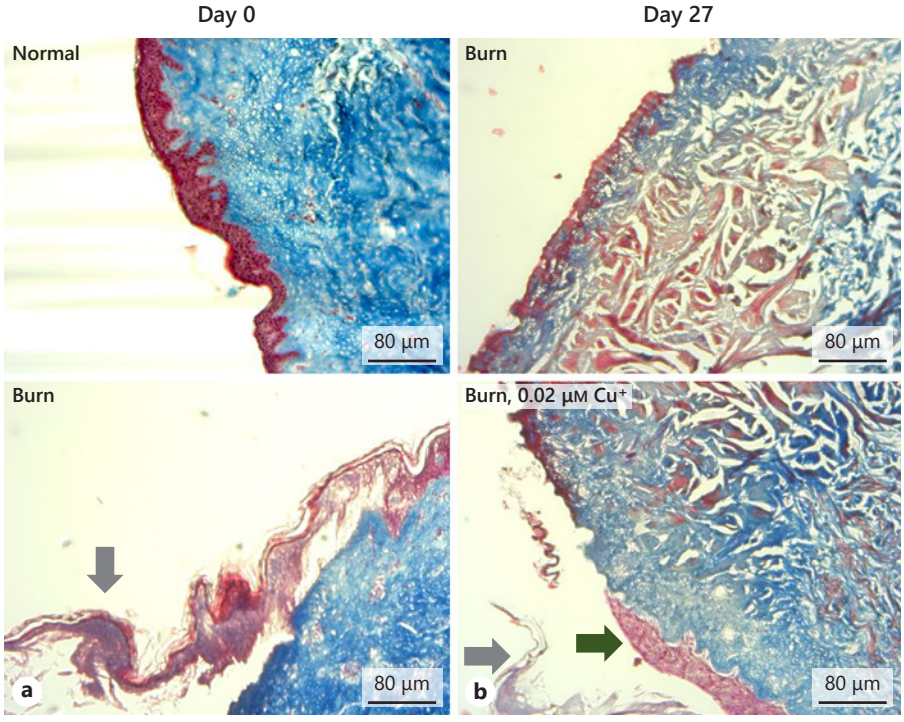

Fig. 7. Masson's trichrome staining of skin explants 27 days after burn. **a** At day 0, burns were inflicted on skin explants. The upper picture represents normal healthy skin. The lower picture represents damaged skin, immediately following the burn infliction. Epidermis is detached from the dermis (gray arrow). Old, separated epidermis is also indicated by the gray arrow at day 27 following the burn infliction (**b**). Re-epithelialization is noted on day 27 only upon copper ion treatments, as indicated by the green arrow. Samples were fixed on indicated time points and histologically stained by Masson's trichrome dye. The blue color represents collagen staining.

inflammatory response involving an oscillation of immune cells to the wound area, it allows detecting local responses of the burn microenvironment. The thermal burn caused an increased secretion of IL-6 and IL-8 to the growth media compared to normal skin. At day 1 following the burn, the levels of IL-6 were dramatically elevated in the burn controls but not in the copper ion-treated samples. The IL-6 secretion decreased to basal level in all samples on day 6. The secretion of IL-8 immediately after burning in the saline-treated samples was biphasic – being first reduced by ~30% on day 1 and increased by ~35% on day 4 after burning. Interestingly, the copper ion-treated burned samples behaved similar to the saline-treated samples with the exception on day 4, in which IL-8 secretion was significantly lower in the copper-treated samples than in saline (control)-treated burn samples.

We have previously demonstrated the kinetic variability of IL-6 and IL-8 secretions in response to another stimulation of skin explants (administration of lipopolysaccharide) in an ex vivo experimental system. The order of cytokine secretion was similar [18]. The prevention of increased burn-induced IL-6 and IL-8 secretion on day 1 and day 4, respectively, is consistent with the anti-inflammatory abilities of copper ions [25–27]. The importance of reducing burn-induced inflammation in the wound healing process was demonstrated in several experimental models in vivo [28–30]. Furthermore, elevated amounts of IL-6 which are associated with the severity of burns were reduced by anti-inflammatory agents which was consistent with additional healing markers in mice [30].

Secretion of TGF-β, which is associated with processes of wound healing by stimulating fibroblasts and infiltrated immune cells [31] was, as expected, elevated in response to the burn damage. However, its level was dramatically inhibited by copper ions on day 4, returning to normal levels on day 6 and onward. The reduced secretion of TGF-β shortly after burning may have contributed significantly to the reduced inflammatory response and deterioration especially of the zone of stasis, preventing the increase in the wound size, which occurred in the saline-treated burn samples only. The increased secretion of TGF-β on day 6 in the copper-treated burn samples may be important, as TGF-β also plays a critical role at advanced stages of wound healing in the context of tissue differentiation, remodeling, and scar formation. Indeed, earlier proliferation of keratinocytes and epithelialization in the copper ion-treated samples occurred, as seen in Figure 5–7. The precise regulation of TGF-β concentration at different stages of wound healing is important, as TGF-β's activity may be dependent on its concentrations [32]. For example, high amounts of TGF-β are associated

with scar formation, and in particular with the formation of keloids and hypertrophic scars [33]. Taken together, our results enable us to speculate the beneficial role for copper ions in preventing scarring or at least the formation of hypertrophic scarring by copper treatment. All this support the recently published findings of wound healing properties of chronic wounds by cuprous oxide-impregnated dressings [16].

Additional pathologies of unhealthy recovery, other than scar formation, are bacterial and fungal infections of the burned wounds. Copper-impregnated dressings [34, 35] and other forms of copper administrations [36, 37] were found to possess antimicrobial capacity, which may also enhance and contribute to burn healing processes outside the laboratory.

Furthermore, others and we have also suggested the dermocosmetic beneficial properties of copper treatment [38, 39]. Ex vivo treatments with copper ions extracted from copper oxide-impregnated dressings on skin resulted in increased expression of collagen and elastin as well as an increase in the amount of their fibers in the dermal tissue [9]. Also, in the copper-treated burned samples, we found an increase in the amount of collagen fibers than in the saline-treated control group, as seen in the dermal layer in Figure 7b. Other clinical studies demonstrated increased elasticity of the skin tissue, reduction of sagging, and overall improvement of the skin well-being following the use of copper-impregnated fabrics [38–43].

Here, cuprous oxide (Cu_2O)- or copper iodide (CuI)-impregnated fabrics were used as a source for copper ions. Topical application of the ions had reduced dramatically inflammation manifestations and prevented the increase in burn size that occurs after the initial damage. We did not find significant differences in regard to the source of the copper ions, that is, from cuprous oxide- or copper iodide-impregnated fabrics. Although burn size at the end of the experiment was not massively improved, due to limitations of the experimental model, this ameliorating effect can have long-term implications, such as healthier scarring. The ex vivo model enabled us to detect local responses of the skin microenvironment that do not include infiltration of immune cells to the burn area. Further long-term clinical investigation is of need to validate the feasibility of copper-containing wound dressing for the treatment of burns.

Statement of Ethics

The study was approved by the Soroka University Medical Center Institutional Review Board, Be'er Sheva, Israel. The skin tissues that were used in this study were from patients scheduled for abdominoplasty who gave their written informed consent to use their skin in the study.

Conflict of Interest Statement

G.B. is the chief scientist of MedCu Technologies, Ltd., which produces copper oxide wound dressings. All other authors do not have conflicts of interest.

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Author Contributions

G.B. and N.O.S. contributed to the conception and design of the work, acquisition, analysis, interpretation of data, and writing of the manuscript. E.S. contributed to interpretation of the data and writing of the manuscript. K.C. conducted significant parts of the lab work.

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