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Comparing the antibacterial and healing properties of medical-grade honey and silver-based wound care products in burns

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ABSTRACT

Burns are a major global healthcare concern, often complicated by the presence of bacteria such as *Pseudomonas aeruginosa* in the wounds. Silver-based dressings are commonly used in the treatment of burns but can cause skin irritation and delay healing time. Medical-grade honey (MGH) provides an interesting alternative. This study investigated the antimicrobial effects and possible cytotoxicity of L-Mesitran Soft (MGH-gel) and its individual components, Medihoney (Manuka), Flammazine (silver sulphadiazine), and silver nitrate ($AgNO_3$) in an *ex vivo* human burn wound model. Bacterial survival and wound healing parameters, including re-epithelialization and keratinocyte proliferation were assessed. L-Mesitran, Flammazine, and $AgNO_3$ reduced *P. aeruginosa* numbers below detection levels. L-Mesitran Soft exhibited a significantly stronger antimicrobial effect compared to Medihoney. The individual components of L-Mesitran contributed significantly to its antibacterial efficacy, thus suggesting synergistic activities. Moreover, L-Mesitran, Flammazine, and $AgNO_3$ slightly inhibited re-epithelialization while Medihoney treatment resulted in a complete lack of re-epithelialization and keratinocyte proliferation. Furthermore, clinical cases illustrated the effectiveness of MGH therapy in infected burns.

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Overall, L-Mesitran Soft had similar effects as silver-based products on bacterial load and epidermal regeneration, but outperformed Medihoney. Therefore, supplemented MGH could be used as an effective alternative to silver-based dressings for *P. aeruginosa*-infected burns.

1. Introduction

Burn injuries contribute significantly to global mortality and morbidity. Previously published data reported that the annual incidence of severe burns was 0.2–2.9/10,000 inhabitants, with a decreasing trend in time. Major risk factors for death were older age and a higher total percentage of burned surface area, as well as chronic diseases. (Multi) organ failure and sepsis were the most frequently reported causes of death. The main causes of early death (< 48 h) were burn shock and inhalation injury [1]. Nowadays, approximately 180,000 deaths are attributed to burn injuries annually, while non-fatal burn wounds are a leading cause of morbidity worldwide [2]. Furthermore, burns greatly affect the patient's quality of life, as patients might suffer from physical symptoms such as itch and pain, or from psychological symptoms including anxiety and traumatic stress [3,4]. The burden of burns measured in disability-adjusted life years (DALYs) was 7,460,448 (95% UI, 5,794,505–9,478,717) in 2019, of which 67% and 33% could be attributed to Years of Life Lost (YLLs) and Years Lived with Disabilities (YLDs), respectively [5]. The economic burden of burn injuries is also substantial. For example, South Africa alone spends an estimated US$26 million each year to provide care for burns caused by kerosene cookstove accidents [2]. Severe burns not only affect the injured area locally, but also induce major immunological and inflammatory changes, and burn shock, all of which can be difficult to control and result in multiple organ failure [6,7]. Due to immunological changes, burn patients are at high risk of wound infection, organ failure, and sepsis mentioned above.

Infection caused by *Pseudomonas aeruginosa* is a major contributor to poor outcomes in burn patients and it has been associated with mortality rates of up to 80% [8]. *P. aeruginosa* takes advantage of the suppressed immune system and loss of skin barrier of the burn patient, and exerts its detrimental effects via several virulence factors, including lipopolysaccharide [9,10]. The increasing prevalence of antibiotic-resistant strains of *P. aeruginosa*, and its ability to form biofilms complicate the treatment strategies even further [10,11]. Silver-based dressings and topical agents, such as silver sulphadiazine and silver nitrate ($AgNO_3$), represent one of the treatment standards for dermal burns. They are used to reduce the bioburden in the wound and act as an antimicrobial barrier. In infected wounds, silver is beneficial for the first few days or weeks, after which non-silver dressings should be used instead [12]. This is due to the toxicity of silver in healthy tissue, which is detrimental to the wound-healing process. Various studies have confirmed that the use of silver dressings indeed impairs wound healing in patients [12,13]. Although systematic reviews have shown that various dressings can shorten wound healing time compared to silver sulphadiazine, only honey based wound dressings showed consistently better results for wound infection [14,15]. The ideal wound dressing would be harmless to healthy tissue, exert a wide-spectrum antimicrobial effect, and work over a longer period of time [12].

Medical-grade honey (MGH) is a honey that is organic, free of pathogens, and eradicated from dangerous micro-organisms by gamma-irradiation to ensure safety and efficacy for medical use [16]. MGH has demonstrated potent antimicrobial activities against a broad spectrum of pathogens, including *P. aeruginosa* [17–19]. The mechanisms underlying this activity include its acidic pH, the osmotic effects caused by its high sugar content, the generation of hydrogen peroxide, and the presence of other bioactive compounds [20]. These multifaceted effects not only contribute to the ability of MGH to treat a broad spectrum of bacteria, but MGH also lacks the risk of resistance [21–23]. Besides its strong antimicrobial effects, MGH also promotes the natural wound-healing process in various ways. These include maintaining a moist wound environment, stimulating autolytic debridement, providing nutrients to the wound bed, and wielding antioxidant and anti-inflammatory effects[18,24]. The overall combined antimicrobial and pro-healing properties make MGH an attractive candidate for the treatment of *P. aeruginosa*-infected burn wounds.

Our previous work demonstrated that L-Mesitran Soft, a MGH-based gel, had similar antimicrobial effects as Flammazine, a silver sulphadiazine cream [17]. However, Flammazine was shown to reduce re-epithelialization significantly in an *ex vivo* burn wound model with human skin compared to L-Mesitran, thereby highlighting the cytotoxicity of silver and the pro-healing effects of MGH. In a recent study by Pleeging *et al.*[18], L-Mesitran Soft was shown to exhibit a strong antimicrobial activity on *P. aeruginosa* biofilm inhibition and eradication using artificial dermis. The antimicrobial activity was likely due to the synergistic activity mediated by the supplements of L-Mesitran Soft. Although the effects of MGH on burn wound infection and healing have been shown before, the antibacterial and healing effects on *P. aeruginosa*-infected burn wounds of the individual components of L-Mesitran Soft compared to other MGH- and silver-based products has yet to be determined.

This study aimed to compare the effect of L-Mesitran Soft and its individual components to several other wound care products, including Medihoney (Manuka), Flammazine, and $AgNO_3$ on the inhibition of *P. aeruginosa* in *ex vivo* burn wound models with human skin. In addition, the effect of the

different treatments on epidermal regeneration and viability was investigated. Lastly, clinical cases of *P. aeruginosa*-infected burn wounds treated with L-Mesitran products are presented.

2. Materials & methods

2.1. Bacteria

P. aeruginosa PAO1 was routinely cultured on Luria Broth (LB, Invitrogen, Paisley, UK) agar at 37 °C. From a proliferating culture in 3 ml LB (OD600 0,3–0,5), bacteria were diluted in 0.85% NaCl to the required colony forming units (CFU)/ml with the assumption that an OD600 reading of 1 corresponds to a bacterial density of 10^9 CFU/ml. CFU/ml was subsequently determined by plating serial dilutions and was on average 1.4 ± 1.2 10^7.

2.2. Burn wound models

A burn wound model[17] was used with small modifications. Healthy skin samples were obtained from adult patients who underwent elective surgery at the Departments of Surgery or Plastic and Reconstructive Surgery of the Red Cross Hospital, Beverwijk. Consent for the use of these anonymized, post-operative residual tissue samples was received through the informed opt-out protocol of the Red Cross Hospital, which was in accordance with the national guidelines (https://www.coreon.org/ accessed on 23 November 2020) and approved by the institutional privacy officers. Split-thickness samples of 0.5 and/or 0.8 mm thickness were harvested using a dermatome (Aesculap AG & Co. KG, Tuttlingen, Germany). A copper plate (4 × 4 mm) attached to a PACE intelliHeat ST50 soldering iron (PACE, Vass, NC, USA) was heated to 80 °C and applied to the epidermal side of the models for 30 s without exerting pressure. The temperature of the copper device was measured by an external digital thermometer (Farnell InOne, Utrecht, the Netherlands).

2.3. Burn wound infection

Burn wound models (0.8 mm thickness grafts) were infected with bacterial suspensions (5 µl of approximately 2 10^7 CFU/ml). After 1 h of incubation at 37 °C, the following treatments were applied: L-Mesitran Soft, raw honey (Uruguayan Organic Honey, in accordance with the medical-grade honey standards[16]), vitamin C & E, mix without honey (all supplied by Mesitran), Medihoney (Manukamed), silver sulphadiazine (Flammazine, Centrafarm B.V., Etten-Leur), 1% silver nitrate (Apotheek Noordwest Ziekenhuisgroep, Alkmaar) in macrogolum (Rode Kruis Ziekenhuis, Beverwijk). Silver nitrate was applied in combination with a gauze to prevent running off. The gauze was either partially saturated for comparison with the other treatment methods or completely saturated to obtain low bacterial counts. Treatments were applied on the bottom of a 6-well plate, on which the infected burn wound samples were placed. Subsequently, 100 µl of treatment was applied topically on the entire skin surface and samples were incubated for 24 h at 37 °C. After treatment, the bacteria in the burn wound models were dislodged in 1 ml PBS by using a TissueLyser LT (Qiagen, Venlo) for 4 min at 45 Hz. Bacterial survival was determined by plating serial dilutions.

2.4. Ex vivo wound healing

Burn wound models (0.5 mm thickness grafts) were cultured air-exposed on metal grids at 37 °C with 5% CO_2. Treatments were applied topically twice a week, for every donor the experiments were carried out in duplicate. Medium consisted of DMEM/Ham's F12 (3:1), 2% fetal calf serum, 1 µM hydrocortisone, 1 µM isoproterenol, 0.1 µM insulin, 10 µM of L-carnitine, 10 mM of L-serine, 1 mM of DL-α-tocopherol, 130 µg/ml of ascorbic acid, a lipid supplement (containing 25 µM palmitic acid, 15 µM linoleic acid, 7 µM arachidonic acid, and 24 µM bovine serum albumin), and penicillin/streptomycin (100 IU/ml penicillin, 100 mg/ml streptomycin). Culture medium was refreshed twice a week and samples were cultured for 2 weeks at 37 °C with 5% CO_2. To evaluate the proliferation of keratinocytes and fibroblasts, the skin samples were incubated with 20 mM of bromodeoxyuridine (BrdU) for 24 h before fixation in Kryofix (50% ethanol, 7% PEG300).

2.5. Histology and immunohistochemistry

Wound samples were processed for paraffin embedding. Sections (5 µm) were deparaffinized and rehydrated for hematoxylin and eosin (H&E) staining, using standard techniques. The newly formed epidermis and total wound area were measured with digital image analysis (NIS Elements Ar software). To evaluate the proliferation of keratinocytes and fibroblasts in the wound model, the incorporation of BrdU was examined. Sections (5 µm) were deparaffinized, rehydrated, and treated with H_2O_2 (1% in PBS) for 20 min. The cellular DNA was denatured in 2 N HCl for 30 min and neutralized with 0.1 M Borax. The sections were incubated with monoclonal anti-BrdU antibody (1:100 in 1% BSA) for 1 h, followed by incubation with poly-HRP anti-mouse (BrightVision, Immunologic) for 30 min at room temperature. Peroxidase activity was detected with 3,3′-diaminobenzidine substrate. All sections were counterstained with hematoxylin. Numbers of proliferating cells in the newly formed epidermis were counted.

2.6. Cytotoxicity assay

Ex vivo excision wound models were prepared from skin grafts using a dermatome (width 7 mm) to remove 0.3 mm of the upper part of the skin graft containing the epidermis [25]. Subsequently, the grafts were cut into pieces of 1 × 1.5 cm using a scalpel and placed on metal grids with medium (see wound healing) at 37 °C with 5% CO_2. Samples were treated topically with 20 µl L-Mesitran, Medihoney (both undiluted) or 1% (v/v) Triton X-100 for 24 or 48 h. Samples were washed in PBS and fixated in 1% (wt/v) paraformaldehyde in PBS for 2 h at 4 °C, followed by overnight incubation in 20% (wt/v) sucrose solution at 4 °C while rotating. Samples were flash frozen in liquid nitrogen and embedded in Tissue Tek O.C.T. compound (Sakura Finetek, Torrance, USA) to prepare 10 µm sections. LDH staining was done as previously described [23].

The penetration depth of any cytotoxicity could not be measured because of scattered staining. Microscopic visualization was performed with a Zeiss Axioskop40FL microscope (Zeiss, Breda, The Netherlands). Images were acquired using a Nikon Eclipse TS2 camera and the NIS-Elements software version 4.4 (Nikon Instruments, Amsterdam, The Netherlands).

2.7. Statistics

Statistical analysis was performed with R (ggplot package, open source). Because a normal distribution cannot be assumed, the non-parametric Mann-Whitney U test (MWU) or the non-parametric paired Wilcoxon test was used to determine significant differences between treatments.

2.8. Case series

In order to showcase the clinical applicability and antibacterial effectiveness of L-Mesitran in treating burn wounds, four patients attending a wound care clinic in South Africa and two patients attending a hospital in Rome (Bambino Gesu' Children's Hospital in Rome) were enrolled. These patients exhibited either confirmed or suspected infections with *P. aeruginosa* or *Staphylococcus aureus* in their burn injuries. Visual documentation, including photographs taken at the initiation of treatment and during subsequent follow-up visits, is presented. Prior to their inclusion in the study, the patients were provided with comprehensive information about the research, and they provided written consent. The study adhered to the principles outlined in the Declaration of Helsinki by the World Medical Association and were approved by local ethical boards without the special number identification.

3. Results

3.1. Supplemented MGH has similar antimicrobial effects against P. aeruginosa as Flammazine and AgNO3, while outperforming Manuka honey

To investigate the antimicrobial effects of the various MGH- and silver-based wound care products, an *ex vivo* human burn wound infection model was deployed. Treatment of *P. aeruginosa* PAO1 in these burn wound models with Flammazine and L-Mesitran Soft reduced the bacterial load below the detection level (≤50 CFU/ml) (Fig. 1A). $AgNO_3$ and Medihoney also reduced the bacterial load to 10^5 and 10^4 CFU/ml, respectively, although the effects were markedly less strong compared to the other products. $AgNO_3$, however, also reduced the bacterial load below the detection level when applied in an oversaturated manner (data not shown). Furthermore, to normalize for any interpatient variations, the log reduction was calculated using the untreated samples per donor (Fig. 1B). Although not significant ($p = 0.063$), L-Mesitran Soft (7.9-log) exhibited stronger antimicrobial effects against *P. aeruginosa* POA1 than Medihoney (4.7-log) (Fig. 1B). Flammazine and saturated $AgNO_3$ reduced the bacterial load by 7.9-log and 3.1-log, respectively. The contribution of the individual components of L-Mesitran Soft, the most active MGH formulation, was also investigated. Of the tested supplements, the raw honey and vitamins C and E did not significantly reduce the bacterial load (Fig. 1A). Remarkably, the mix without honey reduced the bacterial load to almost below the detection level (Fig. 1A) and had a similar log reduction to L-Mesitran Soft (7.6-log) (Fig. 1B). This indicated that these components contributed the most to the antibacterial effect of L-Mesitran Soft. Moreover, while the vitamins did not affect bacterial load, the raw honey reduced the bacterial load by 1.3-log (Fig. 1B). Overall, these results suggest that L-Mesitran Soft has similar antibacterial effects as the silver-based products, while the Medihoney treatment resulted in significantly less reduction of *P. aeruginosa* infection in the burn wound model.

3.2. Burn wound healing is strongly impaired by the treatment of Manuka honey, but not by the other treatments

To determine the effects of the various MGH- and silver-based wound care products on the healing process of burn wounds, re-epithelialization and keratinocyte proliferation were measured in an *ex vivo* burn wound model. Wound healing was not affected by topical treatment with raw honey, vitamins C & E, or silver nitrate, while L-Mesitran Soft, the mix without honey, and Flammazine slightly reduced re-epithelialization (Fig. 2A). Remarkably, treatment of burn wound models with Medihoney resulted in a complete lack of regeneration of the epidermis. Furthermore, the proliferation of basal keratinocytes was unaffected by L-Mesitran Soft, but slightly reduced by treatment with the mix without honey (Fig. 2B, C). In line with the re-epithelialization, Medihoney treatment resulted in no proliferation of the basal keratinocytes. Results with DMEM (included as an additional control) were highly similar to the untreated control.

To visualize cell viability of the epidermis, a LDH staining was performed on the untreated samples and the samples treated with Medihoney and L-Mesitran Soft (Fig. 3). Excisional instead of burn wound models were used, to allow for better visualization of LDH positive cells. Triton-X-100 was included as a control as it is an established cytotoxic agent [26]. Notably, the treatment with Medihoney showed a lack of LDH staining at the edge of the wound area, supporting the lack of re-epithelialization and keratinocyte proliferation. Taken together, these results imply that Medihoney had detrimental effects on the tissue viability in this *ex vivo* human wound model, while the other products only slightly inhibited re-epithelialization.

3.3. Case reports illustrating the effective use of supplemented MGH in infected burn wounds

To establish the clinical significance of these *ex vivo* findings, the efficacy of L-Mesitran Soft in eradicating *P. aeruginosa* and *Staphylococcus spp.* from burn wounds is further demonstrated through multiple case reports. Although wounds infected with *P. aeruginosa* are more related to the *ex vivo* experiments, we aimed to underscore the wide-spectrum antimicrobial activities of MGH. Therefore, the final two clinical cases include *Staphylococcus spp.*-infected burns.

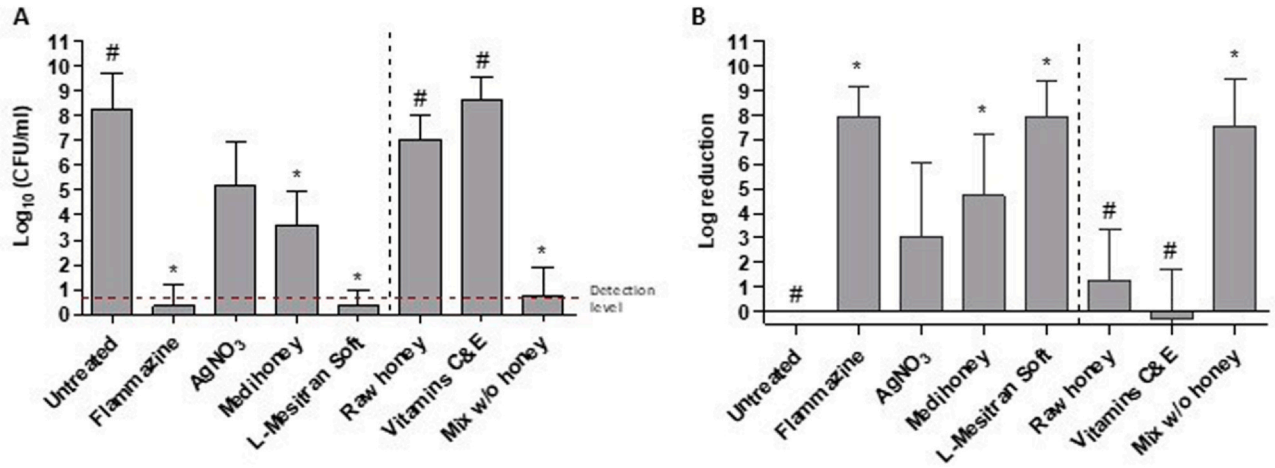

Fig. 1 – Antibacterial effects in the infected burn wound model. Bacterial counts (A) and log reduction (B) after treatment for 24 h of burn wound models infected for 1 h with PAO1. Skin from six donors was used in duplicate. Log reduction was calculated using average results of untreated samples per donor before averaging. Significant differences (Wilcoxon, $p < 0.05$) are indicated: * compared to untreated control, # compared to L-Mesitran; $n = 4–6$.

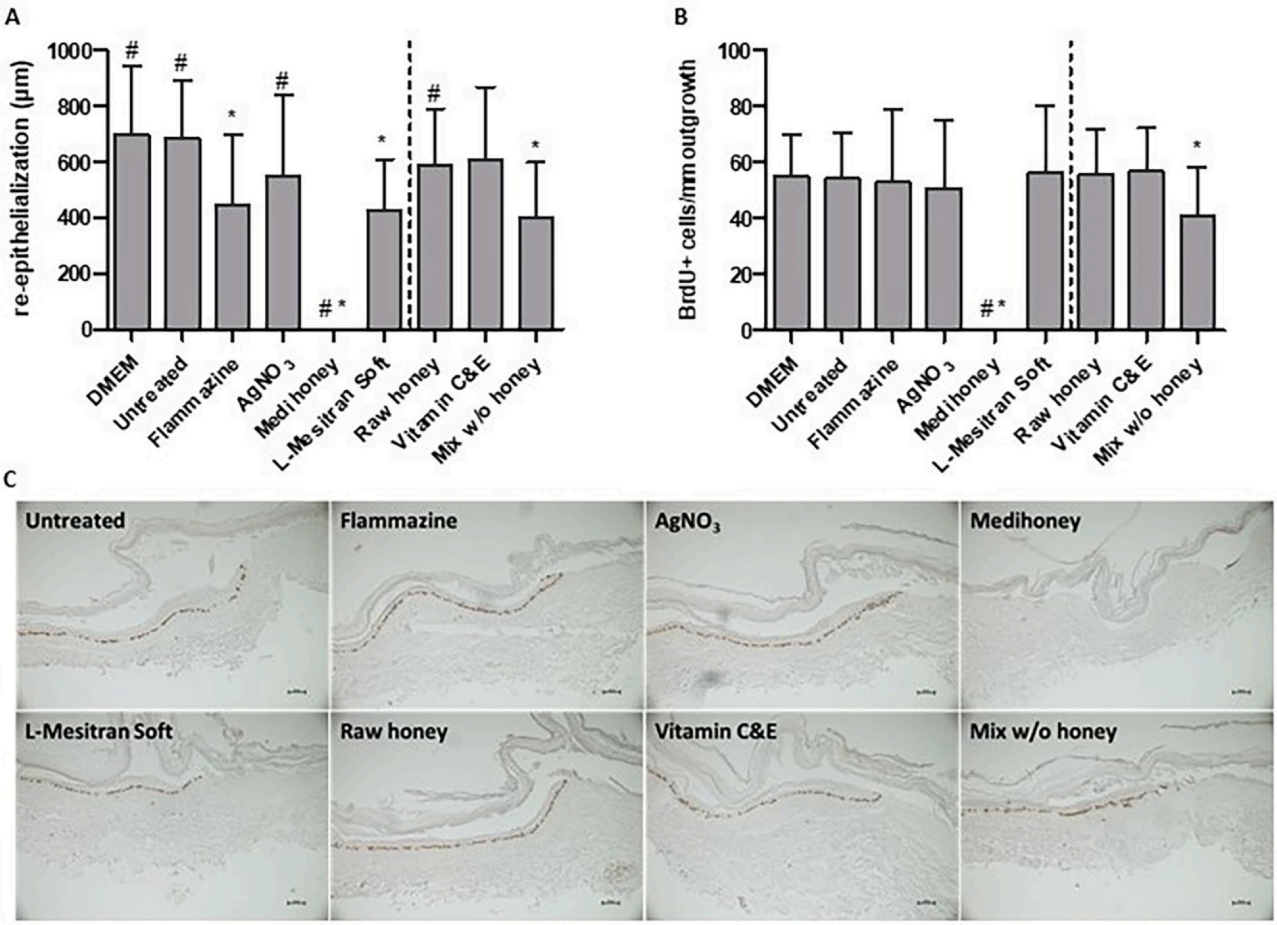

Fig. 2 – Wound healing effects in the burn wound model. A. Re-epithelialization (µm) in burn wound models. B. Proliferation of basal keratinocytes (BrdU+/mm) in the neo-epidermis after staining for BrdU. C. Representative pictures for the BrdU staining. Scale = 100 µm. Significant differences (Wilcoxon, $p < 0.05$) are indicated: * compared to untreated control, # compared to L-Mesitran; $n = 6$.

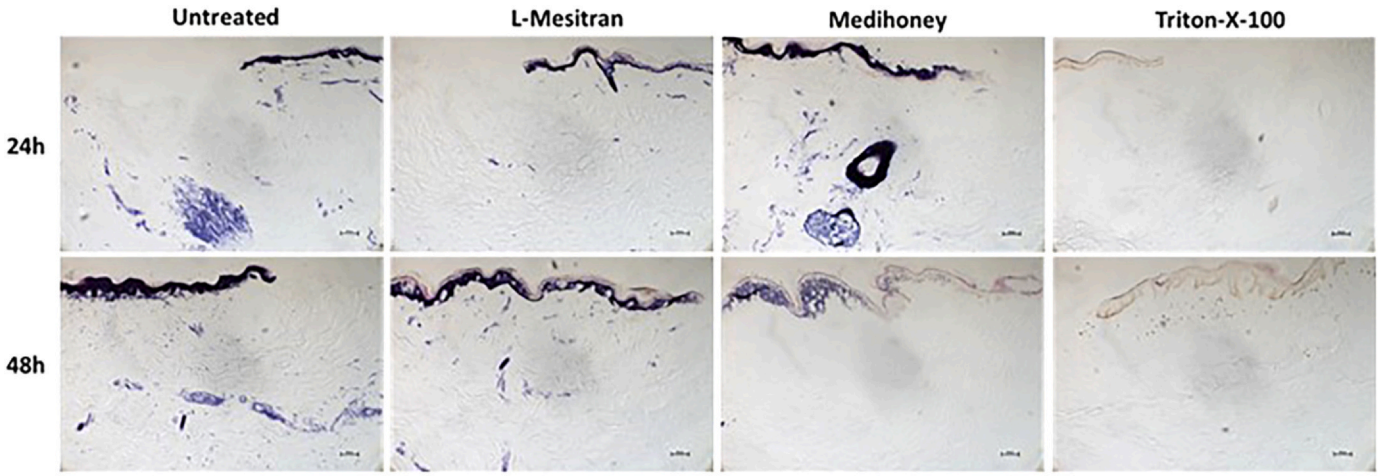

Fig. 3 – Viability burn wound tissue. LDH staining of the *ex vivo* human skin excisional wound model after 24 h and 48 h of treatment with L-Mesitran Soft, Medihoney, or Triton-X-100. Purple-blue staining indicates the viable cells and tissue. Scale = 100 µm.

Case 1. Pseudomonas-infected burn injuries upper body and skin graft wounds leg.

A 71-year-old female patient presented to the clinic with skin graft wounds to her right leg and full-thickness burns to her left arm, left side of her torso, chest, and abdomen (Fig. 4). The burns were caused by a cooking accident in which her nightgown caught fire. Initially, the patient was sent to a private hospital for emergency treatment. After being released, she had outpatient treatment at a private wound clinic but showed no improvement. The patient was subsequently admitted to a government hospital, where a skin graft was performed to patch the defect areas using skin from her right thigh. The wounds were treated with povidone-iodine and sterile gauze, but both the burn injuries and the skin graft wounds failed to heal. The patient scored 7 on the Visual Analogue Scale (VAS) indicating she was in a lot of pain, had financial issues, and lacked social support. Based on the particular discharge and malodor an infection with *P. aeruginosa* was suspected. Both the wounds at the donor site and the burn injuries were present for 3 months at the time that the MGH treatment was initiated. L-Mesitran Ointment, Soft, Tulle, L- Foam, Border, and Active were combined with secondary silicone and foam dressings. Dressings were changed by the wound care specialist in the clinic once a week. Initially, the wounds were treated with a wound contact layer and L-Mesitran Ointment (Fig. 4A, D). Each week, the wounds improved, i.e. showing reduced signs of infection and decreased wound size. Moreover, the patient experienced less pain, scoring 4 on the VAS, and treatment was continued with L-Mesitran Ointment, Soft, and Tulle. After 8 weeks of treatment, the burn wounds showed a marked increase of granulation tissue and re-epithelialization, while the donor site on the leg was almost fully healed (Fig. 4B, E). After 3

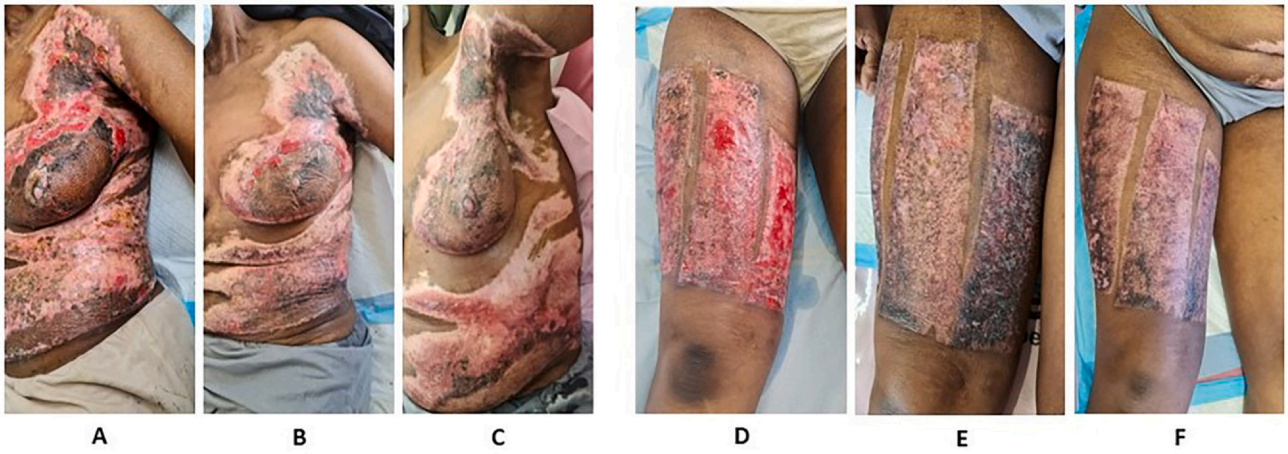

Fig. 4 – Case 1: Infected burn injuries on the upper body and infected skin graft wounds on the right thigh. Wounds at the start of the MGH treatment (A, D), at the 8 weeks follow-up (B, E), and after 13 weeks during the final treatment (C, F).

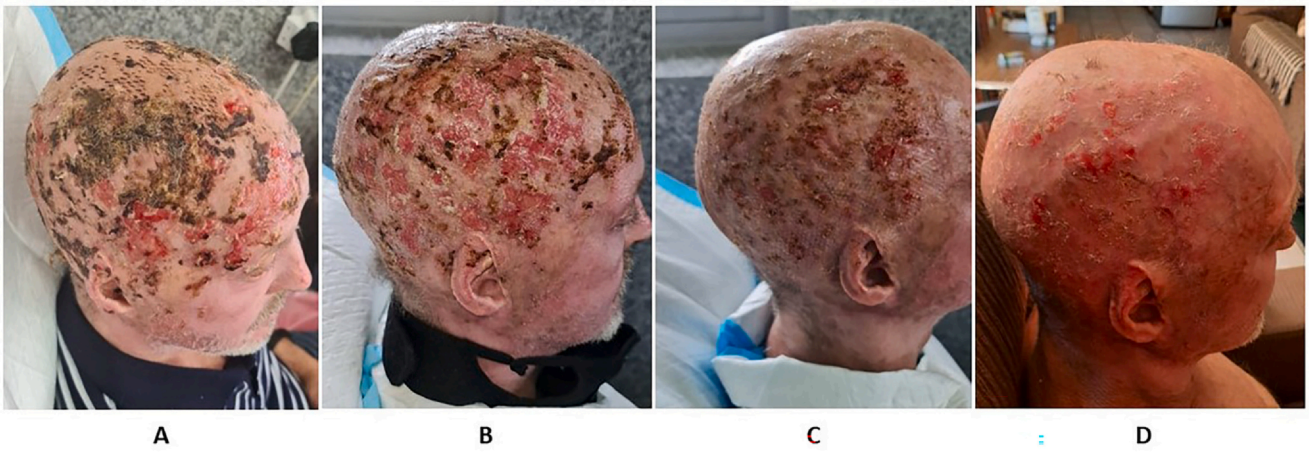

Fig. 5 – Case 2: Infected burns head. A. Wound upon presentation to the clinic and at the start of the MGH treatment. B, C. Clear wound progression after 10 (B) and 24 (C) days. D. Complete healing of the wounds after 56 days of MGH therapy.

months of treatment, the patient scored 0 on the VAS and the wounds were fully healed without any further complications (Fig. 4C, F). The total number of dressing changes was 14.

Case 2. Pseudomonas-infected burn wounds head.

A 57-year-old male patient presented to the clinic with full-thickness burn wounds to his head (Fig. 5). The patient was a smoker and had a history of stage IV melanoma on his nose. He initially sustained burn injuries of various degrees all over his body, which were acquired when an electrical transformer exploded near the patient. The patient caught fire, extinguished the flames with water, and subsequently went to a private hospital for emergency treatment in the intensive care unit (ICU). He was kept in a coma for 3 weeks during which he underwent surgical intervention for serial debridement and skin grafts. Due to the patient's lack of movement, most of his burn injuries healed during his time in the ICU. He was discharged from the ICU to a rehabilitation facility 2 months after the incident. In the rehabilitation center, the patient received physiotherapy for approximately 2 weeks. 2.5 months after the incident, only the wound on his head had failed to heal, so the patient sought help in a wound clinic for MGH treatment. A swab test and culturing confirmed infection with *Escherichia coli* and *P. aeruginosa*. The wounds were treated with L-Mesitran Ointment, Soft, and Tulle on a roll. These products were combined with charcoal, alginate, and silicone dressings. Dressings were changed by the wound care specialist in the clinic twice a week. Upon presentation, the patient's hair was clipped and he was treated with L-Mesitran Tulle on a roll and Ointment combined with an alginate dressing (Fig. 5A). The patient was in moderate pain and he scored 5 on the VAS. He was collaborating well with the treatments prescribed by the healthcare professional and had no financial or social problems. The patient responded well to the MGH products and showed reduced signs of infection and improved healing during each dressing change. Wound debridement, granulation, and re-epithelization became evident after 10 days (Fig. 5B). Treatment continued and the wounds were almost completely healed after 24 days (Fig. 5C). The patient then traveled to another destination for vacation, and he continued the MGH treatment by himself. 56 days after the start of the therapy, the wounds on his head were fully closed, showing minimal scarring, and he scored 0 on the VAS (Fig. 5D).

Case 3. Pseudomonas-infected skin graft wounds on both legs and burn injuries on upper body.

A 27-year-old male patient presented to the clinic with infected burn wounds on his upper body and infected skin graft wounds on both of his thighs (Fig. 6). The patient was steaming his sinuses when he was accidentally pushed into boiling water, which resulted in burn wounds covering large parts of his upper body. He received emergency care at a government hospital, where he also underwent skin graft surgery. Afterward, the patient was transferred to a government clinic for post-hospital wound care where the wounds were treated with povidone-iodine. However, the wounds showed no progression in healing. Upon presentation, the patient was in a lot of pain, scoring 9 on the VAS, and suffered from anxiety. Wounds in both locations showed signs of infection, including a high amount of exudate, pus, and a strong malodor. Based on the symptoms, infection with *Enterococcus faecalis* and *P. aeruginosa* was suspected. The wounds were present for 7 months at the time that the MGH treatment was started. The wounds were treated with L-Mesitran Ointment, Soft, and Tulle combined with cellulose non-woven and aluminized non-adherent dressings. Dressings were changed by the wound care specialist in the clinic twice a week. The patient received analgesic medication for the pain. Initially, the wounds were treated with L-Mesitran Ointment and an aluminized non-adherent dressing (Fig. 6A, D). After each dressing change, the wounds slowly improved and the infection gradually resolved. 1 month after the start with the MGH products, the wounds showed increased granulation and epithelialization tissue (Fig. 6E). The burn injury on his torso also showed improvements, as it was decreased in size and redness (Fig. 6B). Moreover, the wounds were debrided and had a healthier wound bed. Due to these improvements, but also due to the patient's financial

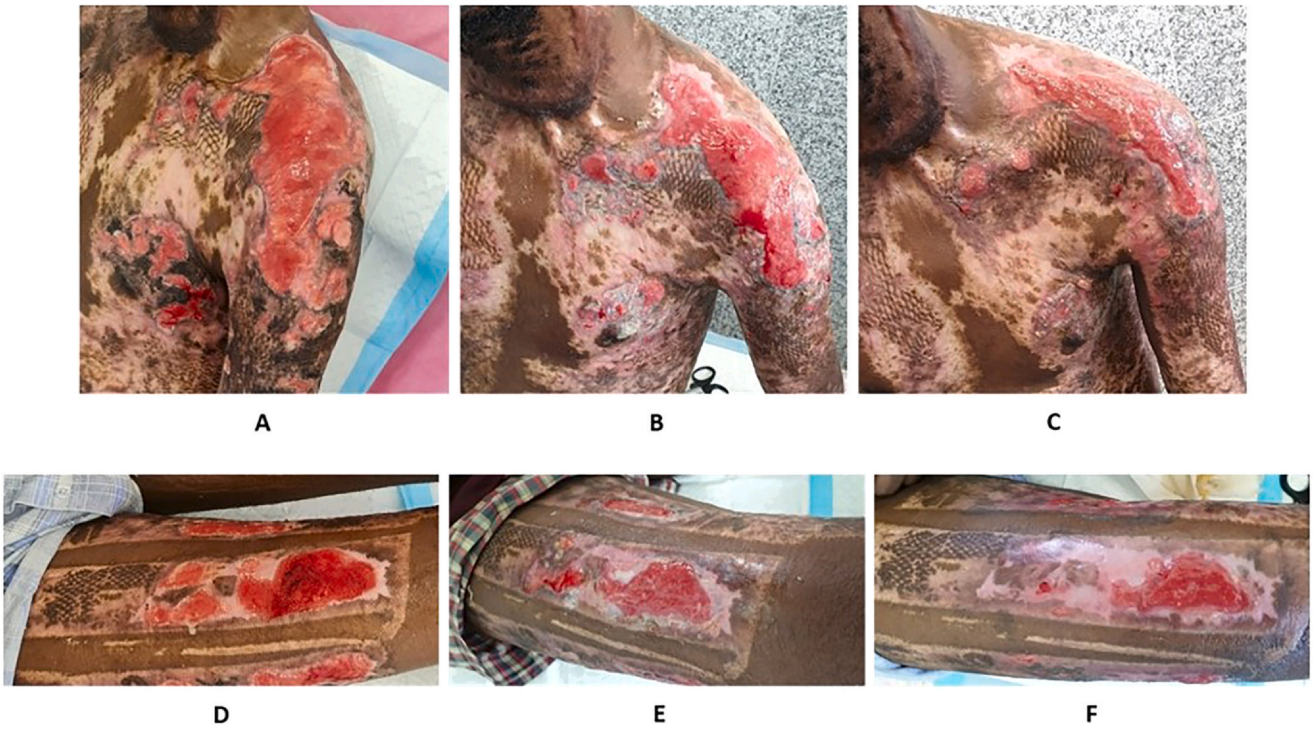

Fig. 6 – Case 3: Infected burn injuries upper body and skin graft wounds right thigh. Wounds at the start of the MGH treatment (A, D). Clear wound progression after 1 (B, E) and 2 months (C, F) of MGH therapy.

situation and transportation, dressing changes were switched to once a week instead of twice a week. Both the burn injuries on his torso and the skin graft wounds showed a significant reduction in size, redness, and exudate after 2 months of treatment (Fig. 6C, F). The patient's quality of life had massively improved as the wound was not malodorous anymore and the pain lessened. The patient did not need analgesics anymore and he scored 0 on the VAS.

Case 4. Pseudomonas-infected burn injury to the foot in a patient with peripheral neuropathy.

A 39-year-old male patient presented to the clinic with a full-thickness burn injury to his right foot (Fig. 7). The patient suffered from peripheral neuropathy caused by type I diabetes mellitus. He sustained his injury while he was taking a foot bath, as the patient did not realize that the water was too hot due to his peripheral neuropathy. After noticing that his foot was swollen, he sought help and got treated by his general practitioner. The patient received treatment with paraffin gauze, povidone-iodine, and oral amoxicillin and clavulanate, but the wound showed no improvement. Two weeks after the injury occurred he was referred to the wound clinic for treatment with MGH. L-Mesitran Soft, Tulle, and Hydro were combined with non-woven swabs and zinc paste bandages. Dressings were changed twice a week by the wound care specialist. Upon presentation, the skin was detached from the underlying tissue (Fig. 7A). Clinical signs of a *Pseudomonas aeruginosa* infection included green exudate and a distinct malodor. Treatment with MGH was started and after 2 days the skin could be removed surgically. After one week of MGH treatment, the wound bed showed more healthy granulation tissue (Fig. 7B). Due to the notable progress observed in the wound after three weeks of treatment, the frequency of dressing changes was reduced from twice a week to once a week. After one month of treatment initiation, a significant reduction in wound size and redness was observed (Fig. 7C). Subsequently, after 2 months the wound achieved complete healing, greatly enhancing the patient's quality of life as reported by the patient himself (Fig. 7D). He received treatment 12 times in total. Notably, a one-year follow-up examination indicated minimal scarring and almost full restoration of pigmentation (Fig. 7E).

Case 5. Staphylococcus-infected burn injuries breast.

An 82-year-old female patient presented to the clinic with burn injuries to the right breast (Fig. 8). The injuries were sustained while the patient was cooking and spilled a saucepan of boiling liquids on herself. The patient was wearing a bra, petticoat, dress, and a chef's bib apron. Unfortunately, despite the guidelines of the Ministry of Health, she did not remove the clothes immediately but with a delay of about 1 h, and therefore the long contact produced partial-thickness and some full-thickness burns. The total surface area of the burn includes the lower quadrants of the right breast, sparing the nipple and 2/3 of the areola. One day after the incident, the patient sought help at the local hospital's ambulatory setting dedicated to burns and complex wounds, and treatment with MGH was started. Clinical signs of infection included local pain, strong exudation, enlarged axillary lymph nodes, and perilesional hyperemia. A wound swab and

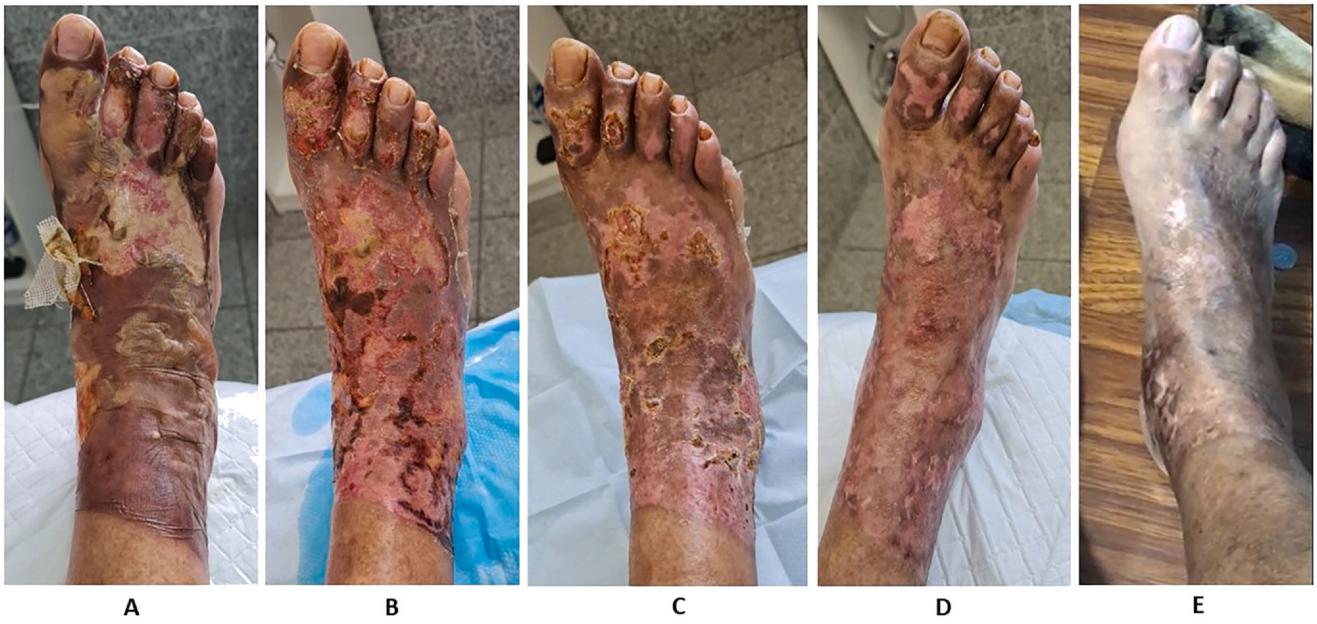

Fig. 7 – Case 4: Burn injury foot in a patient with peripheral neuropathy. A. Wound upon presentation to the clinic and at the start of the MGH treatment. B. 1 week after the start of the MGH therapy, the wound was almost fully debrided. Clear wound progression after 1 (C) and 2 months (D) of MGH therapy. E. 16 months after the start of the MGH treatment, the wound showed minimal scarring and pigmentation had returned.

bacterial culture confirmed infection with methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermidis*. Wounds were first cleansed with cold saline solution and disinfected with a compress of TNT-gauze embedded in polyhexanide dimethylbiguanide, followed by gentle soft debridement using Cutimed Debri-Clean Essity. L-Mesitran Soft was subsequently applied as a primary dressing combined with L-Mesitran Foam and Hydro as secondary dressings. Dressings were changed every 2–3 days by the wound care specialist at the hospital. Upon presentation the wound showed black necrotic tissue (Fig. 8A), which was successfully debrided after 5 days of treatment with MGH (Fig. 8B). 11 days after the start of the treatment, the wound was debrided for 75% and the lesion area was reduced by 50%, clearly showing granulation and re-epithelialization (Fig. 8C). The wound was fully debrided after 14 days of MGH treatment and all signs of infection were resolved (Fig. 8D). Due to the progression of the wound, dressing changes were switched to once a week with L-Mesitran Hydro. The patient fully recovered 22 days after the start of the treatment without experiencing side effects or itching, the latter usually occurring very frequently (Fig. 8E).

Case 6. Abdominal Staphylococcus-infected burn injury in a pediatric patient.

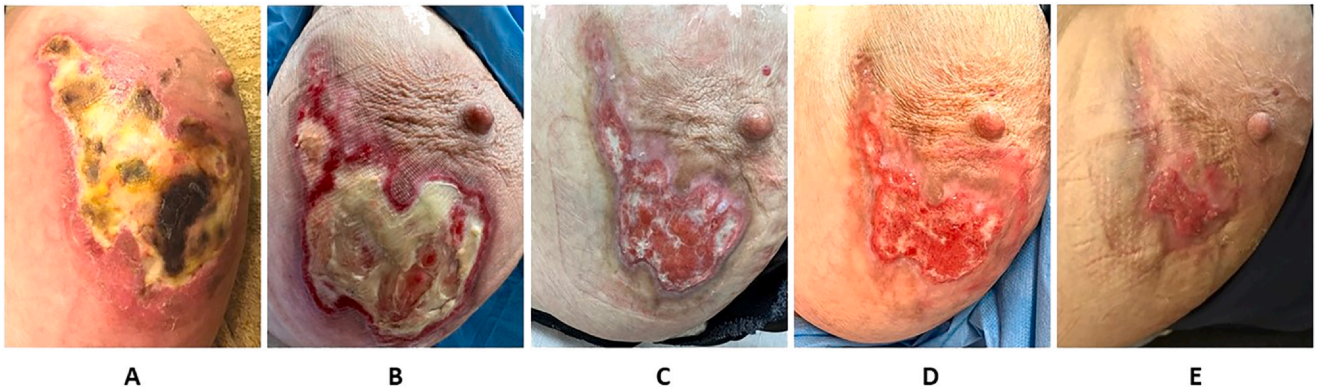

Fig. 8 – Case 5: Infected burn injuries breast. A. Wound at the start of the MGH treatment. B. 5 days after the start of the MGH therapy, the wound showed debridement of the black necrotic tissue. C. Clear wound progression after 11 days of treatment, showing almost full debridement and reduction of lesion area. D. Complete debridement and sterilization of the wound were achieved after 14 days of MGH therapy. E. 22 days after the start of MGH therapy, the wound was fully healed.

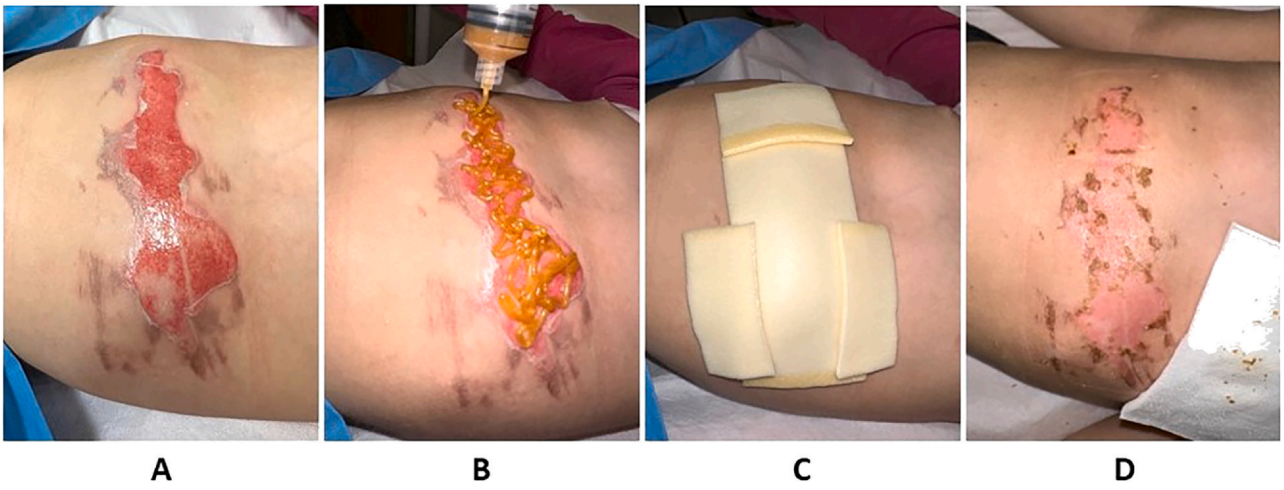

Fig. 9 – Case 6: Abdominal infected burn injury in a pediatric patient. A. Wound at the start of the MGH treatment. Application of L-Mesitran Soft (B) and Foam to the wound area (C). D. 11 days after the start of the MGH treatment the wound was fully healed.

A 3-year and 6-month-old female patient presented to the clinic with an abdominal burn injury (Fig. 9). The wound was sustained after boiling water was spilled onto her. The folds of her clothes were curled up by a waist belt and kept the hot liquids in contact with the skin surrounding the lower part of the chest and the upper third of the abdomen. This skin area contains particularly fragile and delicate skin, also because it has no hairs as appendages, and the prolonged contact with the hot fluids resulted in superficial and deep partial-thickness burns. The patient was treated at home with silver sulfadiazine and paraffin gauze at home after which she was transferred to the burn center of the local pediatric hospital. The patient was treated with painkillers and her parents received immediate medical and psychological counseling. The burned region was transverse and 10×5 cm in size, and appears as a large, already burst blister (Fig. 9A). Clinical signs of infection included local pain and high amounts of exudate. A wound swab and bacterial culture confirmed infection with MRSA. L-Mesitran Soft was applied as a primary dressing combined with L-Mesitran Foam secondary dressings (Fig. 9B, C). Dressings were changed every 3–4 days by the wound care specialist at the burn center. During each dressing change the wound showed marked improvements and after 3 dressing changes, the wound was fully healed and tested negative for infection (Fig. 9D). The total healing time for the patient was 11 days and no complications occurred due to the MGH treatment.

4. Discussion

In this study, we made use of an *ex vivo* human burn wound model to study the effects of MGH- and silver-based wound care products on *P. aeruginosa* infection and skin re-epithelialization. Medihoney and L-Mesitran Soft served as the MGH-based therapies, whereas Flammazine and $AgNO_3$ were indicative of the silver-based treatments. Flammazine, $AgNO_3$, and L-Mesitran Soft all reduced the *P. aeruginosa* infection to below detection levels, while Medihoney exerted significantly lower antibacterial effects. Moreover, Medihoney treatment resulted in a complete lack of re-epithelialization and proliferation of keratinocytes, while L-Mesitran and Flammazine only slightly inhibited re-epithelialization. Additionally, during each experiment, the individual ingredients of L-Mesitran Soft and the mix without honey contributed most to its effects. Lastly, the presented burn wound cases highlighted the effectiveness of L-Mesitran products in the clinic.

MGH has been compared to silver sulfadiazine before in both *in vitro* and clinical settings [17,27]. In an *ex vivo* human burn wound model, the application of L-Mesitran Soft resulted in significantly more re-epithelialization compared to silver sulfadiazine, while exerting similar antimicrobial effects against *P. aeruginosa*[17]. Remarkably, the re-epithelialization effects of L-Mesitran Soft and Flammazine in the current study were not significantly different. This could be due to different formulations of Flammazine and slight differences in methods. In the previous study, the burn wounds were treated for 3 weeks while the current study included a treatment period of only 2 weeks. The use of Flammazine and other silver products is usually advised to be used for only 2 weeks, after which non-silver products should be used [12]. The results of both studies combined indeed suggest that although silver sulphadiazine initially has similar effects on re-epithelialization as MGH, it exerts strong cytotoxic effects if used for longer periods. MGH on the other hand can be used for extended periods of time without posing a risk for healthy tissue. Furthermore, in a clinical study by Baghel *et al.*, burn wound patients treated with honey showed a faster healing time than patients who received a silver sulfadiazine treatment [27]. The same study also showed that all infected wounds treated with honey became sterile within 7 days, while none of the silver sulfadiazine-treated wounds tested negative at that time point. MGH should be used as an alternative to silver dressings as silver still holds several other limitations apart from cytotoxicity. For example, silver could result in discoloration of the skin, also known as argyria.

Other skin reactions, such as rash, in response to silver sulfadiazine have been reported and patients with known hypersensitivity are advised to not use silver sulfadiazine creams [28]. Furthermore, infants under the age of 2 months and pregnant or nursing women should refrain from silver sulfadiazine, as systemic adsorption does occur [29]. These skin reactions have not been reported for the use of MGH and MGH can be safely used for all patients, including pregnant and breastfeeding women, newborns, and premature infants [30–32]. Another major implication is that bacterial resistance has been reported for silver dressings, while MGH lacks the risk of resistance [33].

A striking observation in this study is the difference between the two MGH wound care products: Medihoney and L-Mesitran. Medihoney consists of Manuka honey, which is probably the most well-known and used MGH product. This is probably because Manuka honey was the first honey to be extensively investigated for its antimicrobial properties [34,35]. However, over the past two decades evidence has accumulated that honey from other floral sources has similar, if not better, antibacterial effects [18,34,36–39]. The main difference between the two MGH categories is that Manuka honey exerts its antimicrobial effects via methylglyoxal, while the antibacterial properties of other MGHs are attributed to hydrogen peroxide [40]. Current findings are in line with previous studies that compared Medihoney with L-Mesitran Soft. In one *in vitro* study, the antimicrobial activity of the two MGH products was directly compared against a panel of eleven *Staphylococci* and eleven *Pseudomonas* spp. pathogens [34]. L-Mesitran Soft consistently exhibited potent activity and demonstrated greater effectiveness than Medihoney, despite containing half of the honey concentration. Another study compared the anti-biofilm activities of L-Mesitran Soft to several other MGH products, including Medihoney, using an artificial dermis model [18]. L-Mesitran Soft was superior to all other formulations in inhibiting *P. aeruginosa* biofilm formation and eradicating already formed *P. aeruginosa* biofilms. Moreover, Medihoney treatment resulted in a complete lack of re-epithelialization of the burn wound, which was supported by the LDH staining that showed almost no cell viability at the edge of the wound. In line with our data, 3–5% concentrations of Manuka honey were previously found to result in a progressive reduction in the viability of cells [41]. The cytotoxicity of Manuka has been attributed to methylglyoxal [42]. Based on the aforementioned, MGH from other floral sources has not only stronger antibacterial effects but also exert less cytotoxicity.

Honey exhibits bactericidal effects against a wide spectrum of bacteria, including MRSA and *P. aeruginosa*, which depend on the floral source of the honey [18,43–46], via a variety of mechanisms. For instance, honey impairs the bacterial acquisition of iron required by *Pseudomonas spp.* for replication and host infection [47,48]. Additionally, honey can induce structural changes in the bacterial cells, resulting in the loss of integrity and alterations in shape and surface. This effect may be attributed to the upregulation of *algD* and downregulation of *oprF* gene expression, leading to membrane depolarization, increased permeability, and ultimately cell lysis[49–51]. It also inhibits bacterial adhesion to keratinocytes and certain tissue proteins such as fibronectin, fibrinogen, and collagen, thereby diminishing the virulence of wound pathogens [52]. Furthermore, honey suppresses the expression of flagella-associated genes (*fleQ*, *fleN*, *fliA*, *fliC*), resulting in deflagellation of *P. aeruginosa*. Consequently, this leads to decreased motility, adherence, and overall virulence[53].

Another point of discussion is the contribution of the individual components to the antimicrobial effects of L-Mesitran Soft. L-Mesitran Soft is composed of 40% MGH, vitamins C and E, medical-grade hypoallergenic lanolin, PEG 4000, and propylene glycol which all have been described to have antimicrobial effects [18]. For example, in a randomized controlled trial, the addition of vitamins C and E, and PEG 4000 to honey in the treatment of partial-thickness burns resulted in faster healing compared to honey alone [54]. Additionally, the synergistic effects of vitamin C and honey have been described previously. Supplementation of vitamin C to different types of honey was shown to significantly enhance the antibacterial efficacy against *E. coli* and *P. aeruginosa* in a dose-dependent manner [55]. The same study demonstrated that vitamin C could enhance the antibiofilm activity of honeydew honey against a multispecies biofilm, consisting of *S. aureus*, *Streptococcus agalactiae*, *E. faecalis*, and *P. aeruginosa*, within 24 h [55]. These synergistic effects have been attributed to the ability of vitamin C to generate oxidative stress in bacterial cells by intracellular reactive oxygen species (ROS) production. Honey is rich in minerals which might trigger ROS production by vitamin C via the Fenton reaction [55,56]. Other studies comparing L-Mesitran Soft with its raw honey component also demonstrate the enhanced antimicrobial activity by its supplements [57–59].

In the current study, the mix without honey showed the largest contribution to the observed effects on the burn wound model. This is in contrast to the results of a study focusing on the effects of the individual ingredients of L-Mesitran Soft on *P. aeruginosa* biofilm formation [18]. The vitamins, the raw honey, and the mix without honey all showed similar anti-biofilm effects, while in the current study, the mix without honey demonstrated significantly stronger effects than the raw honey or the vitamins alone. One should note that the current study investigated infected burn wounds in *ex vivo* human skin, while the other study focused on biofilms in artificial dermis. Another point to note is the use of different batches of MGH between the two studies. It is known that the biological activity can vary between different batches of honey [60,61]. Nonetheless, several studies showed consistent antibacterial effects of supplemented MGH even though different batches were used [57–59]. This suggests that supplementation of honey could standardize MGH and aid in maintaining a minimal biological activity. Although the mix without honey contributed the most to the effects in the current study, the raw honey still reduced the bacterial load by 1.25 log suggesting a synergistic effect between individual ingredients against *P. aeruginosa* infection in burn wounds.

It is important to note that our study includes only *P. aeruginosa* strain POA1 in the *ex vivo* experiments. We selected this strain because it is extensively characterized, although multiple other *P. aeruginosa* strains are present in clinical settings. Therefore, future studies could include

clinical isolates of *P. aeruginosa* in determining the antibacterial activity of MGH and silver-dressings in an *ex vivo* human burn wound model. However, during the current study we included clinical cases of *P. aeruginosa*-infected burn wounds to highlight the efficacy of MGH against other *P. aeruginosa* strains as well. One should also note that the antimicrobial effects of $AgNO_3$ were dependent on the manner of application during the burn wound infection experiments. For the presented results, $AgNO_3$ was applied on a gauze which it saturated partially to compare to the other treatments. However, this yielded a markedly lower antibacterial effects than expected. Therefore, we repeated the experiment with a completely saturated gauze, which resulted in a similar, expected bacterial count as the Flammazine treatment (data not shown). The oversaturated gauze is not comparable to the other treatments though. This does implicate that $AgNO_3$ solution might be applied in larger amounts or perhaps more frequently than the other methods. Moreover, the LDH staining was not quantified and only the representative pictures were shown. The viability in the dermis top layer and the penetration depth of any cytotoxicity could not be quantified because of irregular presence of fibroblasts in the dermis. Nonetheless, the images were a valuable addition to the quantitative results of the re-epithelialization and keratinocyte proliferation. Finally, since our study only shows clinical cases using supplemented MGH, future studies could include clinical cases that also show the use of silver dressings or compare MGH and silver in burn injuries.

MGH treatment did not cause any adverse effects in the presented clinical cases and no additional discomfort was reported. The patients described in Cases 5 and 6 fully recovered after 22 and 11 days, respectively. Remarkably, the total healing time was 2–3 months for Cases 1, 2, and 4, probably due to the severity and location of the wounds. The healing trajectory of the patient in Case 3 was not concluded after 3 months. This is because he was non-concordant with his treatment and was very active due to his young age. Despite this, the patient's quality of life massively improved as he no longer experienced malodor and pain. The MGH completely resolved the infections in all cases even though the exact bacterial strain was not quantified in each patient with a swab. In a previous study by Papanikolaou *et al.* hard-to-heal heel pressure ulcers were treated with MGH [62]. Similar to Cases 1 and 3 of the current study, infection was determined by clinical signs, not via wound swabs. Independent of the pathogen in the wound, the MGH treatment completely resolved the infection within 2–3 weeks. Both the case series and the current *ex vivo* study highlight the effectiveness and broad-spectrum antimicrobial activity of MGH.

Multiple studies have previously reported the use of honey-based products for treating wounds and burn injuries [27,32,63,64]. MGH products have been used to successfully treat burn wounds in young children, ranging from the age of 8 months to 15 years [32,63]. Smaropoulos *et al.* treated pediatric burns with MGH-based ointments and dressings [32]. In one case in particular, the patient suffered from an extensive burn with a total body surface area of 35%. Only the burn wound on the patient's leg was treated with MGH, while the other burn wounds on her body were treated with skin grafts and povidone-iodine. Interestingly, the wounds treated with MGH healed markedly faster than the wounds treated with conventional methods, underlining the pro-healing activities of MGH. The same study also included the participation of parents in the nursing process at home. The parents responded positively, reporting that the MGH treatment was easy to apply and stress levels were reduced for their children. Boggust *et al.* made use of MGH-based hydrogels in a series of pediatric burns [63]. The use of the MGH dressings reduced erythema and pain and prevented infection in all cases. Clinicians, parents, and patients provided overall positive feedback. In both studies, the children recovered fast, and the MGH-based treatment was cost-effective and patient-friendlier than the conventional therapy.

5. Conclusion

Overall, supplemented MGH can be used to treat non-healing, infected burn wounds and skin graft-acquired wounds effectively due to its broad-spectrum antimicrobial and pro-wound healing properties. Nevertheless, the type of MGH should be carefully selected as L-Mesitran Soft exhibited significantly stronger antibacterial properties compared to Medihoney, and Medihoney was found to be cytotoxic. L-Mesitran Soft was shown to be similar to silver-based products with regards to antibacterial effects and epidermal viability. However, longer use of silver-based products would lead to decreased epidermal regeneration as opposed to long-term treatment with L-Mesitran. Therefore, supplemented MGH could be recommended as a first-line treatment for severe, *P. aeruginosa*-infected burn wounds.

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CRediT authorship contribution statement

Bouke K.H.L. Boekema, Niels A.J. Cremers: Conceptualization, Resources. **Bouke K.H.L. Boekema, Daniela Chrysostomou, Guido Ciprandi, Niels A.J. Cremers**: Methodology. **Bouke K.H.L. Boekema, Daniela Chrysostomou, Guido Ciprandi, Anouk Elgersma, Marcel Vlig**: investigation. **Bouke K.H.L. Boekema, Linsey J.F. Peters, Niels A.J. Cremers**: Writing – original draft preparation. **Bouke K.H.L. Boekema, Andrea Pokorná, Linsey J.F. Peters, Niels A.J. Cremers**: Writing – review & editing. **Bouke K.H.L. Boekema, Daniela Chrysostomou, Guido Ciprandi, Linsey J.F. Peters**: Visualization. **Bouke K.H.L. Boekema, Andrea Pokorná, Niels A.J. Cremers**: Supervision. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

L.J.F.P and N.A.J.C. are employed by Triticum Exploitatie BV, Maastricht, the Netherlands. However, they were not involved in the design, treatment, and presentation of the results. All other authors declare no conflict of interest.

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Consent

Prior to their inclusion in the study, the patients or their legal representatives were provided with comprehensive information about the research, and they provided written consent. The study adhered to the principles outlined in the Declaration of Helsinki by the World Medical Association and were approved by local ethical boards without the special number identification.

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