Anhydrous Topical Ointment for Management of Infected Wounds

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Short Report

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Abstract

Optimal prevention of microbial infection at the wound site is a priority clinical consideration required to facilitate normal healing process. Topical antimicrobials are commonly used for treatment and prevention of local wound infections. Herein, an anhydrous, hydrophilic, non-greasy, and non-occlusive antimicrobial ointment was formulated by incorporating two main active ingredients, CuO_{(1-x)}ZnO_{x} and (±)-α-bisabolol. CuO_{(1-x)}ZnO_{x} nanocomposite was added to provide a broad-spectrum antimicrobial activity through the release of reactive oxygen species. (±)-α-Bisabolol was added to expedite the wound healing process. Prepared ointment showed very high killing efficacy against a spectrum of bacteria (6Log reduction) and a pathogenic yeast (5Log reduction) within 2h of exposure. Results described here would be of great benefit to professionals as well as for personal use in treating infected wounds.

Introduction

Skin forms the first line of defense against potential injuries and microbial attack. Once skin integrity is compromised due to traumatic or surgical events, skin regeneration occurs following an orderly healing process.\(^1\) Wound healing is an intricate physiological process that is exemplified by a series of four overlapping cellular phases of hemostasis, inflammation, proliferation, and remodeling.\(^2\) However, on some occasions, the regular healing process is impaired and wounds fail to heal in a timely manner.\(^3\) Such wounds failing to heal within 12 weeks period are called chronic wounds. Treatment of chronic wounds is complex and poses a substantial socio-economic burden on the patients.\(^4\) Often, persistent microbial infection and prolonged inflammation are the critical challenges for effective treatment of chronic wounds.\(^5\) To address the current challenges in the field, significant research efforts from both academia and industries are directed towards deeper understanding of wound pathology and treatment optimization to enhance the healing process.

Generally, the medical practitioners advice antibiotics when the classical symptoms of infection are evident. However, inappropriate and excessive use of antibiotics was followed by a rapid emergence of resistant strains of pathogens to various traditionally administered antibiotics.\(^6\) Resistance to conventional antibiotics can be deterrent to usual wound treatment procedures especially threatening the lives of elderly and patients with comorbidities such as diabetes or obesity.\(^7\) Furthermore, compromised vasculature around the wound site can prevent efficient delivery of systemically administered antibiotics. Under such circumstances, topical application of antimicrobials that are effective only in the vicinity of the compromised area is recommended.\(^8\) Silver salts (AgNO\(_3\) and silver sulfadiazine) and silver nanoparticles (Ag NPs) are widely used antimicrobial agents in topical formulations.\(^9\) Many silver containing commercial products are available in the form of creams, ointments, gels, sprays, and wound dressing patches.\(^10\) Silver agents exerts microbiocidal activity through the release of Ag\(^+\) ions, which denatures proteins and disrupt cell wall and membrane functions.\(^11\) However, there are serious concerns regarding cytotoxicity resulting from Ag\(^+\) ions entering the systemic circulation and depositing on internal
tissues and organs. Therefore, effective antimicrobials alternative to silver for topical applications are pursued.

To this end, we report the production of an anhydrous, hydrophilic, non-greasy, and non-occlusive, topical ointment formulation comprising of CuO$_{(1-x)}$ZnO$_x$ nanocomposite as an antimicrobial additive, that is suitable for professional and personal use. The ointment can be directly applied to the wound area, or it can be applied to a bandage that is later reinforced on to the injury site. Components of the ointment were carefully selected to provide coverage of a broad spectrum of activities including antimicrobial, antioxidant, and anti-inflammatory effects.

**Results And Discussion**

The CuO$_{(1-x)}$ZnO$_x$ nanocomposite is a broad-spectrum antimicrobial additive that was described in the previous report. The nanocomposite is composed of primary nanocrystallites of Zn$^{+2}$ ion doped CuO ($\sim$15 nm) phase and pure ZnO ($\sim$10 nm) phase. It exhibited strong antibacterial activity in suspension and when incorporated in medical gauze and acrylic paints against various bacterial species such as *Escherichia coli*, *Listeria monocytogenes*, Methicillin-Resistant *Staphylococcus aureus* (MRSA) and *Salmonella enterica* serovar Typhimurium.

To produce a semi-solid ointment formulation incorporating the CuO$_{(1-x)}$ZnO$_x$ antimicrobial nanocomposite that is also suitable for application over open wounds and burns, several non-occlusive vehicles were initially screened. Such examples include an anionic o/w emulsion based on Novo-Base II, a non-ionic neutral o/w emulsion, and a fat reduced emulsion in gel. However, in all three emulsions no antimicrobial activity was detected. Therefore, a new formulation composed of hydrophilic ointment base was formed by mixing different molecular weight polyethylene glycols and other functional additives that presented high activity and stability was tested (Experimental).

Active ingredients in the formulation were CuO$_{(1-x)}$ZnO$_x$ nanocomposite (0.2%) and (±)-α-bisabolol (1%). CuO$_{(1-x)}$ZnO$_x$ was used to provide a broad spectrum of antimicrobial protection. (±)-α-Bisabolol a natural monocyclic sesquiterpene that is known to possess antioxidant, anti-inflammatory, antimicrobial, and regenerative properties to accelerate the wound healing process. Other ingredients, polyethylene glycol (PEG), polysorbate 80 (non-ionic surfactant), capric triglyceride (lipid material), and glycerin were added to enhance other physical properties such as hydrophilicity, spreadability, stability and homogeneity, moisturizing, and water retention at the wound bed. Shown below is a picture of the prepared ointment with homogeneously dispersed antimicrobial nanocomposite (Fig. 1).

In our study, we selected three representative clinically relevant bacterial strains, *E. coli*, *Pseudomonas aeruginosa*, MRSA, and a pathogenic yeast species, *Candida albicans*, to evaluate the antimicrobial performance of the ointment (Table 1). Antimicrobial tests were performed according to ASTM E2315, a standard test for assessment of antimicrobial activity using a time-kill procedure (a detailed protocol is provided in the experimental section). The ointment exhibited a very high antimicrobial efficacy against
bacterial species (6Log reduction) and against the yeast (5Log reduction) (Table 1). The high antimicrobial efficacy of the ointment is attributed to the CuO\(_{(1-x)}\)ZnO\(_x\) nanocomposite additive. We have previously proposed the mechanism for high bactericidal activity of the nanocomposite as governed by a synergetic combination of electrostatic interaction of nanocomposite with bacterial cell envelope and simultaneous generation of reactive oxygen species (ROS) such as hydroxyl radical (-OH). High concentrations of the ROS damage the functions of lipids, proteins, and DNA to ultimately leading to cell death.\(^\text{13}\)

| Microbial species | Average log reduction |
|-------------------|-----------------------|
| Gram negative     |                       |
| E. coli           | 5.9                   |
| P. aeruginosa     | 6.3                   |
| Gram positive     |                       |
| MRSA              | 6.4                   |
| Yeast             |                       |
| C. albicans       | 4.8                   |

### Conclusions

We reported a successfully preparation an anhydrous, hydrophilic, and non-occlusive antimicrobial ointment formulation comprising of CuO\(_{(1-x)}\)ZnO\(_x\) and (±)-α-bisabolol as active ingredients. Choice of an appropriate ointment base that is based on PEGs and other additives was crucial to get high efficacy against the pathogens, as there was no observed antimicrobial activity in the ointments prepared using other types of bases. Ointment exhibited high efficacy (5 to 6 Log reduction) against clinically relevant Gram-negative bacterial species (E. coli, P. aeruginosa), Gram-positive bacterial species (MRSA) and yeast (C. Albicans). High antimicrobial efficacy of the ointment is attributed to electrostatic interaction between CuO\(_{(1-x)}\)ZnO\(_x\) nanocomposite and bacterial cell envelope and simultaneous generation of ROS. Ointment reported here could be useful either in the hospital setting or personal use for treating infected wounds and to accelerate the healing process.

### Experimental

**Chemicals**: All the chemicals excluding CuO\(_{(1-x)}\)ZnO\(_x\) were purchased from commercial sources and used without further purification. (±)-α-Bisabolol (TCI), PEG-400 (TCI), PEG-4000 (TCI), capric triglyceride (Spectrum Chemical), polysorbate 80 (Spectrum chemical). Synthesis of CuO\(_{(1-x)}\)ZnO\(_x\) was described in a previous publication.\(^\text{13}\)

**Preparation of ointment**: Ointment was produced in two steps. In the first step, 1% CuO\(_{(1-x)}\)ZnO\(_x\) suspension was prepared in PEG-400 using a homogenizer. In the second step, PEG-400 (572 g) and PEG-
4000 (200 g) were mixed at 70 °C and to this added, capric triglyceride (8g), Tween 80 (8g), and (±)-α-bisabolol (10g) while continuously mixing at 70 °C for 30 min. Finally, added 1% CuO(1-x)ZnOₓ PEG-400 (200 g) suspension and mixed for additional 60 min. Brown semi-solid formulation was obtained after slowly cooling the above mixture.

**Antimicrobial activity**: Antimicrobial test was based on ASTM E2315-16. Inoculum was grown from -80°C in 40 mL TSB (Tryptic Soy Broth, Himedia, Mumbai, India) in a 50mL conical tube for 24h @36°C, 100 RPM in a shaker-incubator (ES-60, MIULAB). Cells were washed twice (3000g, 5 min) in saline and concentration estimated using a nephelometer (PhoenixSpec, BECTON DICKINSON, Israel). Bacterial suspension and inoculum adjusted in saline (0.86% NaCl) containing 5% FBS (Fetal Bovine Serum, Biological Industries, Beit Haemek, Israel) to 2x10⁸ cfu/mL.

9.5g ointment was mixed manually to homogenization with 0.5mL FBS to simulate a protein-rich wound exudate environment. After 2h at room temperature the ointment/5% FBS mixture was inoculated with 0.5mL inoculum and vortexed to homogenization. At T₀ and after 2h incubation @30°C, samples of 1g were weighted in a 15mL conical tube and added with 9mL saline. Homogenization was reached after ~30sec vortex, and this “-1” dilution was further serially diluted to “-5” dilution. Using the pour plate method, each dilution was plated in duplicate in molten TSA (Tryptic Soy Agar, HiMedia, Mumbai, India) @45±2°C. Plates were incubated @36°C for 48h (bacteria) of 72h (C. albicans).

When running the test for *C. albicans* the addition of FBS was omitted, both in the inoculum (saline only) and in the test article (10g ointment, w/o FBS).

For calculation of log₁₀ reduction, inoculum count was performed by plating dilutions of the inoculum in TSA as described above. Average duplicate plate counts of the inoculum and counts (T₀/T₂) were multiplied by the corresponding dilution factor and converted to log₁₀ scale. Log₁₀ reduction was calculated by subtraction of log₁₀(Tₓ) from log₁₀(inoculum).

**Declarations**

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**Author contributions**

R.A.S analyzed the data and wrote the manuscript. B.L conducted antibacterial activity experiments. A.F designed and conducted the experiments and supervised the project.

**Competing interests**
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**Figures**

![Image of ointment](image)

**Figure 1**

Ointment formulation incorporating antimicrobial CuO(1-x)ZnOx nanocomposite.