Antibacterial activity and hemocomptability of gelatin and metal oxide for wound healing applications

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ABSTRACT:

Background:

Skin heals normally through the natural skin restoration phenomenon. However, many challenges overcome the proper and rapid wound-healing process. Therefore, it is highly recommended to always develop and use a proper wound dressing that can act immediately to prevent microbial infection and protect the wound. Copper is a trace mineral essential for many wound healing-related processes as Copper stimulates angiogenesis, vascular endothelial growth factor (VEGF) and stimulation of secretion of fibrinogen. Gelatin (GL) is another naturally derived polymer that possess high biodegradability, low irritability, immunogenicity, and antigenicity.

Aim:

To evaluate the Antibacterial activity and hemocomptability of gelatin and metal oxide for wound healing applications.

Materials and methods:

0.3g of Gelatin was dissolved in 50ml of distilled water and stored in 60 degrees for 2 hours.

0.05g of Cu was added to the above solution and stirred for 30 mins.

It was then cooled in -80 degrees Celsius and lyophilized for 24 hours to get a film.

Antimicrobial activity of sample was tested against Staphylococcus aureus and E. coli using Mueller Hinton Agar and the zone of inhibition was measured against the standard(amoxicillin).Hemocompatability assay: The test samples were mixed with 200 µl of RBC solution and final reaction mixture volume was made up to 1 ml by adding sodium phosphate buffer. The reaction mixture was centrifuged again at 2500 rpm for 5 min. The supernatant absorbance was measured at 492 nm and 595 nm keeping sodium phosphate buffer as blank and hemolysis percentage was found.

Results:

The antibacterial action was shown in the form of zone of inhibition. In this study It was observed that the Gelatin and CuO sample exhibited strong activity against E.coli showing the zone of inhibition of 19mm at the dose of 100 uL, while for Staphylococcus aureus the zone of inhibition at 100uL was 14mm.

It was also found that the sample showed less than 5% of hemolysis, therefore indicating that the given sample is hemocompatable. A previous study also concluded that the GNS-reinforced GL could be used as promising base materials for rapid wound-healing purposes.

Conclusion:

The result in the present study obviously declares that natural products are more hemocompatible by displaying lysis less than 5% in concentration range. The extent of hemolysis is an important parameter of toxicity of natural products to the erythrocytes. The given sample also shows antimicrobial activity demonstrating that the Gelatin and copper oxide sample is effective in wound healing.

INTRODUCTION:

Skin is highly susceptible to damage and loss due to some skin diseases, injuries, and accidents. Skin can heal normally through the natural skin restoration phenomenon. However, many challenges overcome the proper and rapid wound-healing.(1) These include possibility of microbial infection, moisture loss from wounds, and scar formation. Consequently, the healing time increases, leading to increasing the risk of more complications of wounds, such as microbial contamination, gangrene, and sepsis, especially in diabetic patients. (2)Wound healing is a complex biological process crucial for tissue repair and regeneration. In recent years, the development of biomaterial-based approaches has gained attention for their potential in enhancing wound healing outcomes. (3)Among these biomaterials, the combination of gelatin, a natural biopolymer, with various metal oxides has emerged as a promising strategy due to its multifaceted properties.(4)

Gelatin (GL) is another naturally derived FDA-approved polymer that possess high hydrophilicity, biocompatibility, and biodegradability and low irritability, immunogenicity, and antigenicity.(3) In addition, it has no carcinogenicity or toxicity. GL is produced through either alkaline or acid hydrolysis of collagen. Due to the presence of several functional groups on its surface, GL is easily chemically modified or cross-linked to other molecules or ligands. Finally, it enhances fluid diffusion and cell proliferation within its structure(5). When combined with specific metal oxides such as zinc oxide, copper oxide, or silver oxide, gelatin-based composites exhibit enhanced antibacterial properties(6). This attribute is vital for preventing and managing infections commonly associated with wounds.Moreover, these gelatin-metal oxide composites demonstrate favorable hemocompatibility, ensuring minimal adverse effects on blood components upon contact.(7) This characteristic is crucial in wound management, as it allows for the promotion of tissue repair while maintaining a suitable environment for blood circulation and clotting.(8)

The unique combination of antibacterial activity and hemocompatibility makes gelatin-metal oxide promising candidates for wound dressings, scaffolds, or other wound care applications. (9)These materials not only provide a barrier against microbial colonisation but also support tissue regeneration, ultimately contributing to improved wound healing outcomes(10). Hence the aim of this study is to evaluate the Antibacterial activity and hemocomptability of gelatin and metal oxide for wound healing applications.

MATERIALS AND METHODS:

Preparation of Gelatin-Metal Oxide Composite:

0.3g of Gelatin was dissolved in 50ml of distilled water and stored in 60 degrees for 2 hours. 0.05g of Cu was added to the above solution and stirred for 30 mins. It was then cooled in -80 degrees Celsius and lyophilized for 24 hours to get a film.

Antibacterial Assay:

- Antimicrobial activity of gelatin metal oxide composite was tested against the strain Staphylococcus aureus, E.coli. Mueller Hinton Agar was utilized for this activity to determine the zone of inhibition. Mueller hinton agar was prepared and sterilized for 15 minutes at 121°C. Media poured into the sterilized plates and let it stable for solidification. The wells were cut using a 9mm sterile polystyrene tip and the test organisms were swabbed. The sample with different concentrations (25µL, 50 µL ,100 µL) were loaded and in the fourth well standard antibiotic amoxicillin was loaded. The plates were incubated for 24 hours at 37°C. After the incubation time the zones of inhibition were measured.

Hemocompatibility Assay:

The test samples were mixed with 200 µl of RBC solution and final reaction mixture volume was made up to 1 ml by adding sodium phosphate buffer. The reaction mixture was centrifuged again at 2500 rpm for 5 min. The supernatant absorbance was measured at 492 nm and 595 nm keeping sodium phosphate buffer as blank and hemolysis percentage was found.

RESULTS:

The antibacterial action was shown in the form of zone of inhibition. In this study It was observed that the Gelatin and CuO sample exhibited strong activity against E.coli showing the zone of inhibition of 19mm at the dose of 100 uL, while for Staphylococcus aureus the zone of inhibition at 100uL was 14mm. It was also found that the sample showed less than 5% of hemolysis, therefore indicating that the given sample is hemocompatable.

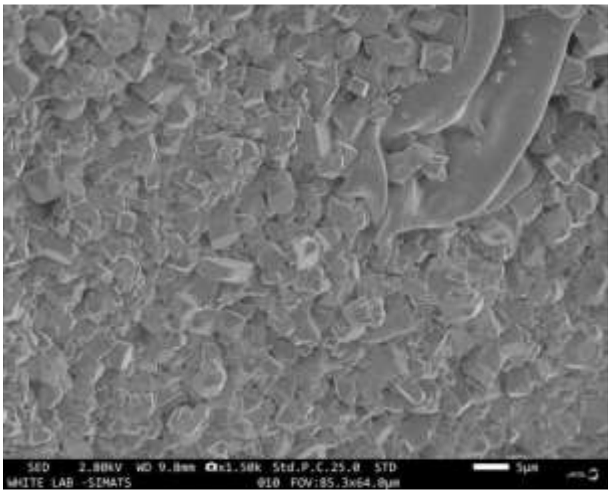

FIGURE 1: Surface morphology of Gelatin-Metal Oxide. The surface obtained an un uniformed formation coating.The surface exhibits petal like morphology.

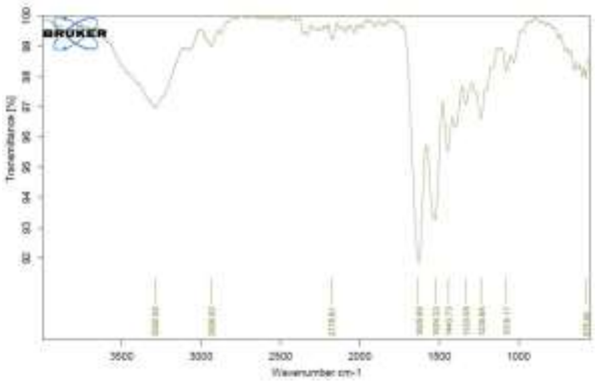

FIGURE 2:The presence of a functional group between 3500-3200 cm-1 in hydroxyl group .1641cm-1 presents the amide group and 1061cm-1 represents carboxyl group. We confirm that the Cu-gelatin composite has been formed.

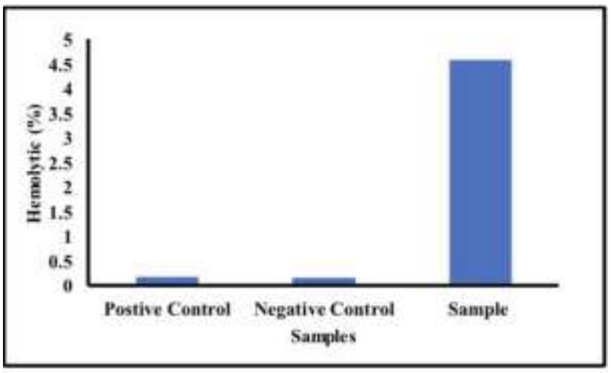

FIGURE 4: As per the ASTM standard.The hemolysis rate will be <5% is non-haemolytic nature. According to the results, we obtained <5% of coated sample.

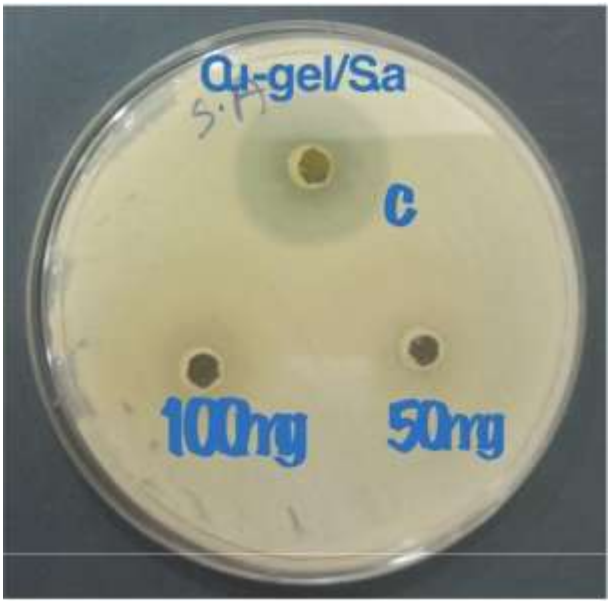

FIGURE 5**: Anti bacterial activity of gelatin metal oxide composite against *E. coli***

Figure 5 shows how gelatin metal oxide inhibited the growth of *E.coli*. At a concentration of 100µL , the zone of inhibition was determined to be most significant (19mm).

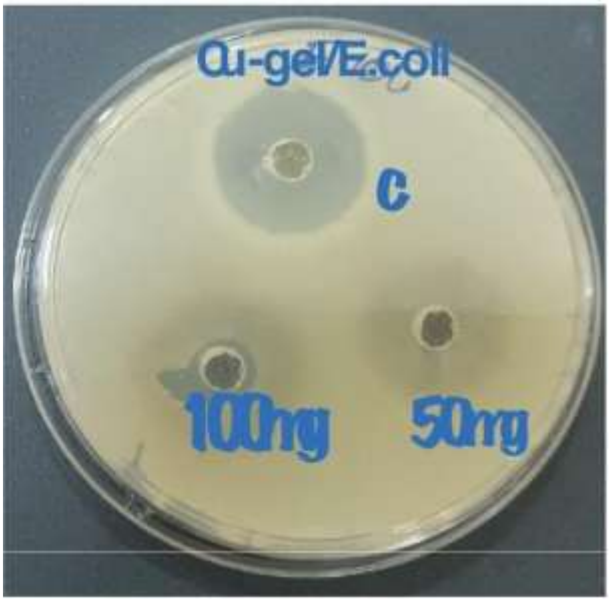

FIGURE 6**: Anti bacterial activity of gelatin metal oxide composite against *E. coli***

Figure 6 shows how gelatin metal oxide inhibited the growth of Staphylococcus aureus. At a concentration of 100µL , the zone of inhibition was determined to be most significant (14mm).

DISCUSSION:

Gelatin, derived from collagen, possesses remarkable properties suitable for biomedical applications. Its biocompatibility, biodegradability, and low immunogenicity make it an excellent candidate for wound healing interventions.(11) Gelatin can be easily modified and combined with other materials to create versatile composites with tailored properties.

Metal oxides such as zinc oxide, copper oxide, or silver oxide have inherent antimicrobial properties. When integrated with gelatin matrices, these metal oxides contribute significantly to the composite's ability to inhibit microbial growth. Silver, in particular, is known for its broad-spectrum antibacterial activity against various pathogens. Zinc oxide and copper oxide also exhibit antimicrobial effects, contributing to the overall efficacy of these composites in preventing infections in wounds.(11,12)

The incorporation of metal oxides into gelatin-based matrices enhances the antibacterial activity of these materials. This property is crucial in wound dressings or scaffolds to prevent microbial colonization and subsequent infections, especially in chronic or non-healing wounds. The controlled release of metal ions from these composites can effectively inhibit bacterial proliferation while supporting the wound healing process.(13)

Another critical aspect of these gelatin-metal oxide composites is their hemocompatibility, ensuring minimal adverse effects on blood components. This characteristic is essential for wound dressings or implants in direct contact with blood, as it prevents clot formation or adverse reactions, thereby supporting proper wound healing and tissue regeneration.(14)

The multifaceted properties of gelatin-metal oxide composites make them suitable for various wound care applications. These biomaterials can be fabricated into dressings, films, hydrogels, or scaffolds tailored to specific wound types. They create an optimal environment for wound healing by simultaneously preventing infections and supporting tissue regeneration.(10,14)

Continued research in this field focuses on optimizing the fabrication methods, controlling the release kinetics of metal ions, and assessing the long-term biocompatibility and efficacy of these composites in clinical settings. (8)Further exploration may involve the incorporation of other materials or bioactive agents to enhance wound healing outcomes.

CONCLUSION:

The result in the present study declares that natural products are more hemocompatible by displaying lysis less than 5% in concentration range. The extent of hemolysis is an important parameter of toxicity of natural products to the erythrocytes. The given sample also shows antimicrobial activity demonstrating that the Gelatin and copper oxide sample is effective in wound healing. Gelatin -metal oxide composites represent a promising avenue in the development of advanced wound care materials. Their combination of antibacterial properties, hemocompatibility, and versatility holds great potential in addressing the challenges associated with wound management and promoting improved healing outcomes in clinical applications.

AUTHORS CONTRIBUTIONS:

Ridha Azimudin: Literature search, data collection, analysis, manuscript drafting.

Dr.Kalaiyarasan M : Aided in conception of the topic, has participated in the study design, statistical analysis and has supervised in preparation and final correction of the manuscript.

ACKNOWLEDGEMENT:

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CONFLICT OF INTEREST:

The author declares that there were no conflicts of interest in the present study.

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