Efficacy and Cytotoxicity of Honey and Honey-Coated Nanoparticles on Wound Healing

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Abstract

In this study, we investigated the efficacy and cytotoxicity of honey, nanoparticles (NPs), and honey-coated nanoparticles (hNPs) as innovative agents for wound healing applications. We paired honey, renowned for its antimicrobial, anti-inflammatory, and antioxidant properties, with nanoparticles of silver selenide (Ag2Se), known for its potent bioactivity. By coating Ag2Se nanoparticles with honey, we aimed to enhance their therapeutic properties while minimizing their cytotoxicity. Using Human Dermal Fibroblasts (HDFa), we conducted cell viability assays and scratch wound healing assays to evaluate tissue regeneration, cellular growth, and antimicrobial effects, specifically assessing the zone of inhibition against Staphylococcus aureus and Escherichia coli. We compared honey samples with varying bioactivity levels to determine their impact on wound healing outcomes. Finally, we analyzed the results using advanced statistical methods to ensure their validity and reproducibility. Our work demonstrated the potential of integrating natural and nanotechnological approaches for improved wound care, paving the way for safer and more effective therapeutic applications.

Introduction

Honey has long been recognized for its wound-healing properties due to its antimicrobial, anti-inflammatory, and antioxidant effects. By maintaining a moist environment, it promotes tissue regeneration, reduces inflammation and infection, accelerates wound healing, and minimizes scarring. Medical-grade honeys like Manuka are especially prized for their consistency and potency, although their availability can be limited by strict production standards. Similarly, nanoparticles such as silver selenide (Ag_2Se) have shown effectiveness in wound healing because of their antimicrobial and regenerative abilities. However, the potential toxicity of nanoparticles remains a major concern. Recent research suggests that coating nanoparticles with honey may enhance their antimicrobial and antioxidant properties while reducing toxicity. Advances in wound healing have highlighted the importance of nanoparticles as effective therapeutic agents, though their clinical use often remains limited by potential toxicity. A previous study found that coating chalcogenide nanoparticles with chitosan reduced cytotoxicity while preserving their beneficial properties[1]. Chitosan, a polysaccharide, shares structural similarities with the sugars found in honey. Similarly, highlighted the importance of natural coatings in improving the efficacy of nanoparticles for wound healing[2]. Honey could serve as an effective coating for nanoparticles, enhancing their antioxidant, antimicrobial, and wound-healing properties.

Objective

Our study aims to evaluate the efficacy and cytotoxicity of silver selenide nanoparticles, both non-coated and coated with honey, to determine whether the honey coating enhances wound healing in Human Dermal Fibroblasts (HDFa).

Hypothesis: If nanoparticles of Ag_2Se are coated with honey, it will lower its cytotoxicity and increase its antimicrobial and wound-healing properties because both of their antimicrobial, antioxidant, and wound-healing properties.

Materials and Methods

Pulsed Laser Ablations in Liquid (PLAL)

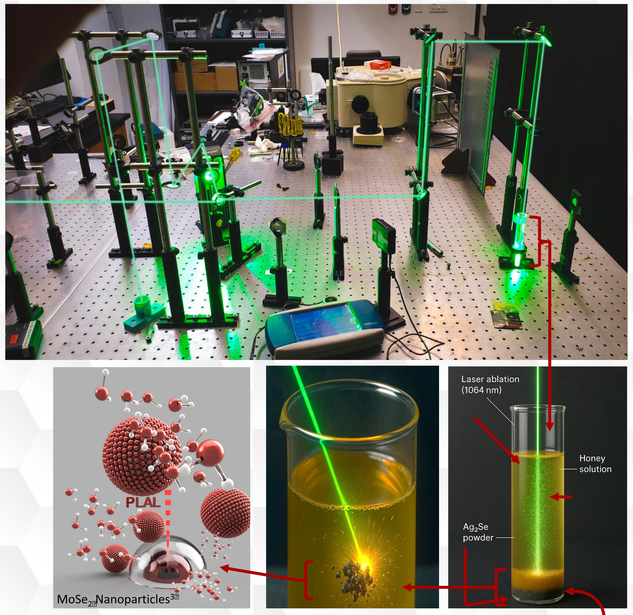


Illustration 1. Pulsed Laser Ablation in Liquid Process

Pulsed Laser Ablation in Liquid was conducted to synthesize non-coated and honey-coated Ag2Se nanoparticles using near-infrared laser light at 1064 nm wavelength. Particle size and zeta potential were measured using Dynamic Light-Scattering (DLS), and Ultraviolet-Visible (UV-Vis) Spectrophotometer was used to ensure the purity of the nanoparticles. The concentration of the nanoparticles was measured using Atomic Absorption Spectroscopy (AAS).

Figure 1. Honey coated nanoparticles

Cytotoxicity Assay

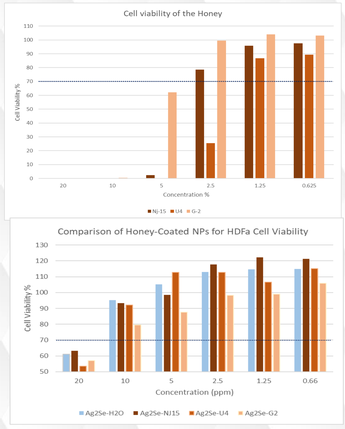
Cytotoxicity of honey, Ag_2Se nanoparticles, and honey-coated nanoparticles (hNPs) was tested on Human Dermal Fibroblasts (HDFa) cultured in Fibroblast Basal Medium. Cells were seeded at 1.0×10^4 per well in 96-well plates and incubated for 24 hours. Test groups included three honey samples (G-2, U4, NJ-15), Ag_2Se nanoparticles, and hNPs prepared with each honey. Honey samples were filtered and diluted to 20%–0.625% concentrations. After 24-hour exposure, CellTiter-Glo reagent was added, and luminescence was measured. Controls included untreated cells (positive) and bleach-treated cells (negative).

Scratch Wound Healing Assay

Scratch wound assay was performed using HDFa cells plated at 5.0×10^4 per well in 24-well plates with FBM. After 24-hour incubation, straight scratches were made with pipette tips, and wells were washed with DPBS. Honey, NPs, and hNPs were added at the highest non-cytotoxic concentration in triplicates. Fibroblast Basal Medium served as the control. Wound closure was imaged every 3 hours for 48 hours using a Zeiss Inverted Microscope, and closure percentages were calculated using live cell-imaging software.

Results

Cytotoxicity Assay



This graph displays the cytotoxicity of silver selenide (Ag_2Se) nanoparticles, both non-coated and honey-coated using low, moderate, and high bioactivity honey. Cell viability, measured by ATP-based luminescence (CellTiter-Glo), increased as nanoparticle concentration decreased. The 1.25% concentration showed the highest viability across all groups and was selected for the scratch assay. Results suggest honey coating enhances nanoparticle biocompatibility.

Figure 2. Comparison of Honey and Honey-Coated NPs on HDFa Cell Viability.

Scratch Wound Healing Assay

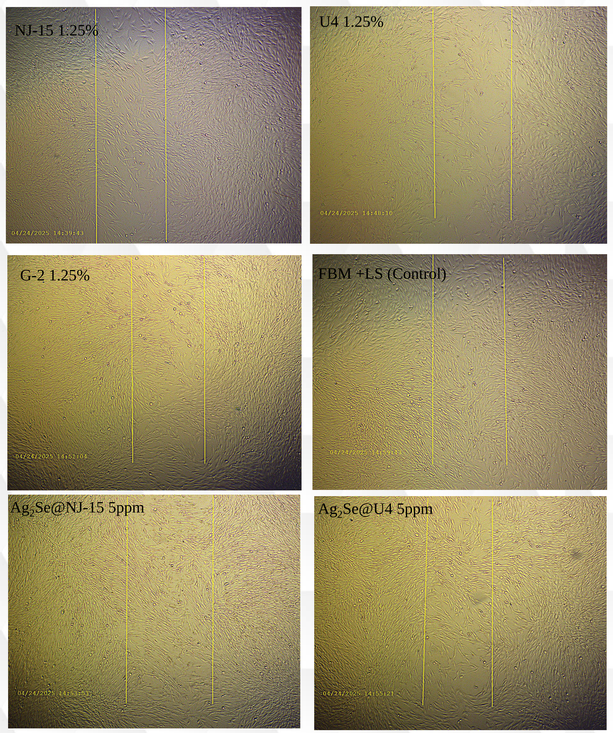


Figure 3. Scratch Wound Healing Assay of Honey, NPs, and hNPs on HDFa after 24 hours

Results

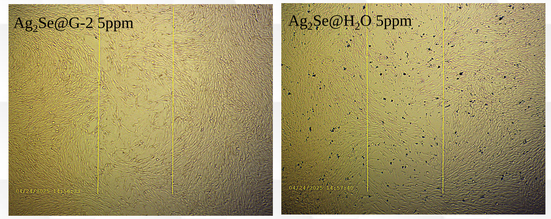


Figure 3. Scratch Wound Healing Assay of Honey, NPs, and hNPs on HDFa

Conclusions

- The synthesis of silver selenide (Ag_2Se) nanoparticles coated with honey improved particle size uniformity, enhanced colloidal stability, and yielded a higher nanoparticle concentration
- These findings support the potential application of honey-coated Ag_2Se nanoparticles in wound care, including their integration into medical devices for infection and inflammation control with improved tissue regeneration.
- Honey-Coated Nanoparticles enhanced cell proliferation and decreased its cytotoxicity, demonstrating the promising therapeutic potential of honey in promoting accelerated wound healing..

Future Implications: Advancing honey-based medical applications holds significant promise, including the innovation of honey-coated medical devices designed to prevent biofilm formation. Accelerating the wound healing process is critical to reducing complications associated with chronic wounds and mitigating the risk of antimicrobial-resistant bacteria, which may develop due to prolonged or untreated wounds.

References

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