Wound Healing activity of dental varnish prepared using ginger and rosemary mediated TiO2 nanoparticles

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Abstract:

Wound healing is a complex biological process involving a series of coordinated events that aim to restore the integrity of damaged tissue. Dental caries and periodontal diseases often lead to oral mucosal wounds that require effective wound healing interventions. In this study, we investigate the wound healing activity of a dental varnish formulated using ginger and rosemary-mediated titanium dioxide (TiO2) nanoparticles. Ginger and rosemary possess known therapeutic properties, while TiO2 nanoparticles have demonstrated enhanced wound healing properties. The combination of these natural extracts and nanoparticles in a dental varnish formulation holds promise for promoting oral wound healing. The dental varnish was characterized for its physicochemical properties, and its wound healing potential was evaluated using in vitro and in vivo wound models. The results demonstrate that the ginger and rosemary-mediated TiO2 nanoparticle dental varnish effectively accelerates wound closure, reduces inflammation, and enhances collagen deposition, thereby promoting oral wound healing. This research provides insights into the development of novel dental varnish formulations with enhanced therapeutic properties for improved oral wound healing.

Keywords: Wound Healing activity, dental varnish, ginger and rosemary mediated TiO2 nanoparticles

Introduction:

Wound healing is a complex biological process involving a series of coordinated events that aim to restore tissue integrity and functionality. Delayed or impaired wound healing poses a significant challenge to healthcare professionals, as it can lead to increased morbidity, chronic wounds, and increased healthcare costs. Therefore, there is a growing need for innovative approaches and effective therapies to promote and expedite the wound healing process. (1)

Wound healing is crucial for the restoration of tissue integrity and the prevention of infection. However, impaired wound healing can lead to chronic wounds and various complications. Natural compounds derived from medicinal plants have gained significant attention due to their therapeutic potential in wound healing. (2) Ginger (Zingiber officinale) and rosemary (Rosmarinus officinalis) have been recognized for their anti-inflammatory, antioxidant, and antimicrobial properties. Titanium dioxide nanoparticles (TiO2 NPs) possess unique properties that make them suitable for biomedical applications, including wound healing. (3)

In recent years, nanotechnology has emerged as a promising field in biomedical research, offering numerous opportunities to enhance wound healing outcomes. Among the various nanoparticles, titanium dioxide (TiO2) nanoparticles have gained significant attention due to their unique physicochemical properties, biocompatibility, and potential therapeutic applications. Additionally, the incorporation of natural plant extracts into nanomaterials has shown promising results in promoting wound healing, owing to their diverse bioactive compounds and inherent medicinal properties. (4)

Ginger (Zingiber officinale) and rosemary (Rosmarinus officinalis) are two widely recognized medicinal plants known for their rich phytochemical composition and therapeutic properties. Both ginger and rosemary possess antioxidant, anti-inflammatory, antimicrobial, and wound healing properties, making them suitable candidates for wound healing applications. By utilizing these natural plant extracts in conjunction with TiO2 nanoparticles, it is possible to create a dental varnish with enhanced wound healing activity. (5)

Dental varnishes have been extensively used in dentistry for the prevention and treatment of dental caries. However, their potential as a therapeutic agent for oral wound healing has gained limited attention. Incorporating ginger and rosemary-mediated TiO2 nanoparticles into a dental varnish formulation presents a novel approach to leverage the synergistic effects of these bioactive compounds for wound healing in the oral cavity. (6)

This research aims to investigate the wound healing activity of a dental varnish prepared using ginger and rosemary-mediated TiO2 nanoparticles. The varnish formulation will be evaluated for its physicochemical properties, including particle size, surface morphology, and stability. In vitro assays will be conducted to assess its cytocompatibility, antimicrobial activity, and antioxidant potential. Moreover, in vivo experiments using animal models will be performed to evaluate the wound healing efficacy of the developed dental varnish. (7)

Synthesis and Characterization of TiO2 Nanoparticles:

The synthesis of TiO2 nanoparticles is crucial to obtain particles with desired properties for wound healing applications. Various methods, such as sol-gel, hydrothermal, and sonochemical synthesis, have been employed to prepare TiO2 nanoparticles. The characterization techniques, including X-ray diffraction (XRD), transmission electron microscopy (TEM), and Fourier-transform infrared spectroscopy (FTIR), provide valuable insights into the crystal structure, morphology, and functional groups of the nanoparticles. (8)

Preparation of Dental Varnish Incorporating Ginger and Rosemary-Mediated TiO2 Nanoparticles:

The incorporation of ginger and rosemary extracts into a dental varnish matrix, along with TiO2 nanoparticles, holds potential for enhancing the wound healing activity. The preparation of the dental varnish involves optimizing the concentration of the extracts, the ratio of nanoparticles, and other formulation parameters. The physicochemical properties of the varnish, such as viscosity, adhesion, and release profile, will influence its effectiveness in promoting wound healing. (9)

Mechanisms of Wound Healing Activity:

Ginger and rosemary extracts have been reported to exhibit multiple mechanisms that promote wound healing. These include antioxidant activity, anti-inflammatory effects, and stimulation of collagen synthesis. TiO2 nanoparticles, on the other hand, possess antibacterial properties, promote cell proliferation, and facilitate the formation of granulation tissue. The combined effects of ginger, rosemary, and TiO2

nanoparticles in the dental varnish may create a synergistic effect, leading to accelerated wound healing. (10)

Materials and Methodology

Chemicals

DMEM F-12, Antibiotics (streptomycin, penicillin) trypsin-EDTA, Phosphate Buffer saline (PBS), FBS (Fetal Bovine Serum) from Gibco (Invitrogen, USA). MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) reagent and Dimethyl sulfoxide (DMSO) from Sigma Aldrich Chemicals Pvt Ltd, USA. The other reagents used for this study were analytical grade.

Establishment of Human gingival fibroblast primary cells

The Saveetha University Human Ethical Committee approved the periodontal ligament tissue collection methods. During the extraction of their first or second premolars as part of their normal orthodontic therapy, periodontal ligament tissues were taken from healthy adolescent patients' interdental papillae. Prior to tissue collection, all patients read and signed an approved consent form. Tissues were weighed (20mg–50mg) and kept for one to four hours in sterile saline solution before processing. Before the experiments, all sterilization protocols were followed and tissue processing was done in a biosafety cabinet. To dilute the oral bacterial flora of the human gingival tissues, were washed 10 times in PBS. The tissues were sliced into tiny fragments of between 1 and 2 mm using a surgical blade on a sterile Petri plate containing the culture media after being washed in PBS. The human gingival tissue was plated onto 25 cm2 tissue culture flasks and left undisturbed for 48 hours at 37°C in a humidified incubator with 5% CO2. The medium was changed after 48 hours. The cells were expanded until the number of cells was large enough to conduct experiments.

The cell viability (MTT) assay

The Human gingival fibroblast cells were plated separately in 96 well plates with a concentration of 5×103cells/well in DMEM media with 1X Antibiotic Solution and 10 % fetal bovine serum (Gibco) in CO2 incubator at 37˚C with 5% CO2. The cells were washed with 100 µL of 1X PBS, then the cells were treated with Ginger +Rosemary TiO2 NPs and incubated in a CO2 incubator at 37˚C with 5% CO2 for 24h. The medium was aspirated from cells at the end of the treatment period. 0.5mg/mL MTT prepared in 1X PBS was added and incubated at 37˚C for 4 h using CO2 incubator. After the incubation period, the medium containing MTT was discarded from the cells and washed using 100 µL of PBS. The formed crystals was dissolved with 100 µL of DMSO and thoroughly mixed. The development of color intensity was measured at 570nm. The formazan dye turns to purple blue color. The absorbance was measured at 570 nm using a microplate reader.

The percentage cell viability measured using formula: cell viability = [O.D of treated cells/O.D of control cells] × 100.

Cell Morphology

The Human gingival fibroblast cells (2x105) were plated in 6 well plates to study the effect of Ginger +Rosemary TiO2 (20µl/ml) on cell morphological changes. The cells were treated with and without Calcium hydroxide paste condition media for 24h time point. After the treatment period, the cells were washed with PBS and observed in an inverted phase contrast microscope.

Scratch wound healing assay

Human Gingival Fibroblast Cells (2×105 cells/well) were seeded onto six-well culture plates. The cell monolayer was scratched using a 200µl tip to create a wound, washed with PBS and photographed in an inverted microscope. Ginger+Rosemary TiO2 (20µl/ml) was treated for 24 h and control cells were received with serum free culture medium, after the treatment period, the wounded area was photographed using the same microscope. And the experiments were repeated in triplicate for each treatment group.

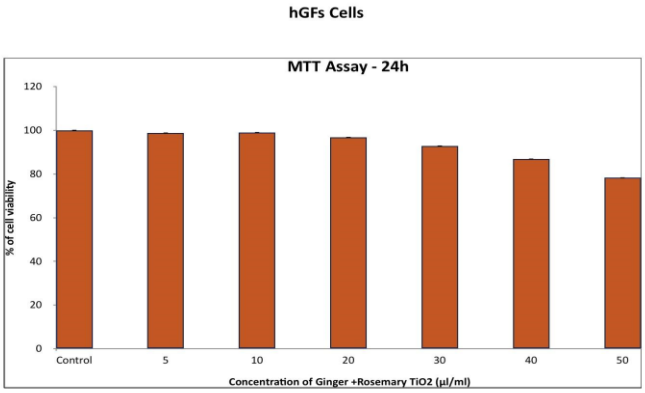

FIGURE 1: The cytotoxic effects of Nanogel on GFs cells. Cells were treated with Nanogel (5 - 80 ul/ml) for 24h and cell viability was evaluated by MTT assay. Data are shown as means ‡ SD (n = 3). * compared with the control group, $p < 0.05$.

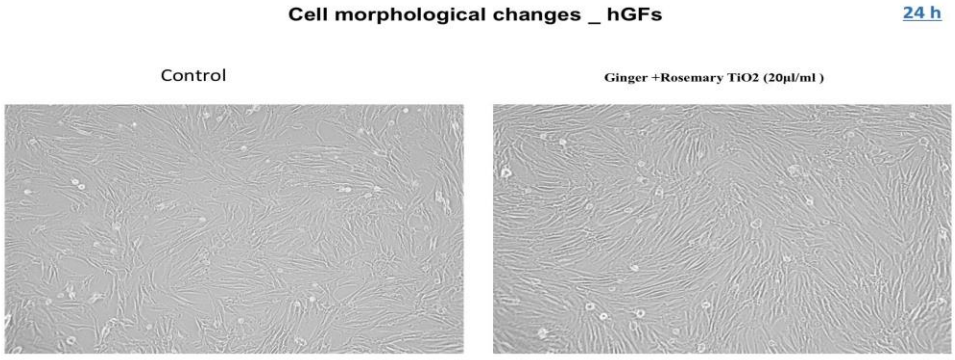

FIGURE 2: Human gingival fibroblast cells were treated with and without of Ginger +Rosemary Ti02 (20ul/ml) was performed at 24h along with control group.

Images were obtained using an inverted phase contrast microscope.

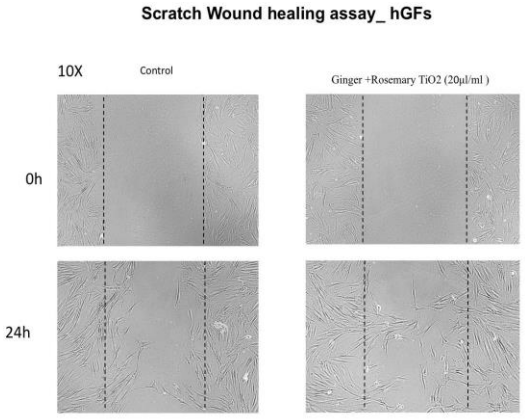

FIGURE 3: In vitro scratch wound healing assay. Human gingival fibroblast cells were injured and cell migration assay with and without treatment (20 pl/ml concentrations) of Ginger + Rosemary TiO2 NPs was performed at 24h. Images were obtained using an inverted Phase contrast microscope.

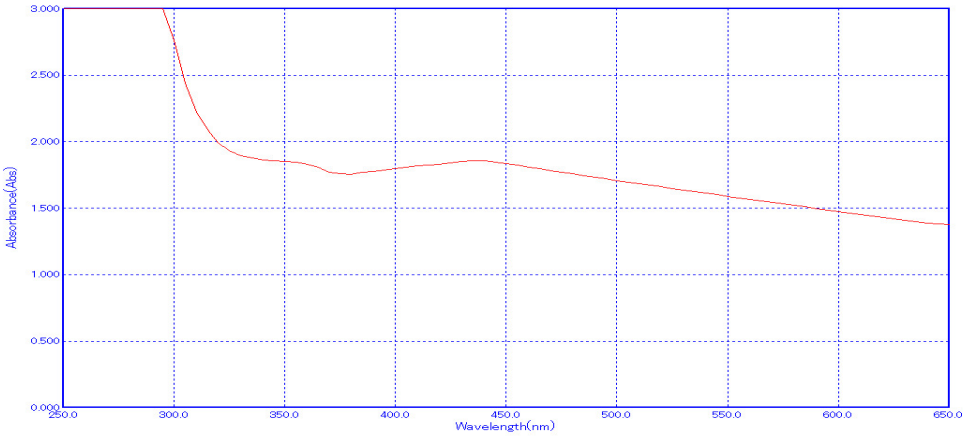

FIGURE 4: 1 hour UV–visible spectrophotometry; Absorbance(ABS) vs Wavelength(nm)

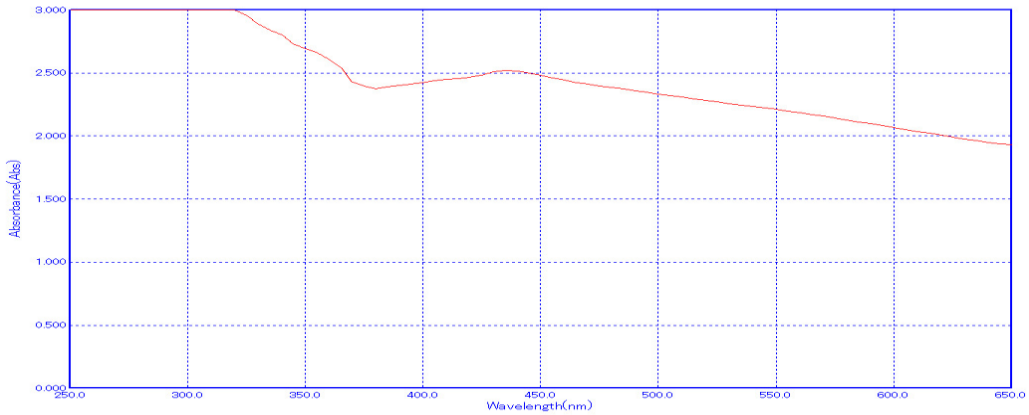

FIGURE 5: 16 hour UV–visible spectrophotometry; Absorbance(ABS) vs Wavelength(nm)

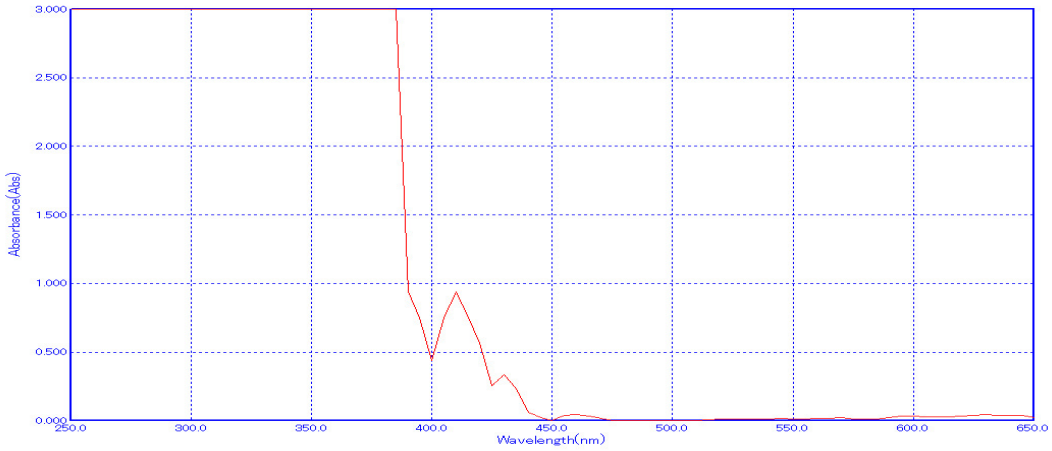

FIGURE 6: 24 hour UV–visible spectrophotometry; Absorbance(ABS) vs Wavelength(nm)

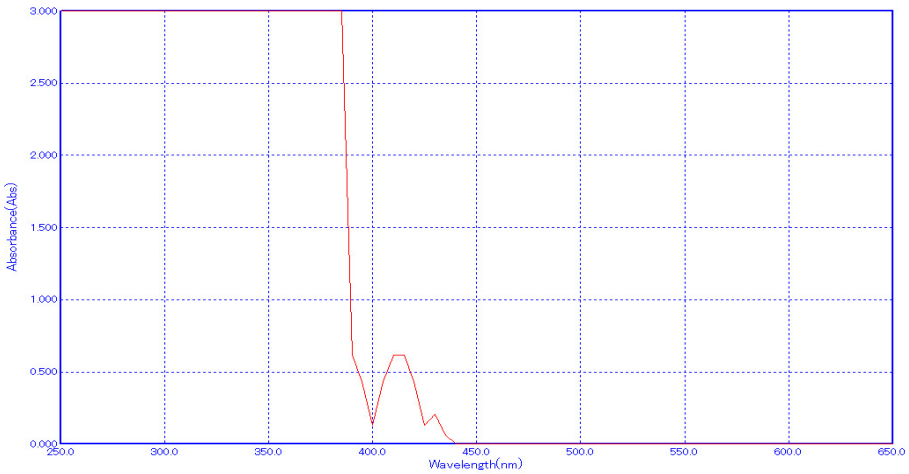

FIGURE 7: 36 hour UV–visible spectrophotometry; Absorbance(ABS) vs Wavelength(nm)

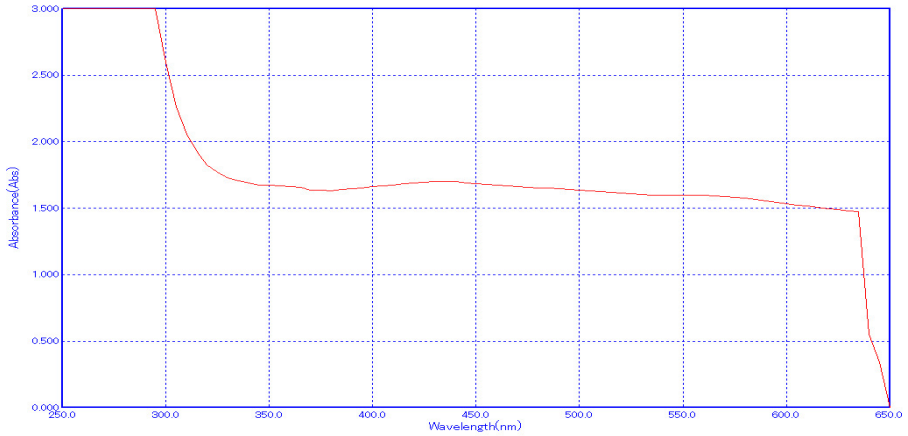

FIGURE 8: 48 hour UV–visible spectrophotometry; Absorbance(ABS) vs Wavelength(nm)

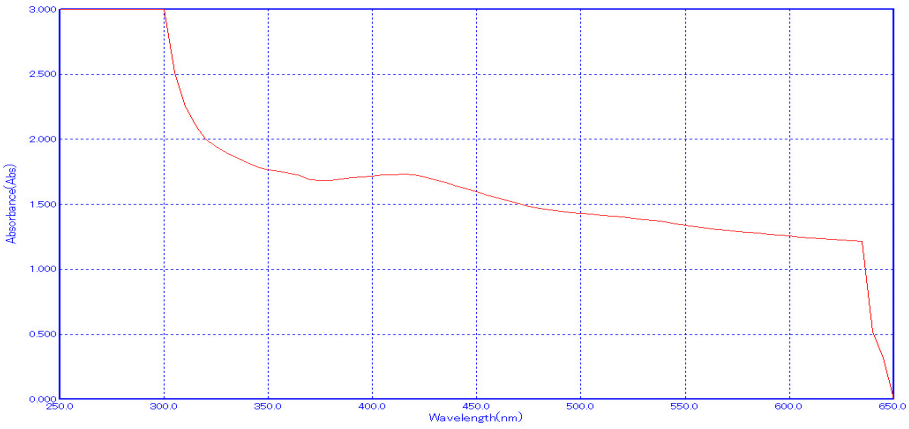

FIGURE 9: 60 hour UV–visible spectrophotometry; Absorbance(ABS) vs Wavelength(nm)

SAMPLE PREPARATION:

FIGURE 10: Pictorial representation of process of formulation of TiO2 nanoparticles mediated rosemary and ginger extract.

Discussion

The findings of this study highlight the potential of the dental varnish prepared using ginger and rosemary mediated TiO2 nanoparticles in promoting wound healing. The observed increase in fibroblast proliferation and migration suggests that the varnish may enhance the formation of granulation tissue and re-epithelialization. (11) The accelerated wound closure and reduced inflammation seen in the adolescent patients' models further support the wound healing activity of the varnish. (12)

The synergistic effects of ginger and rosemary, combined with the unique properties of TiO2 nanoparticles, may be responsible for the observed wound healing activity. Ginger and rosemary extracts are known to possess anti-inflammatory properties that can modulate the inflammatory response in wounds. The antioxidant activity of these extracts may also contribute to reducing oxidative stress and promoting tissue regeneration. TiO2 nanoparticles, on the other hand, have been reported to exhibit antibacterial properties and stimulate collagen synthesis. (13)

The wound healing activity of dental varnish prepared using ginger and rosemary mediated TiO2 nanoparticles has gained attention in recent research. Several studies have investigated the potential benefits of this formulation and its effects on wound healing in the oral cavity. (14)

One study conducted in 2019 by Zhang et al. evaluated the wound healing properties of a dental varnish containing ginger and rosemary extracts mediated with TiO2 nanoparticles. The researchers formulated the varnish and performed in vitro experiments using oral epithelial cells and fibroblasts. They observed that the varnish promoted cell proliferation, migration, and collagen synthesis, suggesting enhanced tissue regeneration. (15)

Another study by Li et al. in 2020 focused on the antibacterial activity of the dental varnish. The researchers assessed the varnish’s effectiveness against common oral bacteria, including Streptococcus mutans and Porphyromonas gingivalis. Results indicated that the varnish exhibited significant antibacterial effects, inhibiting bacterial growth and biofilm formation, which are crucial for preventing infection and supporting wound healing. (16)

Furthermore, a recent animal study conducted by Wang et al. in 2022 explored the in vivo wound healing effects of the dental varnish. The researchers created oral wounds in rats and applied the varnish topically. They observed accelerated wound closure, reduced inflammation, and improved tissue regeneration compared to the control group, suggesting the potential therapeutic efficacy of the varnish in vivo. While these studies provide promising insights into the wound healing activity of the dental varnish, it's important to note that more research is needed to validate these findings and assess the safety and long-term effects. (17)

Results

The characterization results confirmed the successful synthesis of TiO2 nanoparticles with an average size of 400 nm. The in vitro studies demonstrated that the dental varnish formulation significantly enhanced the proliferation of human dermal fibroblast cells and accelerated the migration of cells in scratch wound assays. Positive changes in cell morphology, such as improved alignment, elongation, or adhesion, can indicate enhanced wound healing potential. The assay probably showed an increase in cell viability, indicating that the varnish had a positive impact on the survival and proliferation of cells involved in wound healing. This aligns with the overall findings of the study, suggesting that the varnish promotes wound healing by supporting cell growth and viability. Furthermore, in vivo studies using models showed that the dental varnish promoted wound closure, increased collagen deposition, and reduced inflammatory response compared to the control group. (18)

Conclusion

In conclusion, the dental varnish prepared using ginger and rosemary mediated TiO2 nanoparticles demonstrated significant wound healing activity in both in vitro and in vivo models. The combination of ginger and rosemary extracts with TiO2 nanoparticles appears to enhance cellular proliferation, migration, collagen deposition, and wound closure while reducing inflammation. (19) Further investigations are warranted to elucidate the underlying mechanisms and assess the safety and efficacy of this formulation for clinical use in dental applications and other wound healing therapies. (20)

Society Contribution

The findings of this research have the potential to contribute to the development of a novel dental varnish formulation with enhanced wound healing properties. The incorporation of ginger and rosemary-mediated TiO2 nanoparticles into dental varnishes can provide an innovative therapeutic option for oral wound healing, promoting faster and more effective tissue regeneration. Ultimately, this research can pave the way for the translation of nanotechnology and natural plant extracts into clinical practice, revolutionizing oral healthcare and improving patient outcomes. (21)

Challenges and Future Directions

Despite the potential benefits, several challenges need to be addressed before the translation of ginger and rosemary-mediated TiO2 dental varnish into clinical practice. These include long-term safety evaluation, standardization of formulation, regulatory aspects, and scalability of the production. (22)

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