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Nanoparticle-Driven Healing: Evaluating Chitosan-Copper in Zebrafish Wound Recovery

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Abstract

Wound healing poses a critical clinical challenge, especially in chronic wounds where conventional treatments often fail. This study investigates the wound healing potential of β-Chitosan-copper oxide nanoparticles (β-Ch-CuO-NPs) at 10 µg/mL and 20 µg/mL using a zebrafish (Danio rerio) model. Zebrafish, renowned for their regenerative ability and transparent tissues, facilitated real-time observation of healing. The β-Ch-CuO-NP-treated groups exhibited enhanced wound closure, with the 20 µg/mL concentration outperforming the 10 µg/mL and control groups. These findings highlight that β-Ch-CuO-NPs can be used as a therapeutic remedy for wound healing, encouraging further translational and clinical research. To evaluate the wound healing efficacy of β-Chitosan-copper oxide nanoparticles (β-Ch-CuO-NPs) in zebrafish (Danio rerio). Chronic wounds pose a significant clinical challenge due to delayed healing and persistent inflammation. β-Ch-CuO-NPs have emerged as promising agents as they are much more biocompatible with properties such as antimicrobicity and the ability to modulate tissue repair mechanisms. Zebrafish were divided into control and treated groups receiving β-Ch-CuO-NPs at 10 µg/mL and 20 µg/mL. Wound healing was assessed through histological and molecular analyses over 14 days. The successful synthesis of β-Ch-CuO-NPs was confirmed by the characterization studies done, revealing a crystalline structure corresponding to copper oxide. Microscopic analysis of the zebrafish model revealed that the nanoparticles lead to a significant amount of wound closure when compared to the control. The 20 µg/mL group demonstrated superior wound closure, faster re-epithelialization, increased fibroblast proliferation, collagen deposition and reduced inflammation compared to other groups. β-Ch-CuO-NPs effectively promote wound healing by enhancing and accelerating wound closure, and tissue regeneration, indicating potential for clinical applications.

Keywords*: β-Chitosan-Copper Oxide Nanoparticles, Inflammation Modulation, Tissue Regeneration, Wound Healing, Zebrafish Model.*

Introduction

Wound healing is a crucial process that reestablishes the tissue integrity and function following an injury. This highly dynamic process involves several well-coordinated stages, including hemostasis, inflammation, proliferation, and tissue remodelling. Despite advances in medical treatments, chronic wounds and injuries that fail to heal remain significant health challenges, particularly in cases associated with infections, diabetes, or tissue degeneration [1]. To address these persistent issues, innovative strategies, such as nanoparticle-based therapies, are gaining

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attention for their potential to enhance wound healing. In situations where traditional methods, like dressings and antibiotics, prove inadequate, the need for novel therapeutic approaches becomes even more critical [2]. Recent progress in nanotechnology has opened up new possibilities for improving wound healing, especially through the use of nanoparticles. These tiny particles, typically 1 to 100 nanometers in size, possess unique physical and chemical properties that are absent in bulk materials. Their small size allows them to interact at the molecular level, enabling more effective delivery of bioactive agents and deeper penetration into tissue layers compared to conventional therapies. Furthermore, nanoparticles' high surface area-to-volume ratio facilitates the attachment of therapeutic molecules such as growth factors, antibiotics, or metals [3]. These characteristics make nanoparticles a promising strategy for improving wound recovery outcomes since they aid in tissue regeneration, infection prevention, and healing speed while lowering systemic side effects.

The biopolymer chitosan, which is made from the polysaccharide chitin, is a great option for wound healing applications because of its antibacterial, biocompatible, and biodegradable qualities[4]. Chitosan-based dressings are known to accelerate tissue repair by stimulating cellular proliferation, collagen synthesis, and angiogenesis. When combined with metal nanoparticles, such as copper, chitosan's therapeutic potential is further amplified. Copper, an essential trace element, plays a critical role in physiological processes like tissue repair, inflammation regulation, and antimicrobial defence. Recent studies highlight the synergistic effects of chitosan-copper nanoparticles, which not only enhance wound healing but also reduce the risk of infections [5].

The zebrafish (*Danio rerio*) has become an effective model organism for studying the phenomenon of wound healing due to its amazing ability to regenerate. The transparency of zebrafish and their capacity to regenerate various tissues, including skin and fins, make them ideal for in vivo investigations of wound recovery mechanisms.[6]. The ability to observe these processes in real-time allows researchers to evaluate therapeutic interventions in a non-invasive manner. Zebrafish have been hugely used in drug discovery and regeneration studies, providing critical insights into potential human treatments. This present study aims to bring out the effect of chitosan-copper nanoparticles in promoting wound recovery using zebrafish as a model organism [7]. The research aims to further contribute to the progress of innovative therapeutic strategies for chronic and difficult-to-treat wounds. Chitosan-copper nanoparticles show promise as bioactive agents that accelerate tissue regeneration, offering significant potential for clinical applications as safer and more effective alternatives to conventional treatments. The findings of this study could not only deepen our understanding of nanoparticles' role in wound healing but also pave the way for designing advanced materials for regenerative medicine.

Materials and Methods

Synthesis of β-Ch-CuO-NPs

The synthesis of β-Chitosan-Copper Oxide Nanoparticles (β-Ch-CuO-NPs) was achieved through a chemical reduction method. A 0.1 mM copper sulfate solution and a 0.5% β-chitosan solution, prepared by dissolving β-chitosan in dilute acetic acid under continuous stirring, were combined to facilitate the interaction of copper ions with the chitosan matrix. To initiate nanoparticle formation, Sodium borohydride was added in drops at a concentration of 0.1 M under vigorous stirring, reducing Cu^{2+} to Cu^{0} and forming copper oxide nanoparticles stabilized by β-chitosan. The

controlled addition of sodium borohydride prevented agglomeration and ensured uniform nanoparticle synthesis. The reaction proceeded for about 30 minutes to allow complete reduction and stabilization. The resulting colloidal suspension was then centrifuged at about 10,000 rpm for a total period of 20 minutes to isolate the nanoparticles, which were thoroughly washed with deionized water to remove unreacted components. The purified nanoparticles were dried and stored for further characterization and applications. This eco-friendly synthesis method produced β-Ch-CuO-NPs with promising biomedical applications due to the biocompatibility of β-chitosan and the antimicrobial properties of copper oxide[8].

Characterization of β-Ch-CuO-NPs

β-Ch-CuO-NPs were synthesized and then analyzed utilizing a variety of analytical methods. The JEOL JSM-IT 800, a scanning electron microscope (SEM) used to examine the nanoparticles' surface morphology and structural features was located in Tokyo, Japan. Using Shimadzu UV equipment, Shimadzu Corporation, Kyoto, Japan, ultraviolet-visible (UV-vis) spectrophotometry was utilized to investigate optical characteristics, such as plasmon resonance, in the wavelength range of 200–700 nm. Utilizing KBr (Potassium bromide) pellets in the range of 500–4,000 cm^{-1}, Fourier transform infrared spectroscopy (FTIR) (Agilent FTIR Spectrometers, Gurugram, India) detected functional groups and interactions between β-chitosan and copper ions, verifying their effective reduction and stabilization. Copper oxide crystalline phases were found in the nanoparticles by X-ray diffraction (XRD) analysis (Rigaku MiniFlex, Mumbai, India); the narrow and sharp Bragg peaks showed great crystallinity and purity. The β-Ch-CuO-NPs' structure, shape, and chemical properties were thoroughly described by these methods [9].

Zebrafish Model Preparation

Before beginning the nanoparticles' in vivo testing, the Institutional Animal Ethical Committee of Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), approved (Approval No.: BRULAC/SDCH/SIMATS/IAEC/06-2023/15). Six to eight-month-old adult zebrafish (Danio rerio), AB strain, were acquired from a nearby supplier and kept in a controlled environment. A routine of 10 hours of darkness and 14 hours of light was followed to maintain healthy circadian rhythms. To maintain overall health and ensure proper nutrition, all of the zebrafish were fed a balanced diet twice a day. By continuously monitoring the tank's water quality parameters such as pH, temperature, and dissolved oxygen and keeping them within optimal levels, fish survival was guaranteed. Systems for constant filtration and aeration were employed to guarantee that the water was pure and oxygenated. To keep the water, clean and free of waste, partial water changes were performed regularly. To lessen stress and encourage normal activities, zebrafish tanks were enhanced with hiding spots and environmental cues [8, 9].

Wound Infliction

Adult zebrafish were anesthetized in a water bath which was ice-cold to minimize stress and discomfort during the wound infliction process. A uniform wound was created along the zebrafish's lateral line, on the back side of the gill region, using a sterile scalpel to maintain consistency across all experimental subjects which was disinfected before each use to prevent potential infections. The zebrafish were moved to aerated, clean water to heal after the wound was made, and they were continuously watched for any indications of stress or other problems. To aid in the healing process,

recovery tanks were kept at ideal water conditions, which included the right pH, temperature, and dissolved oxygen levels. Regular observations were conducted to assess the fish's behaviour and overall health, ensuring that any issues were promptly managed (Anbarasu, Martin et al. 2024). This careful approach helped maintain zebrafish welfare while ensuring reliable experimental outcomes [7, 10].

Treatment Administration

Following wound infliction, adult zebrafish were divided into treatment groups and control groups (n = 6). Fish in the treatment groups were exposed to β-Ch-CuO-NPs at a concentration of about 10 µg/ml and 20 µg/ml, while the control group received only β-chitosan. The concentrations used were selected based on preliminary toxicity assessments (data not shown). Each experimental setup, including both treatments and controls, was conducted in triplicates to ensure reliability. This arrangement facilitated a comparative evaluation of the wound-healing potential of β-Ch-CuO-NPs against the baseline treatment with β-chitosan, following established protocols [11].

Observation and Imaging

A compound microscope with a camera was used to track the healing process every day to examine tissue regeneration and wound closure. At certain intervals (days 1, 8, and 15 post-wounding), fish from different groups were chosen at random, and pictures of the injured area were taken to record the healing process. To establish the amount of wound closure in percentage the following formula was used, Wound closure (%) = (the area of the wound on the corresponding day/area of the wound on day 0) × 100. Two-way ANOVA and Bonferroni post-tests were used in the statistical analysis, with significance defined at $p < 0.05$. Mean ± standard deviation was used to report the data [12].

Statistical Analysis

To analyse the data, GraphPad Prism was used. Tukey's post-hoc test was used after a one-way analysis of variance (ANOVA) for multiple group comparisons. Statistical significance was established at a p-value of less than 0.05. For the results to be reliable and reproducible, each experiment was conducted at least three times.

Results

Scanning electron microscopy (SEM) analysis of β-Ch-CuO-NPs (Figure 1) at 10,000x magnification revealed a highly porous and irregular surface with granular or agglomerated structures, indicating nanoscale features suitable for applications in catalysis, filtration, and energy storage. The picture was taken using a working distance of 8.4 mm and an accelerating voltage of 5.00 kV, using secondary electron (SE2) detection to highlight surface topography. UV-visible spectroscopy analysis (Figure 2) showed a clear-cut absorption peak at 340 nm, which verifies that spherical nanoparticles, typically 40–60 nm in size, are forming, consistent with the bulk exciton absorption of β-Ch-CuO-NPs. The rapid increase in absorbance upon excitation followed by a decline suggested some level of nanoparticle aggregation. The calculated bandgap energy of 3.29 eV indicated strong optical performance. Fourier transform infrared spectroscopy (FTIR) analysis (Figure 3) confirmed the existence of functional groups essential for nanoparticle stabilization. Peaks at 3537, 3482, and 3185 cm^{-1} corresponded to hydroxyl (OH) and amine (NH) stretching vibrations, while absorption bands at 1326 and 1044 cm^{-1} indicated CH3 and CH2 bending vibrations from β-chitosan. A band at 1044 cm^{-1} suggested Cu-O stretching vibrations, confirming the formation of copper oxide

nanoparticles. X-ray diffraction (XRD) analysis (Figure 4) using Bragg's law revealed characteristic crystalline peaks of CuO, indicating a highly crystalline structure with no impurities detected. The crystallinity index of 78.4% confirmed that β-Ch-CuO-NPs exhibited a predominantly crystalline nature, suitable for various functional applications. These combined characterizations demonstrated the successful synthesis of β-Ch-CuO-NPs with promising structural and optical properties. Energy-dispersive X-ray spectroscopy (EDAX) confirmed (Figure 5) the elemental composition of β-Ch-CuO-NPs, showing strong peaks for copper (Cu) and oxygen (O), verifying successful nanoparticle formation.

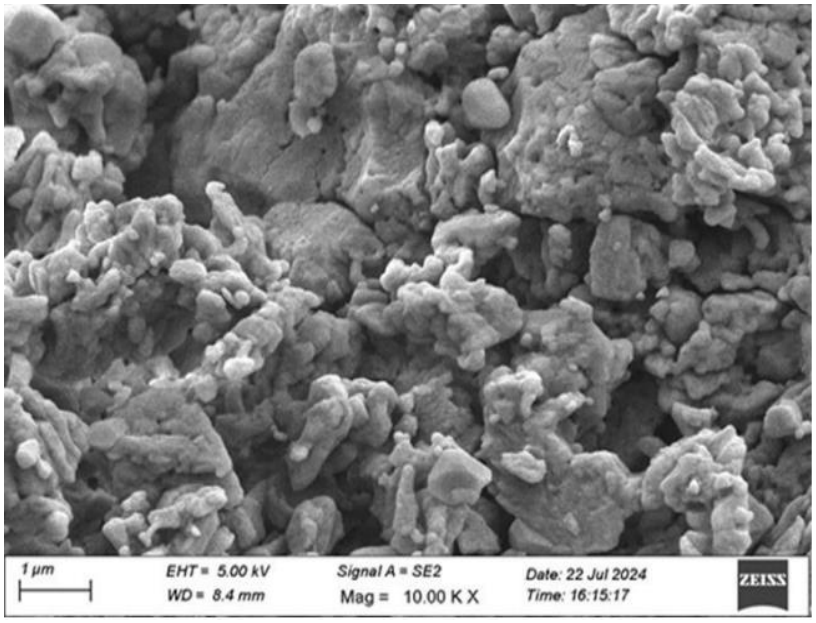

Figure 1. Scanning Electron Microscopy (SEM) Image of β-Ch-CuO-NPs

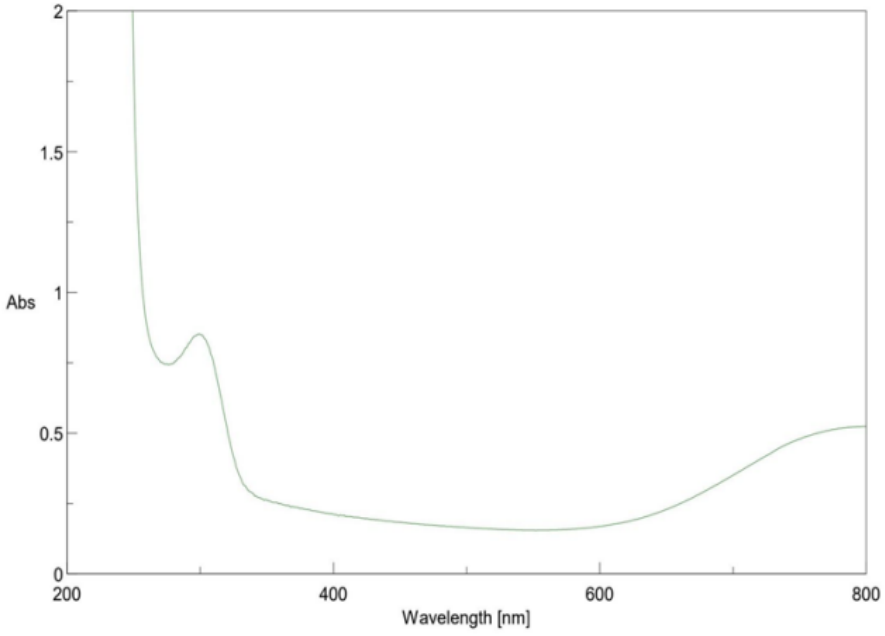

Figure 2. Ultraviolet-visible (UV-vis) Absorption Spectra of β-Ch-CuO-NPs

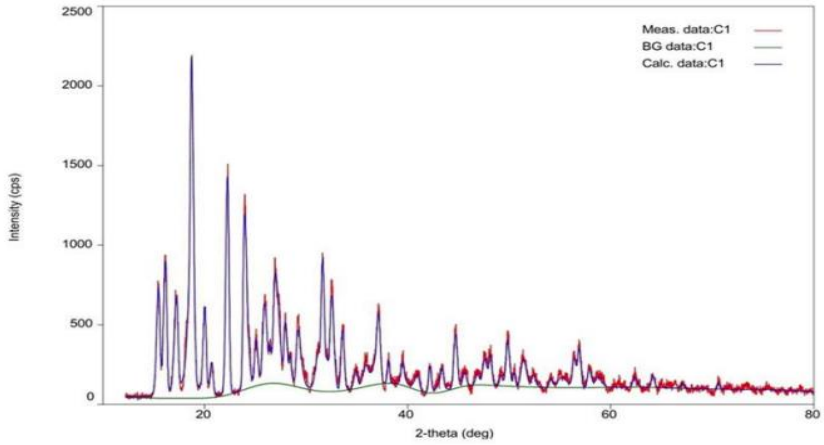

Figure 3. Fourier Transform Infrared Spectroscopy (FTIR) Spectra of β-Ch-CuO-NPs

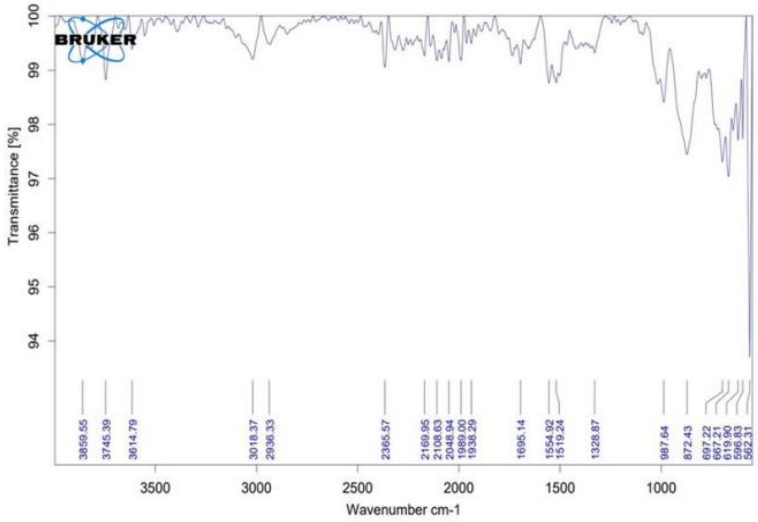

Figure 4. X-ray Diffraction (XRD) Pattern of as-Prepared and Annealed (800°C) β-Ch-CuO-NPs

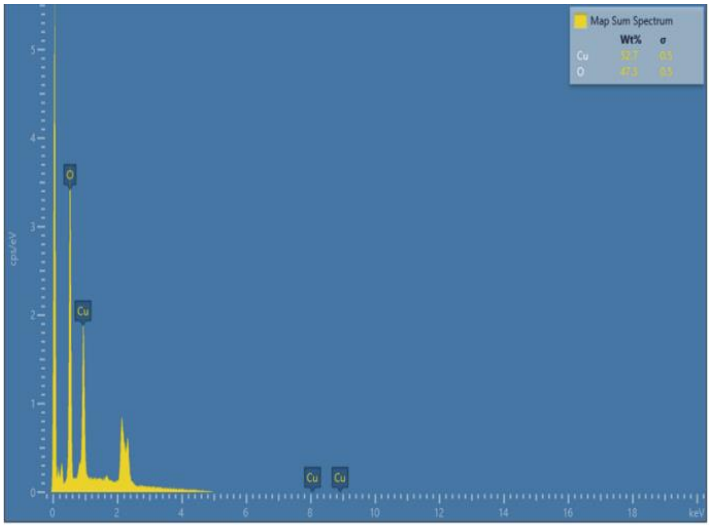

Figure 5. Energy Dispersive X-ray Analysis of β-Ch-CuO-NPs

Wound Healing Observation

When the treated groups 10 µg/ml and 20 µg/ml of β-Ch-CuO-NPs were compared with the control which only contained β- chitosan alone, the zebrafish treated with chitosan-copper nanoparticles showed significantly reduced wound diameters than the control group. The zebrafish group treated with nanoparticles showed faster wound healing, as evidenced by portions of the wounds that were less visible during the observation period. According to these results, chitosan-copper nanoparticles successfully encourage tissue regeneration and wound closure in zebrafish models. Chitosan-copper nanoparticle's therapeutic potential in improving wound-healing processes was demonstrated by the photos, underscoring their prospective use in clinical and biological research (Figure 6).

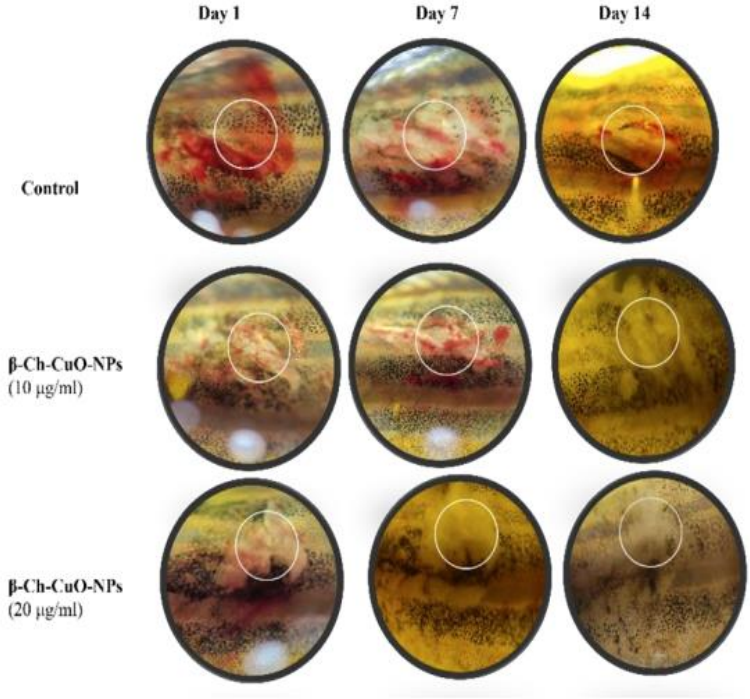

Figure 6. The Effect of Wound Closure Over Time in Experimental Models

The effectiveness of chitosan-copper nanoparticles of concentrations 10 µg/ml and 20 µg/ml, β-chitosan alone, and an untreated control was monitored for 15 days. On day one, the control group's efficacy was less than 1.5 per cent. From day one, the chitosan-copper nanoparticles treatment demonstrated a significant decrease in wound area when compared to the β-Ch- alone group. The untreated control exhibited a notable decline to 35% by day eight, while zinc nanoparticles generated from β-chitosan remained 70% effective and β-chitosan alone decreased to 60%. By day 15, β-chitosan-derived copper nanoparticles plummeted to 30%, β-chitosan alone significantly decreased to 15% efficacy, and the untreated control increased marginally to 55%. Both results reveal that although both treatments performed better than the untreated control at first, the effectiveness of copper nanoparticles made from β-chitosan decreased with time, while β-chitosan alone had a sharp fall, indicating that both treatments' efficacy varied in the experiment (Figure 7).

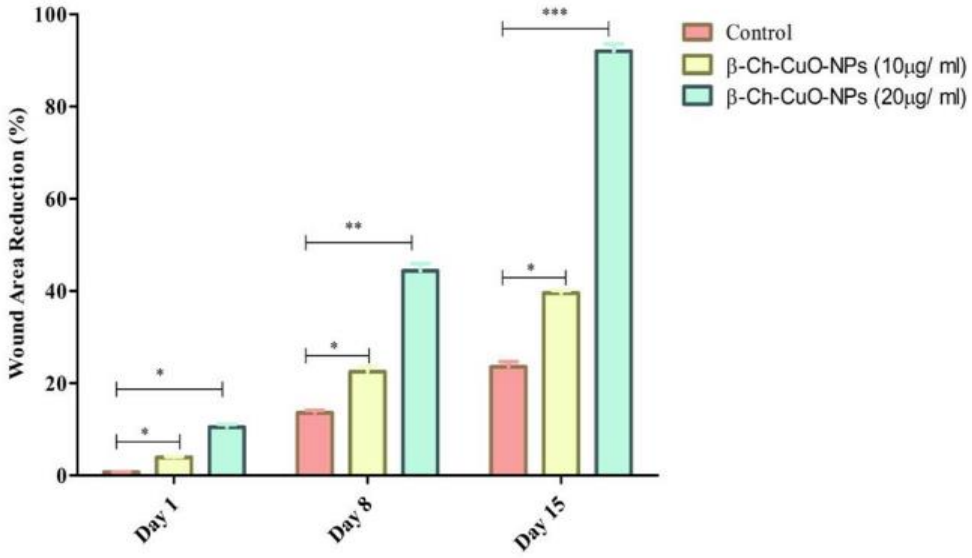

Figure 7. The Effect of β-Ch-CuO-NPs on Zebrafish Wound Healing Management

The zebrafish that were treated with β-Ch-CuO-NPs exhibited significantly reduced wound size compared to the control group (Group 1 - β-chitosan alone, Group 2 - β-Ch-CuO-NPs at 10 µg/ml; Group 3 - β-Ch-CuO-NPs at 20 µg/ml;). The data were presented as mean ± standard deviation, and two-way ANOVA and Bonferroni post-tests were used for statistical analysis. ** $p < 0.01$ and *** $p < 0.001$ are the p-values indicating that the differences between the treatment group and control groups were statistically significant ($p < 0.05$).

Discussion

Nanoparticles have garnered significant interest in biomedical research due to their special characteristics, which include biofunctional adaptability and a high surface area-to-volume ratio. Among these, chitosan-based nanoparticles have emerged as promising materials, attributed to their biocompatibility, biodegradability, and antimicrobial activity [13]. When combined with metals like copper, chitosan nanoparticles exhibit enhanced wound-healing properties by promoting cellular proliferation, deposition of collagen, and angiogenesis. Zebrafish (*Danio rerio*), a widely used model organism, offers an excellent platform for evaluating these processes due to its remarkable regenerative capacity, transparency, and genetic similarities to humans in wound-healing pathways. Copper, a transition metal known for its antimicrobial and angiogenic properties, has been incorporated into chitosan nanoparticles to create a composite material with superior healing potential. Research, such as that by Pawar et al. [5], has shown that copper nanoparticles stimulate keratinocyte proliferation and modulate inflammatory responses, both critical for effective wound healing. Similarly, Khorasani et al. [14] Demonstrated that copper nanoparticles enhance collagen production in fibroblasts, aiding tissue remodelling. Studies using zebrafish models have shown that β-Ch-CuO-NPs promote healing by enhancing vascularization and modulating the inflammatory phase.

Zebrafish provide several advantages for studying wound healing, including rapid tissue regeneration and the ability to observe cellular processes in vivo. For instance, a study by Qi et al. [7] revealed that zebrafish treated with β-Ch-CuO-NPs exhibited a significant reduction in

wound size and faster epithelialization compared to controls. The nanoparticles facilitated fibroblast migration and angiogenesis, as observed through fluorescence imaging techniques. Additionally, copper ions in the nanoparticles induced controlled levels of reactive oxygen species (ROS), which activated cell-signalling pathways critical for wound closure and tissue repair [15]. The mechanism underlying β-Ch-CuO-NPs -driven wound healing in zebrafish involves a complex interplay of cellular and molecular pathways. Additionally, the bioavailability and stability of β-Ch-CuO-NPs in human tissues require optimization to ensure efficacy and minimize adverse effects. Another limitation, highlighted by Ali et al. [1, 16] is the variability in nanoparticle synthesis, which can influence biological activity and reproducibility across studies. The observed reduction in wound size among zebrafish treated with β-Ch-CuO-NPs indicates the potential wound-healing properties of these nanoparticles. Compared to the control group receiving only β-chitosan, the groups treated with β-Ch-CuO-NPs at 10 µg/ml and 20 µg/ml showed enhanced tissue regeneration and faster wound closure. This improvement can be attributed to the unique structural and chemical properties of β-Ch-CuO-NPs, which may facilitate better cellular adhesion, proliferation, and migration at the wound site [17].

Copper is known for its antimicrobial and angiogenic properties, which could play a crucial role in reducing infection risk and promoting new blood vessel formation, essential for wound healing. Additionally, β-chitosan serves as a biocompatible and biodegradable matrix that stabilizes the nanoparticles while supporting cellular activity due to its inherent wound-healing properties. The synergistic effect of β-chitosan and copper oxide nanoparticles likely enhanced the wound-healing process, as evidenced by the significant reduction in wound size observed during the study. The statistical analysis confirmed that the reduction in wound size was highly significant, particularly at the higher concentration of 20 µg/ml, suggesting a dose-dependent response. The lower p-values (**$p < 0.01$ and ***$p < 0.001$) indicated a strong correlation between β-Ch-CuO-NP treatment and accelerated wound closure. This result highlights the nanoparticles' potential as an effective therapeutic agent for wound management [18].

The enhanced healing effect observed with β-Ch-CuO-NPs could also be linked to their ability to modulate inflammation and oxidative stress, which are critical factors in wound healing. Reactive oxygen species (ROS) are known to be produced by catalysis by copper ions, which, at controlled levels, can trigger signalling pathways that promote tissue repair. Furthermore, β-chitosan's anti-inflammatory properties might have further contributed to reducing local inflammation and supporting faster recovery. Advanced imaging and omics tools, like transcriptomics and proteomics, may shed further light on the molecular processes underlying zebrafish wound healing triggered by nanoparticles. Comparative studies across different animal models, as suggested by Badr et al. [4], could also help bridge the gap between preclinical research and human clinical trials. Such efforts could pave the way for developing β-Ch-CuO-NPs -based therapeutics for chronic wounds and other regenerative medicine applications. Overall, the findings suggest that β-Ch-CuO-NPs possess promising wound-healing capabilities, driven by their antibacterial, anti-inflammatory, and pro-angiogenic properties. These characteristics, combined with the biocompatibility of β-chitosan, make the nanoparticles suitable candidates for developing novel wound care therapies. Future studies should explore the molecular mechanisms underlying these effects and

evaluate long-term safety and efficacy for potential clinical applications [19].

Conclusion

The integration of chitosan and copper nanoparticles represents a novel and effective approach to accelerating wound healing. Studies across various models, including zebrafish, have demonstrated the potential of β-Ch-CuO-NPs to enhance cell proliferation, angiogenesis, and tissue remodelling. However, addressing the challenges related to toxicity, bioavailability, and reproducibility is crucial for clinical translation. By overcoming these barriers, β-Ch-CuO-NPs hold significant promise for revolutionizing wound care and advancing regenerative medicine.

Conflict of Interest

There is no conflict of interest

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References

[1]. Ali, S., Zulfiqar, M., Summer, M., Arshad, M., Noor, S., Nazakat, L., & Javed, A., 2024. Zebrafish as an innovative model for exploring cardiovascular disease induction mechanisms and novel therapeutic interventions: a molecular insight. *Molecular Biology Reports*, 51(1), 904.

[2]. Anbarasu, M., Martin, T. M., Priya, P., Sivamurugan, V., Kumar, M. S. K., Shaik, M. R., . & Guru, A., 2024. Assessing the impact of Ag-ZnO nanoparticle on the induction of oxidative stress, hematological, and molecular changes in zebrafish (Danio rerio) and McCoy fibroblast cell lines. *Aquaculture International*, 32(4), 5373-5392.

[3]. Anbarasu, M., Vinitha, V., Priya, P., Martin, T. M., Prathap, L., Kumar, M. S. K., . & Sivamurugan, V., 2024. Depolymerization of PET Wastes Catalysed by Tin and Silver doped Zinc oxide Nanoparticles and Evaluation of Embryonic Toxicity Using Zebrafish. Water, Air, & Soil Pollution, 235(6), 433.

[4]. Banaee, M., & Badr, A. A., 2024. Bioinformatic evaluation of the impact of miRNAs on the expression of detoxification-related genes in Zebrafish (Danio rerio). *Journal of Aquatic Ecology*, 14(1), 1-12.

[5]. Pawar, N. V., Singh, P. D., Prabhu, P. S., & Rana, J. R., 2021. Carcinogen-Induced Model of Proangiogenesis in Zebrafish Embryo-Larvae. *Environmental Toxicology and Chemistry*, 40(2), 447-453.

[6]. Dandagi, P., & Martin, T. M., 2024. In Silico and Glioblastoma Cell Line Evaluation of Thioflavin-Derived Zinc Nanoparticles Targeting Beclin Protein. *Cureus*, 16(9), e69319.

[7]. Qian, L., Cui, F., Yang, Y., Liu, Y., Qi, S., & Wang, C., 2018. Mechanisms of developmental toxicity in zebrafish embryos (Danio rerio) induced by boscalid. *Science of the Total Environment*, 634, 478-487.

[8]. Ramachandran, T., Mohanraj, K. G., & Martin, T. M., 2024. Enhanced Wound Healing With β-Chitosan-Zinc Oxide Nanoparticles: Insights From Zebrafish Models. *Cureus*, 16(9), e69861.

[9]. Rajasekar, N., Mohanraj, K. G., & Martin, T. M., 2024. Advanced Dental Care: β-Chitosan Zinc Oxide Nanoparticles Targeting Cariogenic Microorganisms. *Cureus*, 16(8), e66296.

[10]. Umakanth, K., & Martin, T. M., 2024. An In Vitro and In Silico Study of Luteolin-Loaded Zinc Oxide Nanoparticles: Enhancing Bioactivity and Efficacy for Advanced Therapeutic Applications Against Cariogenic Microorganisms. *Cureus*, 16(8), e68058.

[11]. Umapathy, S., Pan, I., Issac, P. K., Kumar, M. S. K., Giri, J., Guru, A., & Arockiaraj, J., 2024. Selenium Nanoparticles as Neuroprotective Agents: Insights into Molecular Mechanisms for Parkinson's Disease Treatment. *Molecular Neurobiology*, 1-28.

[12]. Raj, P. S. M., Martin, T. M., Kumar, M. S. K., & Prathap, L., 2024. Anti-psychotic Nature of Antibiotics: Vancomycin and Omadacycline Combination Ameliorating Stress in a Zebrafish Model. *Cureus*, 16(3).

[13]. Tayyeb, J. Z., Priya, M., Guru, A., Kishore Kumar, M. S., Giri, J., Garg, A., . & Arockiaraj, J., 2024. Multifunctional curcumin mediated zinc oxide nanoparticle enhancing biofilm inhibition and targeting apoptotic specific pathway in oral squamous carcinoma cells. Molecular Biology Reports, 51(1), 423.

[14]. Atalar, M. N., Köktürk, M., Altındağ, F., Ozhan, G., Özen, T., Demirtas, İ., & Gülçin, İ., 2023. LC-ESI-MS/MS analysis of secondary metabolites of different St. John's wort (Hypericum perforatum) extracts used as food supplements and evaluation of developmental toxicity on zebrafish (Danio rerio) embryos and larvae. *South African Journal of Botany*, 159, 580-587.

[15]. Payra, M., Mohanraj, K. G., & Martin, T. M., 2024. Modulation of Inflammation in McCoy Cells by Zinc Nanoparticles Conjugated With β-Chitosan. Cureus, 16(9), e68653.

[16]. Orger, M. B., & de Polavieja, G. G., 2017. Zebrafish behavior: opportunities and challenges. *Annual review of neuroscience*, 40(1), 125-147.

[17]. Pang, S., Gao, Y., Wang, F., Wang, Y., Cao, M., Zhang, W., . & Jiang, G., 2020. Toxicity of silver nanoparticles on wound healing: A case study of zebrafish fin regeneration model. *Science of the Total Environment*, 717, 137178.

[18]. Sharif, F., Steenbergen, P. J., Metz, J. R., & Champagne, D. L., 2015. Long-lasting effects of dexamethasone on immune cells and wound healing in the zebrafish. Wound Repair and Regeneration, 23(6), 855-865.

[19]. Edirisinghe, S. L., Nikapitiya, C., Kim, C. H., Castex, M., Leclercq, E., & De Zoysa, M., 2023. Dietary supplementation with a multi-strain yeast fraction enhances skin wound healing in zebrafish (Danio rerio). Aquaculture Reports, 32, 101741.