CHAPTER 4: SUMMARY, CONCLUSIONS AND FUTURE PROSPECTS

4.1 SUMMARY AND CONCLUSIONS

The global need for research on advanced wound healing materials is a pressing concern, driven by the intricate biological processes involved in wound repair and the persistent clinical challenges associated with chronic and complex wounds. Traditional wound care approaches often prove insufficient in addressing the multifaceted nature of tissue regeneration, inflammation, and infection control. Advanced wound healing materials, including polymeric nanoparticles, nitric oxide-releasing platforms, and metal oxide nanocomposites, emerge as promising candidates to overcome these challenges. Understanding the underlying molecular and cellular mechanisms of these materials can unveil novel therapeutic targets, enabling precise modulation of the wound healing process. Moreover, the interdisciplinary nature of this research, combining elements of nanotechnology, biochemistry, and material science, holds the potential to revolutionize conventional wound care strategies. This scientific pursuit not only deepens our understanding of fundamental biological processes but also paves the way for the development of innovative, targeted, and efficient interventions that can significantly impact global healthcare.

This scientific imperative transcends the laboratory, evolving into a societal necessity with profound implications for individuals and healthcare systems worldwide. Chronic wounds, often linked to conditions such as diabetes, vascular diseases, or traumatic injuries, impose a substantial burden on patients' quality of life and strain healthcare resources. By investing in advanced materials research, we embark on a transformative journey to alleviate the suffering of millions affected by persistent wounds, fostering improved patient outcomes and well-being. Additionally, the development of innovative wound healing solutions has broader implications for healthcare equity, ensuring that individuals across diverse socio-economic backgrounds have access to cutting-edge treatments. As we advance in the understanding and application of these materials, we take a significant step towards addressing global health disparities, promoting inclusivity, and offering transformative solutions for individuals facing the debilitating consequences of chronic wounds.

An optimal wound-healing process necessitates intricately coordinated and regulated interactions between the immune and biological systems, given that long-term wounds present severe clinical challenges, rendering patients susceptible to potentially life-threatening microbial infections. Consequently, imperative efforts are directed towards developing innovative treatments and therapeutic strategies. In this study, we devised a formulation based on polymeric (N-acryloyl-glycine) nanoparticles (PNAG NPs) to expedite skin restoration. PNAG NPs synthesized via the mini-emulsion radical polymerization technique exhibited a diameter of approximately 35 nm and were incorporated into an ointment base for application in wound healing.

The resultant PNAG nanoformulation demonstrated characteristics such as biocompatibility, hemocompatibility, proliferation enhancement, and migration facilitation. *In vivo* assessments revealed a remarkable ~31% increase in wound healing efficiency compared to the control group. Periodic monitoring of inflammatory markers, namely TNF-α and IL6, exhibited temporal fluctuations in their levels. TNF-α levels followed a similar pattern in both the PNAG treatment group and the control, while the IL6 expression was lower in the PNAG treatment group. The elevated level of IGF-1 in the PNAG treatment group correlated with increased angiogenesis and subsequent accelerated wound healing.

This investigation illustrated that PNAG NPs orchestrate rapid coordination between cell proliferation and migration, exert anti-inflammatory effects, and facilitate simultaneous regeneration of skin tissues without the need for external synergistic factors such as drugs, genes, or cells. In conclusion, the intriguing findings suggest that PNAG NPs hold promise in overcoming existing challenges in wound healing and may serve as a potential material for regenerative applications.

Endogenous gasotransmitter nitric oxide (NO) is a central signalling molecule that modulates wound healing by maintaining homeostasis, collagen formation, wound contraction, anti-microbial action and accelerating tissue regeneration. Optimum delivery of NO using nanoparticles (NPs) is clinically challenging; hence, it draws significant attention in wound healing. A novel polymeric nanoplatform loaded with sodium nitroprusside (SP NPs) has been fabricated and used for wound healing to obtain the sustained release of NO in therapeutic quantities.

SP NPs induced excellent proliferation (~300%) of mouse fibroblast (L929) cells have been observed. With the increase in the SP NPs dose, at 200 µg mL^{-1} concentration, a 200% upsurge in proliferation is observed along with enhanced migration, and only 17.09 h is required to fill the 50% gap compared to the control group, which requires 37.85 h. Further, SP NPs showed an insignificant impact on the coagulation cascade, revealing safer wound-healing treatment when tested in isolated rat RBCs. Additionally, SP NPs have excellent angiogenic activity at 10 µg mL^{-1} dose. Excitingly, formulated SP nanoformulation is non-irritant, non-toxic and does not produce any skin sensitivity reaction on rat's skin. Further, an *in vivo* wound healing study revealed that within 11 days of treatment with SP nanoformulation, 99.2 ±1.0% of the wound was closed, while for the control group, only 45.5 ±3.8% was repaired. These results directed us that due to sustained NO release, the SP NPs and SP nanoformulation are paramount with enormous clinical potential for the regeneration of wound tissues.

The third objective of my thesis revolves around the biological assessment of metal oxide antimicrobials (ZnO, ZnO-SnO_2, ZnO-Ag_2O/Ag, and ZnO-CuO) and their composites to explore their potential applications in treating complex microbial-infected wounds. Addressing the global public health threat of antimicrobial resistance, as highlighted by the World Health Organization (WHO), is paramount. Numerous metals, especially in their "nanoparticle form", are increasingly employed for their antimicrobial properties, preventing adhesion, and treating microbial infections. The burgeoning demand for metal and metal oxide nanoparticles stems from their physiochemical properties, making them invaluable in meeting diverse biomedical needs.

In our preceding investigation, we demonstrated the outstanding antimicrobial efficacy of zinc oxide (ZnO) and its composite nanoparticles — (ZnO, ZnO-SnO_2, ZnO-Ag_2O/Ag, and ZnO-CuO) — against various microbes. These composite nanoparticles, particularly in combination with polymeric nanoparticles, exhibit significant potential in inhibiting microbial growth at wound sites. Notably, their antimicrobial prowess is evident even at a remarkably low dose of 0.010 μg mL^{-1} against Gram-positive/ Gram-negative bacteria and fungal strains.

Microscopic analysis through TEM images reveals the mesoporous nature of these nanoparticles, with a crystalline structure having a size below 10 nm in diameter. These metal oxides are synthesized through the solvothermal reduction method using $NaBH_4$. XRD confirms their crystallinity. Their antibacterial activity is dose and time-dependent, showcasing effectiveness against a broad spectrum of microbes. Hemocompatibility assessments indicate acceptable levels of haemolysis, with zinc oxide-copper oxide exhibiting the lowest haemolytic nature. Cytotoxicity evaluations using MTT assays in

L929 and PC12 cell lines unveil differential compatibility, directing us towards potential applications in tissue regeneration for wound healing.

Scratch wound healing assays underscore the varied proliferative and migratory activities of metal oxide nanocomposites, with zinc oxide-stannous oxide leading in both aspects. Evaluation through the chicken embryo membrane assay indicates angiogenic potential, with zinc oxide-copper oxide displaying the highest activity. Overall, the nanocomposites exhibit haemolytic activity, which may be attributed to their low particle size, ensuring therapeutic concentrations below 10 μg mL^{-1}. This aligns with their antimicrobial efficacy, demonstrating superior cell compatibility with L929 compared to PC12 cells, thus affirming their migratory and proliferative nature. In conclusion, these metal oxide nanocomposites present multifaceted attributes that hold promise for therapeutic applications in tissue regeneration.

In conclusion, the imperative for global research on advanced wound healing materials stems from the intricate biology of wound repair and the persistent challenges associated with chronic wounds. Traditional approaches often fall short, prompting the exploration of innovative materials like polymeric nanoparticles, nitric oxide-releasing platforms, and metal oxide nanocomposites. The interdisciplinary nature of this research holds transformative potential, with deepened insights into fundamental processes and the development of targeted interventions. Beyond the laboratory, the societal impact is profound, addressing the burdens imposed by chronic wounds on individuals and healthcare systems globally. As we invest in advanced materials research, a transformative journey unfolds, offering cutting-edge solutions that foster inclusivity and alleviate the suffering of millions. The promising findings from formulations like PNAG NPs, SP NPs, and metal oxide nanocomposites underscore their potential in revolutionizing wound care, presenting a multifaceted approach that holds enormous clinical promise. This journey signifies a pivotal step towards addressing global health disparities, promoting inclusivity, and offering transformative solutions for individuals grappling with the consequences of chronic wounds.

4.2 <u>FUTURE SCOPE OF PRESENT WORK</u>

The thesis delves into the development of a poly(N-acryloyl glycine) nanoparticle (PNAG NP) based formulation for effective skin restoration. The formulation, exhibiting biocompatibility, hemocompatibility, and enhanced wound healing efficiency in *in vivo* assessments, suggests a promising avenue for addressing long-term wounds and microbial infections. The correlation between changes in inflammatory markers and increased angiogenesis further supports the potential of PNAG NPs in regenerative medicine. Another future prospect highlighted in the thesis involves the use of polymeric nanoplatforms loaded with sodium nitroprusside (SP) nanoparticles for the sustained release of nitric oxide (NO) in wound healing. The observed excellent proliferation and migration of cells, along with angiogenic activity, position SP NPs as a clinically significant solution. The non-irritant, non-toxic nature of the SP nanoformulation, coupled with its remarkable wound closure efficiency in *in vivo* studies, underscores its clinical potential for regenerating wound tissues.

Beyond PNAG and SP NPs, the exploration of metal oxide antimicrobials and their composites, as detailed in the thesis objectives, opens up a promising future in the field of wound care and microbial infection treatment. The focus on addressing global public health concerns related to antimicrobial resistance, as emphasized by the World Health Organization, highlights the relevance of this research. The demonstrated antimicrobial efficacy of zinc oxide and its composites, particularly in combination with polymeric nanoparticles, suggests potential applications in inhibiting microbial growth at wound sites. Moving forward, the synthesis methods, such as solvothermal reduction using

NaBH4, and the characterization techniques, including TEM images and XRD analysis, provide a foundation for further advancements in the development of metal oxide nanocomposites. The dose and time-dependent antibacterial activity, coupled with haemocompatibility assessments and cytotoxicity evaluations, contribute to a comprehensive understanding of their biological effects. The observed varied proliferative and migratory activities, as well as the angiogenic potential of the metal oxide nanocomposites, offer opportunities for their integration into tissue regeneration applications for wound healing. The alignment of antimicrobial efficacy with superior cell compatibility, as affirmed by scratch wound healing assays and chicken embryo membrane assays, strengthens the potential therapeutic applications of these nanocomposites.

In conclusion, the diverse aspects covered in the thesis, ranging from metal oxide nanocomposites to PNAG NPs and SP nanoformulation, collectively contribute to a comprehensive understanding of their potential applications in treating complex microbial-infected wounds. The findings pave the way for future research and development in the pursuit of innovative and effective therapies for wound healing and infection control.