

Abstract

Advanced wound healing research addresses severe clinical issues by developing novel treatments to optimize the process and reduce vulnerability to fatal microbial infections. Effective wound healing requires proficient orchestration between immune and biological systems. This study aimed to formulate poly(N-acryloyl glycine) nanoparticles (PNAG NPs) to enhance skin restoration. PNAG NPs, about c.a. 35 nm in diameter, were synthesized using mini-emulsion radical polymerization and incorporated into an ointment. TNF- α and IL6 levels in the PNAG nanoformulation treated group shifted from 4.10 ± 3.18 to 5.17 ± 3.27 pg mL⁻¹ and 11.93 ± 2.34 pg mL⁻¹ to 10.33 ± 3.31 pg mL⁻¹, respectively, indicating reduced inflammation while IGF-1 levels increased from 25.41 ± 13.56 ng mL⁻¹ to 64.22 ± 16.93 ng mL⁻¹, correlating with enhanced angiogenesis and improved wound healing. PNAG NPs also facilitated coordinated cell proliferation, migration, anti-inflammatory responses, and simultaneous skin tissue regeneration without external synergistic factors. The cytocompatible and hemocompatible PNAG nanoformulation demonstrated 31% higher wound healing efficiency compared to the control group *in vivo*.

Further, the regenerative potential of PNAG NPs was potentiated by harnessing the regenerative power of Nitric Oxide (NO) by the loading of NO donor [Sodium nitroprusside (SNP): a well-known NO donor]. The SNP loaded PNAG NPs are termed as SP NPs and can release NO in sustained manner for more than 24 hours, crucial for wound healing. SP NPs promoted significant proliferation (~300%) and migration of L929 cells. Haemocompatibility studies in isolated rat blood components confirmed the safety of SP NPs. The SP nanoformulation showed excellent angiogenic activity, non-toxicity, and can closed wounds significantly faster ($99.2 \pm 1.0\%$) within 13.23 days than the control group ($45.5 \pm 3.8\%$) *in vivo*, demonstrating its clinical potential for tissue regeneration.

Further, the metal oxide (ZnO, ZnO-CuO, ZnO-Ag₂O, ZnO-SnO₂) antimicrobial nanoparticles had been synthesized by keeping in mind that these will be applied in the treatment of complex infected skin wounds. Given the threat of antimicrobial resistance, nanoparticles were utilized for their properties. ZnO and its metal composites, including ZnO-CuO, ZnO-Ag₂O/Ag, and ZnO-SnO₂ NPs, showed excellent antimicrobial properties against various microbes. These nanoparticles have their particle size below

10 nm, demonstrated mesoporous structures and nanocrystallinity, confirming their antibacterial activity in a dose- and time-dependent manner. Hemocompatibility assessments revealed low haemolysis, with ZnO-CuO exhibiting the lowest haemolytic nature. Cytocompatibility studies showed compatibility with L929 cells, particularly for ZnO and ZnO-SnO₂ nanocomposites. Cell migration and proliferation evaluations in scratch wound healing model highlighted the superior performance of ZnO-SnO₂, followed by ZnO, ZnO-Ag₂O, and ZnO-CuO. ZnO-CuO nanocomposite showed the highest angiogenic potential evaluated *in ovo*, suggesting its potential application in tissue regeneration through wound healing. In our work PNAG NPs demonstrated efficacy in coordination of cellular response, anti-inflammatory actions, and angiogenesis which collectively accelerating the skin regeneration. Further, sustained NO release via SP NPs exhibited remarkable proliferation and migration capabilities, proving their clinical potential and metal oxide nanocomposites, especially ZnO-CuO, showed promising antimicrobial, hemocompatible, and cell-compatible properties, making them valuable for therapeutic wound healing applications.