8. Conclusion

The current hypothesis focused on improving the efficacy of antimicrobials and facilitating the active annihilation of the biofilm protected microbial colonies by applying nanoformulation based strategy along with the biofilm degrading substance either in conjunction or conjugated with nanoparticles. Two different strategies were adopted to treat the biofilm abundant in different constituents principally found in the different disease conditions.

Almost all the biofilms have eDNA as a principal component and associated with disease progression and complication. The solid lipid nanoparticles of silver sulfadiazine (SSD-SLNs) were successfully prepared and incorporated the SSD-SLNs along with DNase-I in chitosan gel. The combination of DNase-I along with SSD-SLNs was postulated to improve the antibacterial activity of SSD against biofilm embedded microbes by disrupting the extracellular DNA of EPS and reduce the dose dependent fibroblast toxicity of SSD following the encapsulation in SLNs, thus accelerate wound healing. In support of hypothesis, the combination of SSD-SLNs and DNase-I significantly improved the antimicrobial efficacy and exhibited significantly higher biofilm eradication potential as compared to SSD, besides attenuating the dermal fibroblasts toxicity. Moreover, the proposed strategy was able to accelerate the wound healing in-vivo and demonstrated complete re-epithelialization of skin confirming the superiority of the proposed co-administration in comparison with clinically approved therapy. As discussed in the introduction, many studies has reported the nanoparticles as a potential option for wound therapy, reducing fibroblast toxicity but none of the formulation was effective against the biofilm associated wounds. However, the findings of the current research suggested that the optimized nanoformulation has superior

response in terms of wound healing, reduced fibroblast toxicity along with enhanced biofilm elimination as compared to the previously investigated nanoformulation.

Further, AgLase functionalized chitosan nanoparticles of ciprofloxacin were developed to dismantle the alginate rich biofilm of the *P. aeruginosa* principally found in lungs of the cystic fibrosis patients. The optimized nanoparticles demonstrated excellent antimicrobial and anti-biofilm inhibition potential with highest depletion rate of microbial cell count in biofilm and simultaneously depleted the biofilm thickness to maximum extent. All the findings collectively support that AgLase-CIPR-CH-NPs can actively disrupt the biofilm EPS and enhance antibiotic susceptibility against the biofilm embedded microbes without causing blood and local toxicity to lung epithelia. However, the strategy needs to be evaluated first in diseased animals.

Number of research described the nanoparticle and AgLase as the effective way for treating mucoid *P. aeruginosa* lung infection in cystic fibrosis. However, the current study suggests that functionalization of AgLase to nanoparticles can improve the stability and activity of enzyme and also facilitate the drug delivery as well. Therefore it can be an effective and better alternative option to treat the biofilm-associated infections in disease like chronic wound and cystic fibrosis.

However, some concern like low EE particularly with ciprofloxacin loaded chitosan nanoparticles should be addressed. Apart from that, the efficacy of proposed strategies and the stability of the enzymes needed to be evaluated in different physiological condition to confirm its robustness and suitability as the microenvironment of the biofilm varies depending on the site of infection, bacterial strain and pathophysiological condition of the patients in terms of the composition, pH, oxygen level, nutrients availability and others.