6 SUMMARY AND CONCLUSIONS

6.1 Summary

Infections caused by MDR bacteria are the leading cause of chronic wound conditions, which contribute to an increase in mortality. Moreover, the burn wound-induced infections develop a biofilm around the wounded area, which causes resistance to the standard antibiotic therapy regimen prescribed for treating burn wounds and their associated infections. When it comes to burn wounds, *A. baumannii, and K. pneumoniae,* are among the most frequently isolated, and *P. aeruginosa* and *S. aureus* bacteria are most commonly co-isolated. Additionally, the pathogenic bacteria create biofilms surrounding wounds, which slow down healing and raise mortality rates, necessitating extended hospital stays. Therefore, a more profound treatment strategy in the form of bacteriophage was developed that overcame antibiotic resistance. Bacteriophage therapy shovers multiple advantages against standard wound healing treatment through their high specificity, low toxicity to eukaryotes, and self-replicating phenomena. In the present study, *A. baumannii* (BHU/AB/39), *K. pneumoniae* (BHU/KP/657), *S. aureus* (BHU/SA/4193), and *P. aeruginosa* (BHU/PA/1956) multidrug-resistant bacteria were isolated and confirmed by an antibiotic susceptibility test. Further, we have isolated bacteriophages against these bacteria named BPABΦ1, BPKPΦ1, BPSAΦ1, and BPPAΦ1, and also amplified and purified them. The specificity of the bacteriophages was assessed by conducting spot tests to determine their lytic range. The results revealed that all phages exhibited a narrow host spectrum, indicating their selectivity towards the host bacterium. Further, from the one-step growth curve analysis of phage BPABΦ1, BPKPΦ1, BPSAΦ1, and BPPAΦ1 latent period and burst size were found to be 102, 129, 75, and 112 PFUs per infected host cell, respectively. Based on morphology analysis, BPABΦ1 and BPKPΦ1 were found to

be from *Corticoviridae* and *Siphoviridae* families, respectively while the BPSAΦ1, and BPPAΦ1 both were found to belong to *Caudoviricetes* family.

Further, to deliver the phages effectively into the affected infectious wound site, the individual phage-loaded chitosan microparticles (BPABΦ1-CHMPs, BPKPΦ1-CHMPs, BPSAΦ1-CHMPs, and BPPAΦ1-CHMPs) and mixed phage loaded chitosan microparticles (MBP-CHMPs having a combination of BPSAΦ1 and BPPAΦ1) were formulated, which were found to have the desired quality attributes *viz.* particle size within 1-5 µm, homogeneous PDI, positive zeta potential, and highest possible entrapment efficiency. The microparticles were further converted into gel to improve their spreadability and applicability around the wound. Subsequently, *in vitro* release tests were conducted on microparticles and gels, revealing a sustained release profile. Furthermore, the investigation of chitosan microparticles using scanning electron microscopy demonstrated spherical morphology, whereas the examination of gels showed an interwoven crosslinked structure. Additionally, the study demonstrated the antibacterial and antibiofilm efficacy of BPABΦ1-CHMPs, BPKPΦ1-CHMPs, BPSAΦ1-CHMPs, and BPPAΦ1-CHMPs against *A. baumannii, K. pneumoniae, P. aeruginosa,* and *S. aureus* bacterial strains. Furthermore, it has been shown that MBP-CHMPs exhibit a high degree of efficacy in eliminating the biofilm formed by polybacterial strains (*P. aeruginosa* and *S aureus*).

To assess the potency of the developed formulation against infected burn wounds, animal studies were conducted, and the depth and oxygen saturation of wounds were monitored using USG/PA imaging. Additionally, the histopathological study validated the process of re-epithelialization in wounded skin. Further, bacteriophage gel showed effectiveness in infectious wound closure which was observed by diminishing the inflammation and abscess formation and enhancing the formation of fibroblast and collagen. In addition, no skin irritation was found after the wound healing study. In

addition, histopathological examinations validated the findings of *in vivo* wound healing investigations utilizing USG/PA imaging. The ultrasound images provided clear visual evidence of the heightened extent of damage to the skin layer on the third day. Subsequently, as the burn wound underwent the process of recovery, there was a gradual reduction in the depth of the wound, which became evident by the fourteenth day. Since the 21st day following the burn incident, the depth of the wound has been significantly reduced to a minimal level through the application of a bacteriophage solution. In addition, the percentage of sO2 gradually increased as the treatment duration increased, and it was observed to be higher in the groups receiving the bacteriophage therapy. Most notably, in the first week after a burn, hypoxia was shown to be higher in the burn's center than in its periphery, which may be correlated to dermal injury and poor blood flow. The animal study and histological analysis confirmed that BPs-CHMPs and BPs-CHMPs-gel enhanced epidermal healing and granulation surrounding the burn region, demonstrating the improved wound-healing efficiency of bacteriophage formulation. This may be because BPs are highly selective for antibiotic-resistant strains, effectively inhibiting the proliferation of invading bacteria at the location of the burn wound. The stability of the lytic activity of phage by microparticle entrapment has been seen to last for four months, therefore demonstrating its adequacy for personalized therapy. Our research raises awareness and is a modest step towards bacteriophage formulations as personalized therapy. However, there is still much room for improvement in areas such as piolet scaling, genome sequencing, kinetic data generation, and clinical trials.

6.2 Conclusions

The proliferation of antibiotic-resistant bacteria poses an urgent threat to the current inventory of antibiotics. Biofilm-related burn wound infections caused by AMR bacterial strains can be difficult to treat with clinically available antibiotics.

Bacteriophages are extensively present biological entities in the natural environment and have proven notable efficacy in combating and eradicating bacteria that have developed resistance to multiple drugs. BPs therapy has evolved as an effective personalized therapy in such cases; however, the loss of biological activity with time and the treatment of wound infections involving more than one MDR strain (poly bacterial infections) are the major challenges. In this work, we have developed the different bacteriophage-loaded chitosan microparticles by using a direct ionic gelation method. The microparticles were formed by crosslinking and ionic interactions between the chitosan-free amine group and the phosphate group of the sodium TPP. Further, the developed microparticles were dispersed into the "SEPINEO™ P 600" by gentle stirring. SEPINEO™ P 600 is a readily available vehicle, which, upon gentle stirring after the addition of water, forms gels. Further in the present work, we also successfully demonstrated the loading of single and more than one BPs (acting on a different host) into the CHMPs without compromising their biological activity and efficacy in poly-bacterial infections. The developed formulation was able to sustain the release of the BPs for a longer period and the stability study showed that the prepared formulations maintained their biological activity for up to four months. A series of studies demonstrated the superior efficacy of the developed formulation as compared to the marketed formulation; SSD (1%) which is considered a gold standard for treating burn wound infections. The proposed formulation strategy can certainly be used for personalized therapy where the available therapy options are not effective.