

# **Bacteriophages formulation strategy against burn wound bacterial infections**

## **Abstract**

Antimicrobial Resistance (AMR) is one of the growing challenges that occurs when bacteria, viruses, fungi, and parasites no longer respond to medicines. Common bacterial species responsible for nosocomial chronic wound infections include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*. In addition, the infected bacteria form biofilms around wounds, delaying wound healing and increasing mortality rates which require prolonged hospitalization.

To overcome these challenges, bacteriophage (BP) therapy has been developed as an alternative to the existing antibiotic therapy. It was observed that BPs are self-replicating viruses that can lyse multidrug-resistant bacteria without infecting the eukaryotic cells. Although BP has been proven to be effective against MDR bacterial infections, maintaining BP biological stability (lytic activity) due to environmental conditions (either during processing, formulation development or at the site of application) is difficult. Moreover, there has been limited growth in the quantification of mixed BP, which acts on different host bacterial strains.

After considering all these challenges, we have developed BP-loaded chitosan microparticles laden gel (BP-CHMPs-gel) for the treatment of MDR biofilm-mediated burn wound bacterial infections. Further to provide a local action, the developed CHMPs were incorporated into a gel, which was accomplished using SEPINEO™ P 600 which improves the stability, applicability, and texture of the final formulation. Henceforth, in the current study, we have isolated phages (BPKPΦ1, BPABΦ1, BPPAΦ1, and BPSAΦ1) against MDR *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Staphylococcus*

*aureus* and developed the BP-loaded microparticle-laden gel to assess the therapeutic effect of lytic bacteriophage formulation on the biofilm-associated infection. The isolate phages are classified into different classes (*Corticoviridae*, *Siphoviridae*, and *Caudoviricetes* classes) based on morphology observed by cryo-TEM. The prepared microparticles were spherical and found to have a particle size within the range of 1-5 $\mu$ m, demonstrating sustained release for up to 12 h. Interestingly, the biological activity of the entrapped phages was maintained for even more than 4 months. Further the phage loaded microparticles BPAB $\Phi$ 1-CHMPs, BPKP $\Phi$ 1-CHMPs, BPSA $\Phi$ 1-CHMPs, BPPA $\Phi$ 1-CHMPs showed biofilm eradication efficacy of 94.46 $\pm$ 2.19, 79.23 $\pm$ 2.08, 88.43 $\pm$ 1.04, and 81.31 $\pm$ 0.53%, respectively. Furthermore, the mixed phage (BPSA $\Phi$ 1 and BPPA $\Phi$ 1) loaded microparticles were found effective against poly bacterial infection caused by *S. aureus* and *P. aeruginosa* with 85.68 $\pm$ 0.28% of biofilm eradication efficacy. The formulation's efficacy against infected burn wounds was tested in animals, and USG/PA imaging monitored wound depth and oxygen saturation. Re-epithelialization in damaged skin was confirmed by histopathology. The results demonstrated that the phage formulations treated groups showed a decrease in wound volume and >80% of wound closure in comparison with infection control groups. Collectively, data suggest the superiority of phage therapy to treat biofilm-mediated wound infections due to MDR singular and polybacterial infection.