OPTIMIZATION OF A MULTIPLE WATER-IN-OIL-IN-WATER NANOEMULSION ENCASING BACTERIOPHAGES FOR INHALATIONAL ANTIBIOTHERAPY

Castro, L.M.[1], Azevedo, A.F.[1], Moura, A.[2,3], Matos, C.M.[1], Moutinho, C.G.[1,2,*], Teixeira, J.A.[2], Azeredo, J.C.[2], Balcão, V.M.[1,2]

[1]Bioengineering and Biopharmaceutical Chemistry Research Group (GIBQB), Fernando Pessoa University, Rua Carlos da Maia nº 296, P-4200-150 Porto, Portugal. [vbalcao@ufp.edu.pt]

[2]IBB - Institute for Biotechnology and Bioengineering, Centre for Biological Engineering, Universidade do Minho, Campus de Gualtar, P-4710-057 Braga, Portugal. [jateixeira@deb.uminho.pt]

[3]Jean Piaget Health College, Alameda Jean Piaget, Vila Nova de Gaia, P-4405-678 Gulpilhares, Portugal.

Infectious bacterial diseases still remain the main cause of human premature deaths, especially in developing countries. The emergence and spread of pathogenic bacteria resistant to many chemical antibiotics (multidrug-resistant strains) have created the need for the development of novel therapeutic agents.

Bacteriophages have proven to be an interesting and effective alternative in the management of persistent bacterial infections where conventional chemical antibiotherapies fail. The lethality and specificity of bacteriophages for specific bacteria, their ability to replicate within bacterial hosts and safety of these human-friendly viruses makes them highly lethal antibacterial agents, besides being efficient and relatively cost-effective.

Group A streptococci (GAS) are serious human pathogens that cause infections ranging from mild pharyngitis, tonsillitis, to chronic rheumatic heart disease and, in some cases, severe streptococcal toxic shock syndrome and necrotizing fasciitis. The frequency and severity of GAS infections has been increasing over the last decades, which has promoted extensive research on the improvement of naturally occurring antimicrobials as alternatives to their conventional chemical counterparts.

In this research effort, development and optimization of a biotechnological process for the inhalational administration of a bacteriophage was pursued, using strategies of nanoencapsulation within lipid nanovesicles. This method of targeting may have a high potential for the treatment of bacterial infections of the respiratory tract, caused mainly by *Streptococcus pyogenes*. As a *proof-of-concept* for the nanoencapsulation strategy, and since there is not yet available a strictly lytic bacteriophage cocktail for *Streptococcus pyogenes*, a well-defined and characterized bacteriophage was utilized, viz. bacteriophage T4.

Water-in-oil-in-water (W/O/W) multiple emulsions are nanosystems in which dispersions of small water droplets within larger oil droplets are themselves dispersed in a continuous aqueous phase. Due to their compartimentalized internal structure, multiple emulsions present important advantages over simple O/W emulsions for encapsulation of biomolecules, such as the ability to carry both polar and non-polar molecules, and a better control over releasing of therapeutic molecules. Bacteriophage T4 was accordingly entrapped within W/O/W multiple nanoemulsions, aiming at mimicking the multifunctional design of biology, optimized with several lipid matrices, poloxamers and stabilizing layer compositions. Physicochemical characterization of the optimized bacteriophage-encasing nanovesicle formulations encompassed determination of particle (hydrodynamic) size, size distribution and particle charge (Zeta potential), via Dynamic Light Scattering analysis, surface morphology via Cryo-SEM, and thermal analysis via DSC, whereas antimicrobial activity of the nanoemulsions produced were evaluated via the "spot-test" using appropriate bacterial cultures.

***Corresponding author:** Carla Moutinho [carlamo@ufp.edu.pt]