

# Development of an inhalational therapeutical system based on bacteriophages to treat pharyngo-tonsillitis: a nanoencapsulation approach.

Castro, L.M.<sup>1</sup>, Azevedo, A.F.<sup>1</sup>, Moura, A.<sup>2,3</sup>, Santos, S.<sup>2</sup>, Matos, C.M.<sup>1</sup>, Moutinho, C.G.<sup>1,2</sup>, Teixeira, J.A.<sup>2</sup>, Azeredo, J.C.<sup>2</sup>, Balcão, V.M.<sup>1,2,\*</sup>

<sup>1</sup>Bioengineering and Biopharmaceutical Chemistry Research Group (GIBQB), Fernando Pessoa University, Rua Carlos da Maia nº 296, P-4200-150 Porto, Portugal. [15204@ufp.edu.pt]

<sup>2</sup>IBB - Institute for Biotechnology and Bioengineering, Centre for Biological Engineering, Universidade do Minho, Campus de Gualtar, P-4710-057 Braga, Portugal.

<sup>3</sup>Jean Piaget Health College, Alameda Jean Piaget, Vila Nova de Gaia, P-4405-678 Gulpilhares, Portugal.

Inflammatory diseases that occur in the pharynx and involving both the adenoids and tonsils are important not only for being very frequent, but also because they often require minor surgery for their resolution. These structures have immunological functions leading to production of antibodies, and work in the local immunity of the pharynx and protection of the entire body. The most common etiologic agent of sore throats is *Streptococcus pyogenes*, an important pathogen of the beta-hemolytic group A which causes streptococcal pharyngitis. The emergence of antibiotic-resistant bacterial strains and the poor penetration of chemical antibiotics in bacterial biofilms raise the need for safe and effective options of antimicrobial treatment. The application of bacteriophages (or cocktails therefrom) has been proposed as an alternative (or complement) to conventional chemical antibiotics, allowing the release of natural predators of bacteria directly on these biofilms. The major advantage of bacteriophage-based antibiotherapy relative to its conventional chemical counterpart is that bacteriophages replicate at the site of infection, being available in abundance where they are needed the most. When compared with chemical antibiotics, bacteriophages have other important advantages: (i) strong tissue permeability, (ii) bacteriophage concentration remains high at the focus of infection, continuously increasing with bacterial (host) presence, (iii) elimination of the focus of infection occurs only after eradication of the host bacterium, (iv) bacteriophages are fully compatible with antibiotics and may act synergistically, (v) they are specific against the target bacteria, (vi) have a superior ability to penetrate bacterial biofilms, inducing production of enzymes that hydrolyze the biofilm polymeric matrix, (vii) although bacteria can develop resistance to bacteriophages, isolation of new lytic bacteriophages is much simpler and cheaper than developing a new chemical antibiotic. In this research effort, development of a biotechnological process for the inhalational administration of a bacteriophage cocktail (endotoxin free) was pursued, using strategies of nanoencapsulation within lipid nanovesicles (as forms of protection for the bacteriophage against the immune system) to treat infectious pathologies such as pharyngo-tonsillitis caused by *Streptococcus pyogenes*. This method of targeting may have a high potential for the treatment of bacterial infections of the respiratory tract, since inhalation therapy is considered to be favorable to certain respiratory infections because the aerosol is delivered directly at the site of infection, accelerating the action of bacterial predators. Additionally, a smaller amount of bioactive substance is needed, thus preventing or reducing possible side effects. 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 compartmentalized 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. T4 bacteriophage was 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 size, size distribution and particle charge, via Zeta potential analysis, surface morphology via CRYO-SEM, and thermal analysis via DSC.