High potential polymer carriers to enhance antibiotic efficiency in bacterial biofilms

Cystic fibrosis (CF) is a frequent genetic disease resulting in chronic and difficult-to-treat pulmonary infections and inflammation that needs a lifelong treatment with aggressive antibiotic therapy by oral, intravenous or inhaled routes. The most abundant bacterial species in the lungs of CF patients are *Staphylococcus aureus* and *Haemophilus influenzae*, both are among the first pathogens to colonize the lungs at early age, progressively followed by *Pseudomonas aeruginosa, Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans* and *Burkholderia cenocepacia* complex (*Bcc*). Thereby, infections with *P. aeruginosa* and *Bcc* are associated with high mortality due to the recurrent formation of antibiotic-resistant biofilms in the abnormal CF mucus. Biofilms are microbial communities embedded in a self-produced polymeric matrix. Both, biofilm matrix and mucus hinder an efficient treatment with inhaled antibiotics, such as tobramycin, aztreonam lysine and colistin that represent the cornerstone of guideline-based treatment of CF.

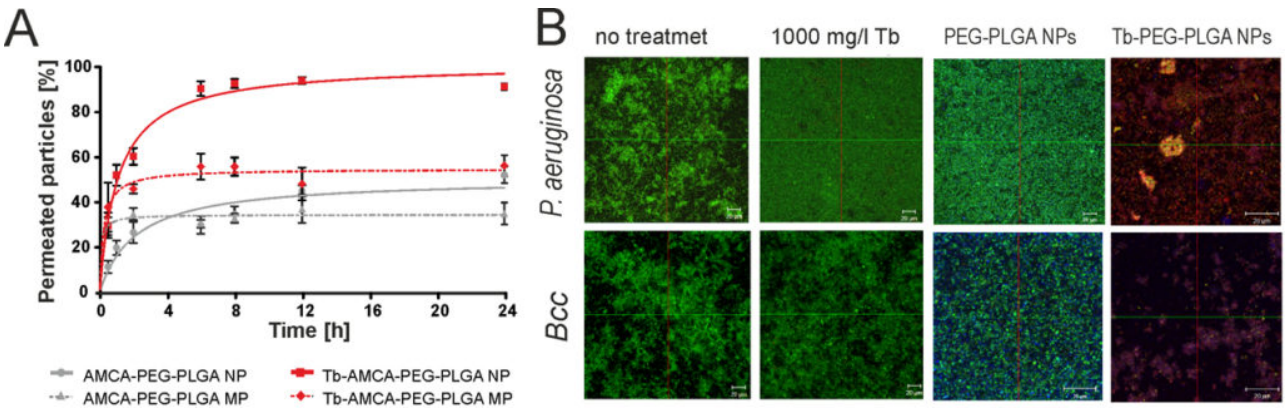

Fig. 1. A. *In vitro* permeation kinetics of AMCA-labelled PEG-PLGA nanoparticles (NP), Tb-PEG-PLGA NP, PEG-PLGA microparticles (MP) and Tb-PEG-PLGA MP through artificial mucous (AM) by the ThinCert™ chamber assay. The percentage of permeated particles across the mucus layer over a time period of 24 h was analysed by fluorescence measurement (mean ± SE, n = 6). B. CLSM images of *P. aeruginosa* and Bcc biofilms under static conditions in AM treated with PBS (no treatment) or with 1000 mg/l free tobramycin (Tb) or with 500 mg/l empty PEG-PLGA NP or with 500 mg/l Tb-PEG-PLGA NP (corresponding to 0.77 mg/l Tb). To visualize the particles in fluorescently live/dead (green/red) stained biofilms, a part of the PLGA was covalently bound to blue fluorescent 7-amino-4-methyl-3-coumarinylacetic acid (AMCA).

In our study, tobramycin (Tb) was encapsulated by poly(D,L-lactide-co-glycolide) (PLGA) and (poly(D,L-lactide-co-glycolide)-co-poly(ethylene glycol)diblock (PEG-PLGA). Nanoparticles (NPs) with 225-231 nm, and microparticles (MPs) with 896-902 nm in size and negative zeta potentials (ZP) were produced and their physicochemical properties determined *in vitro*. The incorporation of

Tb increased the ZP of the particles to nearly neutral values (-11 mV) and enhanced the permeation of the particles through artificial mucus (AM). The Tb-PEG-PLGA NPs showed the most efficient permeation properties (Fig. 1A) indicating a high potential to overcome the mucosal barrier in CF patients.

These NPs and MPs (500 mg/l) were used to treat matured biofilms of *P. aeruginosa* and *Bcc* in AM under static and fluidic conditions. The used *P. aeruginosa* strain ATCC 27853 was susceptible to Tb while *Bcc* ATCC 25416 was resistant to Tb according to the minimal inhibitory concentrations (MICs were 0.25 mg/l and 16 mg/l, respectively) determined in planktonic bacteria. The minimal biofilm eradication concentration (MBEC) of pure Tb that was defined as reduction of viable cells by three log10 magnitudes was >1000 mg/l in *Bcc* and *P. aeruginosa*, which is not a feasible concentration for treating patients. The encapsulation strongly enhanced the effectiveness against biofilms of both pathogens under static and fluidic experimental conditions. The biofilm-embedded bacteria were killed by less than 0.77 mg/l encapsulated Tb, whereas 1000 mg/l of free Tb, was still ineffective in killing the biofilms as determined in live/dead stained biofilms by confocal scanning microscopy (CLSM) (Fig. 1B). No cytotoxicity was detected *in vitro* in human lung epithelial cells with any particle formulation for concentrations between 10 mg/l and 1000 mg/l particles.

Aminoglycosides inhibit protein biosynthesis by binding to the bacterial ribosome. They cross the outer membrane of gram-negative cells via Mg^{2+}-dependent self-promoted uptake that depends on the transmembrane electrical potential. The restored activity against *Bcc*, which is intrinsically resistant to Tb due to its inert outer membrane, strongly supported the idea that PEG and/or PLGA destabilize the outer membrane synergistically supporting the uptake of Tb into the metabolically inactive biofilm-embedded pathogens.

In our opinion, the biodegradable and biocompatible PEG-PLGA nanoparticle formulation that have been already approved as drug carriers for inhaled application by regulatory authorities, might improve inhaled CF therapy in the future by enhancing the antimicrobial activity of aminoglycosides and minimizing critical side effects.

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Publication

Polyester-based particles to overcome the obstacles of mucus and biofilms in the lung for tobramycin application under static and dynamic fluidic conditions.
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