Nebulized polysaccharide-based polyplexes unlock efficient pulmunary RNAi therapy

Sofia Bonsignore*, Emanuela Fabiola Craparo, Salvatore Emanuele Drago, Cinzia Scialabba, Gennara Cavallaro

Lab of Biocompatible Polymers, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Via Archirafi 32, Palermo, 90123, Italy.

*Presenting Author Email: sofia.bonsignore@unipa.it

Translating local siRNA administration into effective chronic lung disease treatments relies on designing smart nanovectors that successfully overcome physiological barriers to enable site-specific cellular internalization[1]. In this study, to develop low-toxicity graft copolymers, the natural, biocompatible polysaccharide pullulan (PULL) was selected as a starting material due to its hydroxyl-rich backbone, which is suitable for chemical tailoring [2]. Following controlled acid hydrolysis to tune its molecular weight, the resulting PULL backbone was functionalized with 1,2-bis(3-aminopropylamino)ethane (bAPAE) to yield a cationic derivative and decorated with all-trans-retinoic acid (ATRA) to introduce hydrophobic character alongside potential anti-inflammatory activity[3]. Potentiometric titration revealed a pH-dependent multi-step protonation behavior for PULL-bAPAE, supporting both nucleic acid complexation at physiological pH and buffering capacity to drive endosomal escape, while the amphiphilic PULL-bAPAE-Ret copolymer exhibited self-assembly behavior at low concentrations. Full complexation of siRNA was achieved at low polymer-to-siRNA weight ratios, specifically 6 for PULL-bAPAE and 5 for PULL-bAPAE-Ret, corresponding to N/P ratios of 1.45 and 1.17, respectively. The resulting polyplexes demonstrated N/P dependent stability in mucin dispersions and strong protection against RNase enzymatic cleavage. Evaluation of aerosol performance using the BEURER IH55 vibrating mesh nebulizer confirmed structural integrity after nebulization, with minimal alteration in hydrodynamic size and high recovery yields for both components. Aerodynamic characterization confirmed the suitability of the formulation for targeted lower-airway delivery. In vitro studies demonstrated increased cellular uptake, efficient endosomal escape, and enhanced gene knockdown for PULL-bAPAE-Ret compared to PULL-bAPAE, with post-nebulized polyplexes maintaining significant gene silencing efficacy. In conclusion, these results establish rationally engineered amphiphilic PULL copolymer as promising, highly adaptable carrier for

pulmonary siRNA delivery, combining optimal physicochemical features, biological efficacy, and suitable aerosol properties for the localized treatment of chronic lung diseases.

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