Design of lectin-functionalized lipid nanocarriers as therapeutics for pulmonary biofilm associated infections

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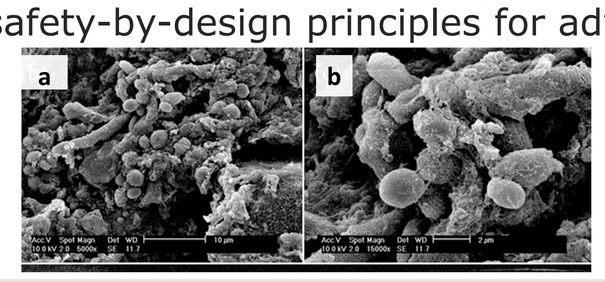
INTRODUCTION & HYPOTHESIS

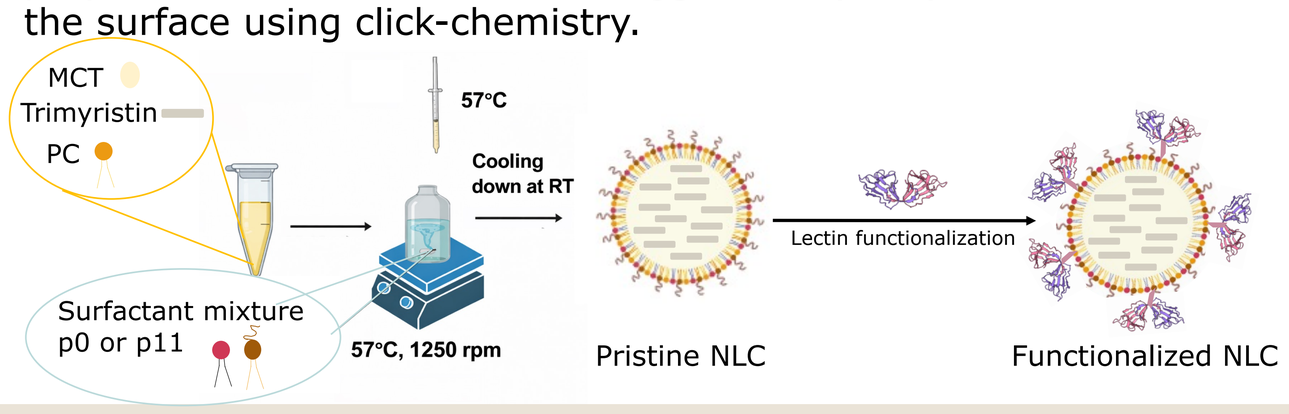
Pathogenic bacteria transition from planktonic cells to biofilm communities, which are common in chronic lung infections such as cystic fibrosis, chronic obstructive pulmonary disease (COPD), and ventilator-associated pneumonia[1]. Encased within an extracellular matrix, biofilms enhance antibiotic resistance and facilitate gene transfer, contributing to persistent infections[2]. To address rising antimicrobial resistance, this work develops lectin-targeted lipid nanoparticles against respiratory pathogens, guided by safety-by-design principles for advanced nanomaterials[3].



120-h postintubation endotracheal tube: a) SEM image at 5000x; b) SEM image at 15000x focusing in a cluster of bacteria cells (Adapted from [1])

METHODOLOGY

Nanostructured Lipid Carriers (NLC) based on trimyristin, medium chain triglyceride (MCT) and phosphatidylcholine(PC) with varying surfactant composition were synthesized using solvent injection method, building on their previously demonstrated antibiofilm properties[4]. *Galanthus nivalis* agglutinin (GNA) was attached to the surface using click-chemistry.



RESULTS

PHYSICOCHEMICAL CHARACTERIZATION

Two prototypes using different surfactants, encoded as NLC-p0 and NLC-p11, were developed (table 1). Analysis of independent batches (4-6 batches) demonstrated reproducibility and stability for both prototypes (Figure 1A). Fluo-NTA showed homogeneous probe distribution within particles (Figure 1B), with no leakage under physiological conditions, enabling investigation of biotransformation in pulmonary fluids and bacterial and cell culture media.

Table 1. NLC surfactant composition and physicochemical characterization by DLS

NLC prototype	Surfactant composition	Size (nm)	PDI	Z potential (mV)
NLC-p0	PEG stearate 40 Poloxamer F68	160 ± 4	0.180	-30 ± 3
NLC-p11	Tween 80 Poloxamer F68	170 ± 8	0.200	-26 ± 2

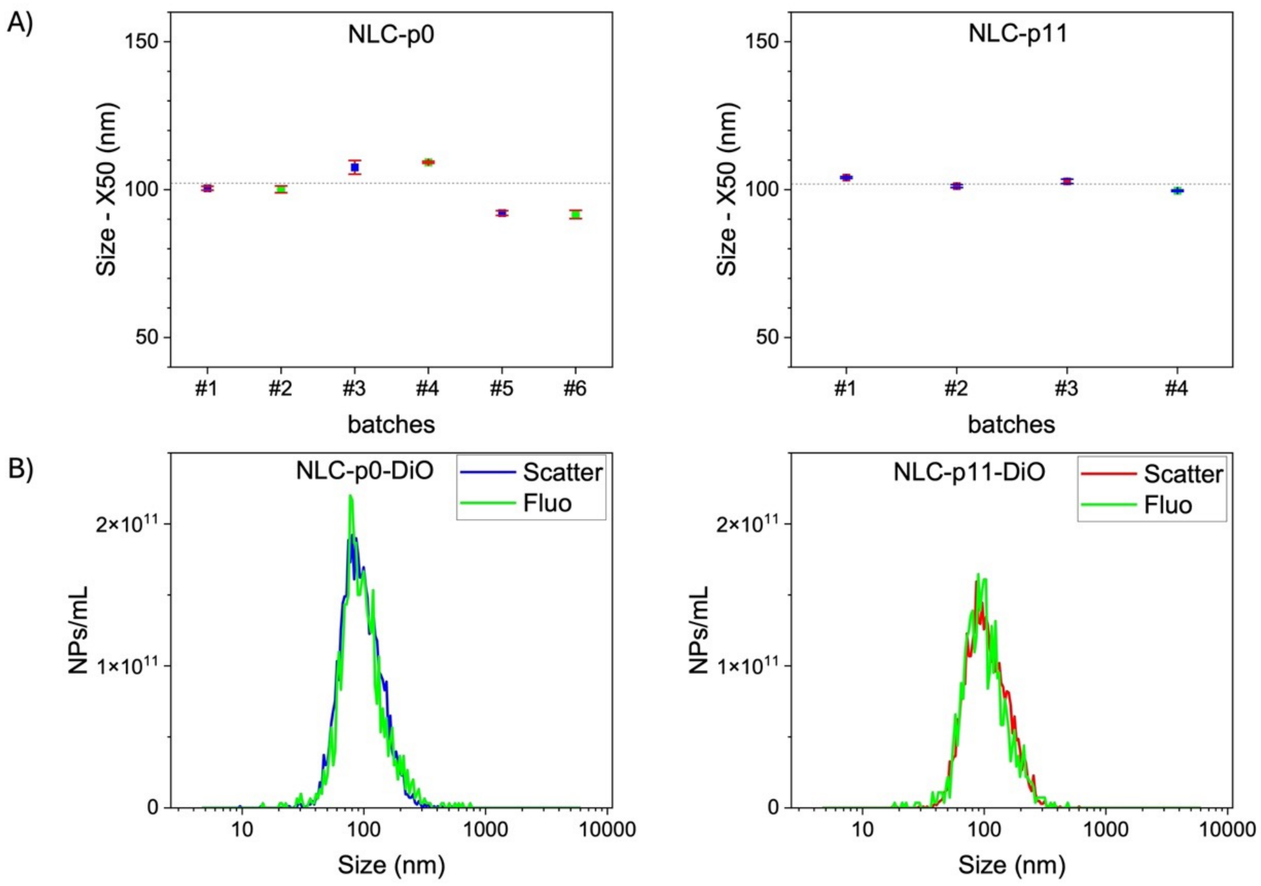


Figure 1. Median size (X50) obtained by NTA for different batches of NLC-p0 and NLC-p11 (A); size distribution by NTA of fluorescent NLC-p0 and NLC-p11 in scatter (blue, red) and fluorescent mode (green) (B).

REFERENCES: [1] Heidari, A., *et al*. J. Biomed. Opt. 20(12), 126010; [2] Liu, H.Y., *et al.,* NPJ Antimicrob Resist 2, 27 (2024) [3] Malaekeh-Nikouei, B., *et al.*, J. Drug Deliver. Sci. Tech., 60, 10188 (2020); [4] PCT/ES2025/070765.

BIOLOGICAL CHARACTERIZATION

Co-cultures of alveolar epithelial cells (A549), endothelial cells (EA.hy926), and monocyte-derived macrophages (dTHP-1), maintained on inserts at an air–liquid interface (ALI) were used as a model. Cellular secretion of cytokines (IL-6 and IL-8) was measured to evaluate pro-inflammatory responses to the NLC, whereas LDH release was used as an indicator of cytotoxicity. The release of cytokines induced by NLC-p0 appears to be linked to batch contamination rather than its composition (Fig. 2A & 2B). NLC-p0 showed mild cytotoxicity, while NLC-p11 showed a significant cytotoxicity (Fig. 2C). The inclusion of a fluorescent probe do not affect the cytotoxicity. We also determined that double autoclaving (Fig. 2D, MN-2A & MR-2A) is the most effective procedure for reducing endotoxin content.

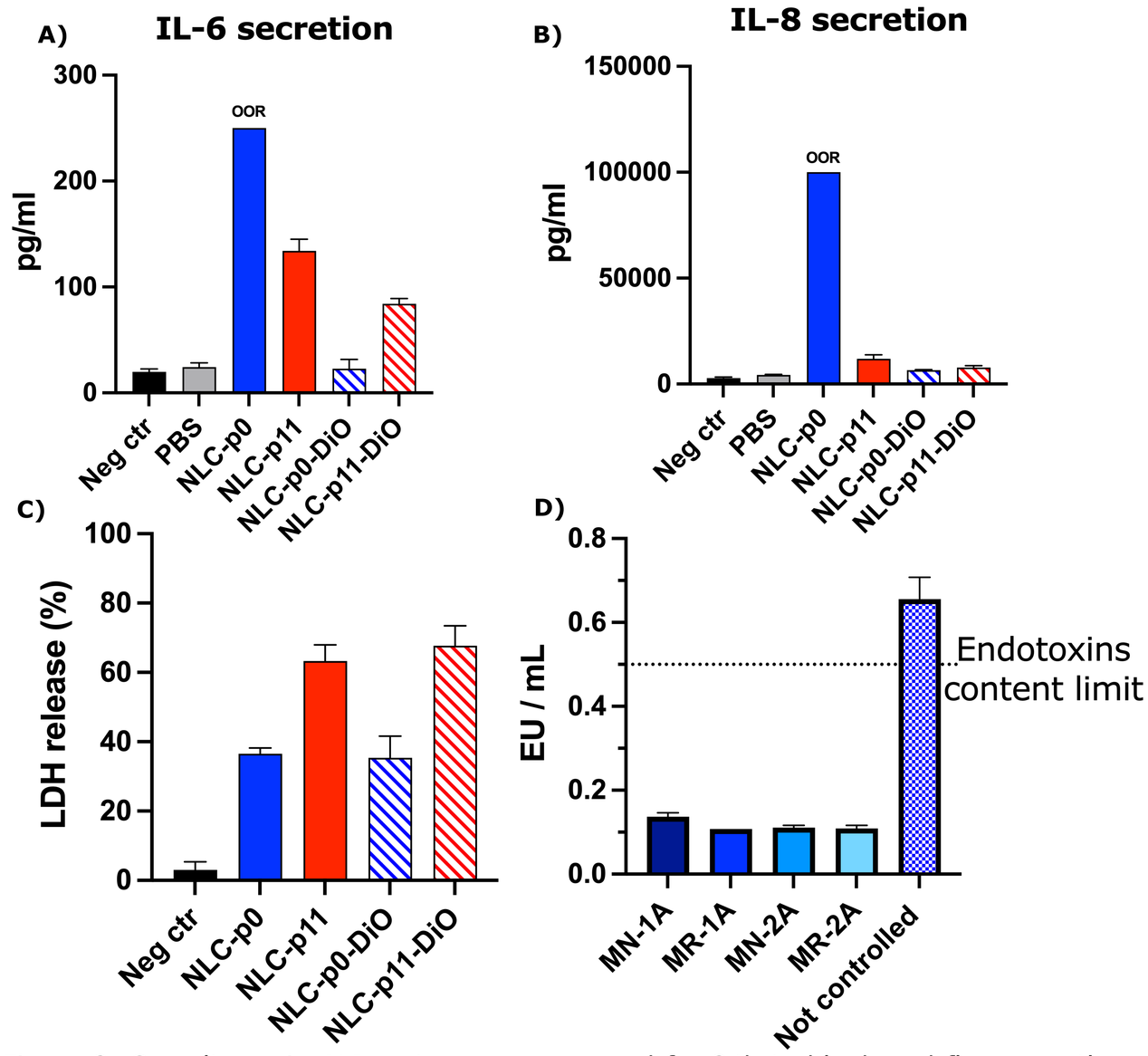


Figure 2. Co-cultures in ALI system were exposed for 24h to blank and fluorescently labelled (DiO) NLC-p0 and NLC-p11 at 119 µg/cm². Negative control: Cell culture medium. Cellular secretion of IL-6 (**A**), IL-8 (**B**) and LDH release (**C**). Out of range (OOR): Treatments above the highest curve concentration. (**D**) Endotoxin levels in NLC-p0 synthesized using single-use (MN) or reusable (MR) TFF membranes and one (1A) or two (2A) autoclave cycles. (All data from a single experiment in triplicate).

CONCLUSIONS AND ACKNOWLEDGEMENTS

Physicochemical analyses on NLC developed confirmed reproducibility, batch uniformity, and stability in biological environments. Also, they remain consistent after incorporation of fluorescent probes. Functionalization with lectins is expected to expand their uses. ALI coculture results indicate that NLC-p0 exhibits superior cytocompatibility with lowest LDH release. Therefore, NLC-p11 requires further modification to improve biocompatibility without compromising its functional properties.

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