Supporting Information

Surface charge determines the lung inflammogenicity: a study with polystyrene nanoparticles

Jeong Eun Kim^{1,*}, Sunay V. Chankeshwara^{2,*}, Frank Thielbeer², Jiyoung Jeong¹, Ken Donaldson³,

Mark Bradley², Wan-Seob Cho¹

¹Lab of Toxicology, Department of Medicinal Biotechnology, College of Natural Resources and Life Science, Dong-A University, Busan, Republic of Korea, ²School of Chemistry, West Mains Road, University of Edinburgh, Edinburgh, UK, ³ELEGI/Colt Laboratory, Centre for Inflammation Research, University of Edinburgh, Edinburgh, UK

*Both authors contributed equally to this work.

Synthesis of polystyrene NPs

Chemicals: Fmoc-Ser(t-Bu)-OH and Fmoc-Asp(t-Bu)-OH were purchased from GL Biochem (Shanghai, China). Fmoc-Arginine(Boc)₂-OH was purchased from Novabiochem (Merck, Darmstadt, Germany). Fmoc-HN-PEG₂-OH was purchased from IRIS Biotech GmbH (Marktredwitz, Germany). Fmoc-Met-OH, glutaric anhydride, 4-sulfobenzoic acid potassium salt, 2,20-azobis(2-amidinopropane) dihydrochloride (V-50), divinylbenzene (DVB), methanol, diisopropylcarbodiimide (DIC), piperidine, pyridine, sodium acetate, styrene, *N,N*-Diisopropylethylamine (DIPEA) and triethylamine were purchased from Sigma-Aldrich (Gillingham, UK). Acetic anhydride, magnesium sulfate (MgSO₄), sodium hydroxide (NaOH), sodium chloride (NaCl), and tris(hydroxymethyl)aminomethane were purchased from Fisher Scientific (Loughborough, UK).

Ethyl 2-cyano-2-(hydroxyimino) acetate (Oxyma) was purchased from Apollo Scientific (Stockport, UK). Dimethylformamide was purchased from Rathburn (Walkerburn, UK). Phosphate buffered saline (PBS) was purchased from Oxoid (Basingstoke, UK). Trifluoroacetic acid (TFA) was purchased from Acros Organics (Geel, Belgium). 4-Vinylbenzylamine hydrochloride (VBAH) and 3',6'-Dihydroxy-5-(4-vinylphenyl)-3H-spiro[isobenzofuran-1,9'-xanthen]-3-one (polymerisable fluorescein derivative) were synthesized following literature procedures (Thielbeer et al. 2011).

Aminomethyl-functionalized NPs: Styrene (3.1 mL, 25 mmol, freshly washed with 4 × 25% NaOH and 4 × brine), divinylbenzene (DVB) (0.08 mL, 0.5 mmol, 2 mol % with regards to styrene, freshly washed with 4 × 25% NaOH and 4 × brine), *p*-vinylbenzylamine hydrochloride (VBAH) (0.15 g, 0.88 mmol, 3 mol %), polymerisable fluorescein derivative (0.38 g, 0.88 mmol, 3 mol %), 2,2-azobis (2-methylpropionamidine) dihydrochloride (V-50) (0.04 g, 0.15 mmol), and MgSO₄ (0.01 g, 0.075 mmol) were added to N₂-purged water (50 mL) and stirred for 15 min under a N₂ atmosphere. The reaction mixture was stirred at 80 °C for 15 h. The particles were collected by centrifugation (15 min at 30 000 g) and washed with DMF (3 × 25 mL), MeOH (3 × 25 mL), and water (3 × 25 mL). The hydrodynamic diameter of the particles was determined to be 215 nm by dynamic light scattering (Malvern Zetasizer Nano-ZS, Malvern Instruments, Worcestershire, UK). The amino loading of the particles was determined as 31 μmol/g by a quantitative Kaiser test with a solid content of 2.3 ± 0.04% (23 mg/mL).

Acetylated fluorescent NPs: Precursor fluorescent NPs (aminomethyl-functionalized NPs) (23 mg, 1 mL, 31 μmol, 1 equiv) were washed with DMF (3 × 2 mL), and the supernatant was removed. A solution of acetic anhydride, pyridine, and DMF (2:3:15 (v/v/v), 2 mL) was added and the reaction mixture was shaken for 30 min. The NPs were centrifuged, the supernatant was removed, and the acetylation reaction was repeated. The reaction mixture was removed and washed with DMF (3 × 1 mL) with centrifugation (30 000 g for 15 min). The resultant NPs suspended in 5% aqueous NaOH solution and the reaction mixture was shaken for 30 min, the supernatant was removed, and the

procedure was repeated. Finally, the NPs were washed with MeOH (3×25 mL), and water (3×25 mL) and reconstituted with water (1 mL).

Carboxylated fluorescent NPs: Precursor fluorescent NPs (aminomethyl-functionalized NPs) (23 mg, 1 mL, 31 μ mol, 1 equiv) were washed with DMF (3×2 mL), and the supernatant was removed. A solution of glutaric anhydride (17 mg, 0.15 mmol, 5 equiv), DIPEA (26 μ L, 0.15 mmol, 5 equiv) in DMF (1.5 mL) was added and the reaction mixture shaken for 30 min. The NPs were centrifuged, the supernatant was removed, and the reaction was repeated. The reaction mixture was removed and washed with DMF (3×1 mL) MeOH (3×1 mL), and water (3×1 mL) with centrifugation (30 000 g for 15 min) and reconstituted in water (1 mL).

Modification of aminomethyl fluorescent NPs- general procedure for *GD*F-PLNP, *ZI*F-PLNP, *OH*F-PLNP, *PEG*F-PLNP, and *SO_{3H}*F-PLNP by Oxyma/DIC coupling: Ethyl 2-cyano-2-(hydroxyimino) acetate (oxyma)/diisopropylcarbodiimide (DIC) couplings were carried out. The acid (4-sulfobenzoic acid potassium salt (36 mg, 0.15 mmol, 5 equiv), Fmoc-HN-PEG₂-COOH (58 mg, 0.15 mmol, 5 equiv), Fmoc-Asp(tBu)-OH (62 mg, 0.15 mmol, 5 equiv) and Fmoc-Ser(tBu)-OH (58 mg, 0.15 mmol, 5 equiv) were pre-activated in DMF (1 mL) by the addition of Oxyma (21 mg, 0.15 mmol, 5 equiv) and DIC (19 mg, 26 μ L, 0.15 mmol, 5 equiv) for 5 min. The pre-activated acid was added to the suspension of amino-functionalized NPs in DMF (23 mg, 1 mL, 31 μ mol, 1 equiv), and the reaction mixture was heated to 60 °C by microwave irradiation (Initiator 60 microwave, Biotage) for 20 min. The coupling was repeated three times. Upon completion, the NPs were collected by centrifugation (Eppendorf, 5430R) and washed with DMF (3×1 mL).

Fmoc-Deprotection of NPs for *ZI*F-PLNP and *OH*F-PLNP: The NPs were washed in DMF (3×2 mL) and the supernatant removed. A solution of 20% piperidine in DMF (2 mL) was added to the NPs and the reaction mixture was shaken for 20 min. The NPs were centrifuged, the supernatant was removed, and the procedure was repeated.

***t*-Butylester and *t*-Butyloxycarbonyl carbamate deprotection for *GD*F-PLNP, *ZI*F-PLNP and *OH*F-**

PLNP: Functionalized NPs with t-Bu and t-Boc protecting groups on side chains of amino acids were washed in DMF (3×2 mL) and resuspended in a solution of 10% TFA in DMF (2 mL). The reaction mixture was shaken for 4 h followed by centrifugation of the NPs, removal of the supernatant, and washing with DMF (3×2 mL) with centrifugation (30 000 g for 15 min). Finally, the NPs were washed with MeOH (3×1 mL), and water (3×1 mL) and reconstituted with water (1 mL).

Figure S1. The morphology of NH_2F -PLNPs analyzed by scanning electron microscopy.

Figure S2. The distribution of *F*-PLNPs in the lung at 24 h after pharyngeal aspiration into rats.

REFERENCE

Thielbeer F, Chankeshwara SV, Bradley M. 2011a. Polymerizable fluorescein derivatives: synthesis of fluorescent particles and their cellular uptake. *Biomacromolecules* 12(12):4386-4391.