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Alkylglyceryl chitosan nanoparticles for drug delivery across the blood–brain barrier

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Targeting therapeutic compounds to the central nervous system (CNS) via systemic administration requires crossing the blood–brain barrier (BBB). This is currently one of the most challenging problems in CNS drug development. A series of alkylglyceryl chitosans with systematically varied degrees of grafting were prepared through synthetic steps that involved the protection of amino moieties via the formation of phthaloyl chitosan. These alkylglyceryl-modified chitosans were formulated into nanoparticles via a standard ionic gelation technique using sodium tripolyphosphate; the stability and size distribution profiles of nanoformulations were determined using dynamic light scattering. The mean diameter of the particles was found to range between 200 and 350 nm, with the zeta potential between +37 and +41 mV. The stability of nanoformulations was investigated under physiological conditions: it was found that an increase in pH from 4 to 7.4 resulted in a raised hydrodynamic diameter of particles and in a corresponding decrease of their zeta potential. A further chemical modification involving a partial quaternisation of the alkylglyceryl-modified chitosan improved the stability of the formulation at neutral pH, as shown by the changes in the zeta potential and particle size. Preliminary *in vitro* tests using mouse-brain endothelial cells demonstrated no toxicity and an efficient uptake and indicated that butylglyceryl chitosan and butylglyceryl N,N,N-trimethyl chitosan nanoparticles are promising formulations for BBB targeting.

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A study of the interaction of novel, coated microparticles with alveolar macrophages and their application in tuberculosis treatment via inhalation

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Introduction: Mycobacterium tuberculosis (MTb) is a pathogenic mycobacterium and the main causative agent of tuberculosis infection in humans. Current treatment involves a multi-dose drug regimen for a minimum of 6–9 months. Approximately 80% of all MTb cases affect the pulmonary region. Despite this fact therapy is currently based on oral and parenteral formulations [1]. Aerosol delivery of anti-tubercular agents aims to reduce the systemic toxicity associated with conventional therapy, to maximise local concentrations of therapeutics in the alveolar region and target alveolar macrophages (AM), the niche environment of the MTb bacilli. We have bioengineered novel, inhalable microparticles designed to efficiently target drugs intracellularly to alveolar macrophages using opsonic coatings. The aims of this study were: (i) to determine the effect of the coatings on the uptake and intracellular trafficking of the microparticles in AMs and (ii) to assess the effect of coated and uncoated microparticles on macrophage activation. *Materials and methods*: Poly-lactide-co-glycolide (PLGA) microparticles were manufactured using a solvent evaporation method and coated with a number of opsonic proteins. THP-1 cells were differentiated using phorbol 12-myristate-13-acetate (PMA) into a macrophage-like cell and where necessary infected with MTb. Non-infected or infected cells were treated with fluorescently labelled microparticles, fixed and counterstained using LAMP-1 and DAMP. Their uptake and intracellular trafficking was visualised using confocal laser scanning microscopy (CLSM). THP-1 blue cells were used to assess the effect of the microparticles on AM activation. This cell line produces a reporter protein when NFkB is activated. These cells were also differentiated using PMA and subsequently treated with microparticles. *Results*: The coated microparticles were efficiently internalised by infected THP-1 cells and showed some degree of co-localisation with MTb after 1 h. Microparticle-treatment led to significant activation of NFkB. The degree of activation was found to be microparticle size and coating dependent. *Conclusion*: Opsonic coating of inhalable microparticles significantly increases their uptake into TB-infected AMs and facilitates co-localisation with the mycobaterium. Previous work by us and others has shown that empty microparticle treatment of MTb infected cells can decrease mycobacterial viability. The increase in NFkB expression associated with microparticle treatment may explain this phenomenon via induction of pro-inflammatory cytokines important for mycobacterium control. Overall this work suggests that microparticles may have immunopotentiator applications in MTb control.

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Development of a high throughput method for screening of novel nanotechnologies for siRNA transfection of airway cells using high content screening (HCS)

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Introduction: RNA interference (RNAi) is an endogenous system in eukaryotic cells whereby sequence-specific RNAs are able to bind and degrade their complementary mRNA. Properly applied, this system could potentially be used to control and treat a wide range of respiratory diseases including cystic fibrosis, lung cancer and inflammatory lung disease. However, siRNA delivery problems encountered in the lungs include poor airway mucus penetration, insufficient cell uptake, poor cell-type specific targeting and rapid clearance. To overcome these problems, we have developed a range of novel nanotechnologies for transfection of airway epithelial cells and alveolar macrophages. The aim of this study was to develop a high throughput method for screening novel nanotechnologies for siRNA transfection of airway cells using high content screening (HCS). *Materials and methods*: A range of polyethyleneimine-polyethyleneglycol (PEI-PEG) polymers was synthesised and complexed with fluorescent siRNA (fl-siRNA) and