## A75

## 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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## A76

## 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.

*Reference*

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## A77

## 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

used to transfect an airway epithelial cell line, Calu-3 cells. A range of targeted, mannosylated liposomes were also synthesized, fl-siRNA was encapsulated therein and these complexes were used to transfect an alveolar macrophage-like cell line, THP-1. Each of the systems was characterised for size, zeta-potential and encapsulation efficiency prior to transfection. To determine the efficiency of fl-siRNA transfection facilitated by these nanoparticles a protocol was specifically designed to qualitatively and quantitatively monitor siRNA uptake using InCell 1000 high content screening. *Results*: A number of the PEI-PEG nanoparticles significantly increased siRNA uptake into Calu-3 cells and a number of the mannosylated liposomes were capable of efficiently transfecting alveolar macrophages, a particularly difficult to transfect cell type. *Conclusion*: HCS facilitated the screening of a large number of novel nanoparticles rapidly and comprehensively for siRNA delivery efficiency, providing both high quality cell images and quantitative data on siRNA uptake, thereby avoiding the need for separate microscopy and quantification studies.

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## A78
## Endosomal DNA release studies using giant unilamellar vesicles as model endosomal membranes

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Endosomal DNA release is one of the main barriers to successful non-viral gene delivery, since the inability of DNA to escape from the endosome at an early stage leads to its degradation through trafficking to the lysosomal compartment. It is therefore essential to understand the interactions between commonly used gene delivery vectors and endosomal membranes. While membrane interactions are often studied using small unilamellar vesicles (SUVs) as model bilayers, it is proposed that giant unilamellar vesicles (GUVs) present more realistic models due to their larger size, their superior lipid packing due to reduced surface curvature and the ability to visualise them using light or confocal microscopy. GUVs composed of a mixture of neutral or neutral and negatively charged lipids, representing early or late stage endosomal membranes respectively were prepared by electroformation in calcein, followed by the addition of cobalt chloride to quench background fluorescence. GUVs were then observed by confocal fluorescence microscopy before and after the addition of lipid:DNA complexes composed of equimolar mixture of dimethyldioctadecylammonium bromide (DDAB) with the helper lipid dioleoylphosphatidyl-ethanolamine (DOPE) incorporating a 10 mol% rhodamine-labelled DOPE at a 4:1 lipid:DNA charge ratio. Furthermore, in order to visualise the DNA in relation to the encapsulated calcein (green) and the lipid (red), 4′,6-diamidino-2-phenylindole (DAPI) was added to highlight the DNA blue. Both endosomal models formed spherical GUVs approximately 10–90 μm in diameter and were visible as green calcein-encapsulating vesicles. Upon the addition of lipid:DNA complexes to the early endosomal model, a large number of GUVs were shown to lose fluorescence due to calcein leakage, which was concentration dependent first order kinetics. This was also associated with visible alignment of the lipid (red) and the DNA (blue) around the GUV with possible pore formation and DNA translocation across the endosomal membrane. When lipid:DNA complexes were added to the late endosomal membrane model (which incorporated a small percentage of anionic lipid), calcein leakage and pore formation on the surface of the GUV membranes were clearly visible. Additionally, and exclusively to this model, however, a high number of GUVs were shown to deform after the addition of the complexes with or without calcein leakage. This was thought to be due to electrostatic interactions between the cationic DDAB and the anionic lipid domains of the endosomal membrane. In conclusion, it is thought that DDAB-DOPE:DNA complexes interact with both early and late endosomal membranes, causing pore formation and DNA translocation across the membrane, however the nature of the interaction changes as the endosomes traffic from early to late stages.

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## A79
## Characterisation of a cytosolic shuttle based upon ricin toxin

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We have cloned and codon optimised both modified ricin B chain (containing N-terminal 6 His and V5 motifs) and disarmed ricin A chain (containing either a deletion (deleted amino acids 177–183) or mutation (amino acids 177–183 mutated to Gly) within the active site). These molecules were expressed in *Escherichia coli* BL21*DE3 and affinity purified from *E. coli* lysate using Talon affinity resin. Following an initial round of characterisation by SDS PAGE and Coomassie brilliant blue staining, Western blotting (using commercially available anti-ricin A or B chain antibodies as well as antibodies specific for N- and C-terminal epitopes) was successfully used to confirm the production of both species of molecule. Both ricin A and B chains were tested for toxicity against a panel of cell lines either individually, after mixing the A and the B chains, or after re-folding using published protocols. Having ascertained that, relative to wild-type ricin A chain, minimal toxicity was displayed by the disarmed A chain analogues, further controls were undertaken to investigate the character of the recombinant B chain. These studies are reported here and show that the recombinant B chain demonstrates both lectinic activity and the ability to translocate to the Golgi, being localised to GM130 positive structures as depicted by epifluorescence microscopy. Further, crude subcellular fractionation and Western blotting of Vero cells exposed to refolded ricin toxin containing disarmed A chain show the disarmed A chain in the cytosol and the differential sedimentation of the B chain within membrane delimited structures. This data suggests the potential of these materials as cytosolic delivery vehicles suitable for use with gene medicines such as antisense oligonucleotides or RNAi agents.

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