A15
Endocytic DNA and siRNA delivery mediated by pH sensitive peptides

Mia S.W. So[1], Katarzyna Witt[2], A. Yun Lan[2], James Mason[2], Jenny K.W. Lam[1,*]
[1] *Department of Pharmacology & Pharmacy, LKS Faculty of Medicine, The University of Hong Kong, 21 Sassoon Road, Hong Kong*
[2] *Pharmaceutical Biophysics Group, Pharmaceutical Science Division, King's College London, Franklin-Wilkins Building, SE1 9NH, UK*

*Corresponding author.
E-mail: jkwlam@hku.hk (J.K.W. Lam).

Amphipathic peptides have emerged as promising candidates for both DNA and siRNA delivery. Consisting of both hydrophilic and hydrophobic domains, they bind to nucleic acids and at the same time provide pH dependent membrane destabilising activity, promoting endosomal escape. The aim of this study was to investigate the efficiency of such peptides in delivering siRNA and plasmid DNA, and to improve our understanding of how the structural difference between the peptides could affect the uptake mechanism and intracellular trafficking of the system. A series of structurally related histidine-rich amphipathic peptides (LAH4-L1, LAH6-X1L, LAH6-X1-26 and LAH6-X1-W) were investigated. The LAH peptides are 25–26 amino acids in length and comprise cationic lysines to allow electrostatic interaction and complexation with the negatively charged nucleic acids. Each of the peptides also contains four or six histidine residues. With a starting pK_a around 6.0, the imidazole group of histidine may allow buffering and subsequently destabilise endosomes, thus enhancing endosomal escape of the nucleic acids. The LAH peptides demonstrated pH responsive character whish is classically manifested as a conversion from an alpha helical conformation at neutral pH to a disordered conformation at acidic pH. Differences in the number of charges and the hydrophobicity in the four peptides affect the nature and pH dependence of this transformation. Luciferase reporter gene studies showed that the *in vitro* DNA transfection efficiency of the LAH peptides were comparable to commercially available lipofectamine in both A549 and MCF-7 cells. These peptides, in particular LAH6-X1L, also showed high resistance to serum in MCF-7 cells. In addition, both LAH4-L1 and LAH6-X1L mediated significant knockdown of GAPDH enzyme in siRNA transfection studies in the presence of serum. Live cell confocal imaging was carried out to study the intracellular trafficking of the peptide/nucleic acid complexes. Co-localisation experiments were performed with LAH6-X1L- DNA/siRNA complexes and dextran in A549 cells, with the nucleic acids labelled with rhodamine (red), nucleus labelled with Hoechst (blue) and dextran lablled with Alexa-fluor-488 (green). Dextran is known to be internalised through fluid-endocytosis and end up in endososmes and later lysosomes. At an early stage (within the first hour of post-transfection) there was a high level of co-localisation between LAH6-X1L complexes and dextran (shown as orange in colour). At later stages (300 minutes post-transfection), the degree of co-localisation significantly reduced as the siRNA (red) and dextran (green) was shown to be clearly separated from each other. Our results indicate that the histidine-rich peptides offer great promise as siRNA delivery vectors with the ability to promote endosomal/lysosomal escape.

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Role of polymer architecture on polycation induced cell death: systematic study on molecular mechanism

Zuzana Kadlecova*, Elisa Corbacella, Florian Maria Wurm, Harm Anton Klok
Ecole Polytechnique Fédérale de Lausanne (EPFL) Laboratory of Polymers, STI - IMX - LP MXD 112 (Batiment MXD), Station 12, CH-1015 Lausanne, Switzerland

*Corresponding author.
E-mail: zuzana.kadlecova@epfl.ch (Z. Kadlecova).

More than 3000 references can be found in the literature that relate to the application of polycations for drug or gene delivery. However, systematic studies that try to correlate polymer molecular weight, architecture and/or composition with polycation induced cell toxicity are scarce, and the underlying biomolecular mechanisms remain largely unknown. In this contribution new findings are presented on the mechanisms of polycation induced cell death and its correlation with the polymer architecture and degradation rate. For our studies, we firstly synthesized a polymer library based on L-lysine monomer units. The library contained linear, hyperbranched and dendritic L-lysine analogues in a broad range of molecular weights. We then investigated the effect of molecular weight (Mn), degree of branching and polydispersity on the mechanism of short and long term cell toxicity *in vitro*. The molecular mechanisms underlying cell death at various stages of cell exposure to polycation were identified. The onset and extent of these specific modes of cell death were shown to be dependent on the size and degree of branching of the polycations. Simultaneously the *in vitro* degradation profile for analogues was assessed and correlated with the process of cell death. For the first time the factors contributing to the differential toxicity profile of the L-lysine analogues are analyzed and discussed.

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A novel 3D model for the study of functionalised-nanoparticle penetration into human tissue

H. Child*, C.C. Berry
The Centre for Cell Engineering, Joseph Black Building, The University of Glasgow, Glasgow, Scotland, G12 8QQ, United Kingdom

*Corresponding author.
E-mail: h.child.1@research.gla.ac.uk (H. Child).

The advancing field of nanotechnology is progressing rapidly towards the development of multifunctional nanoparticles for use in biomedicine. These nanoparticles benefit from functional biomolecules attached to their surface and can act as unique carrier systems. However, the impermeable nature of both the plasma and nuclear membranes hinders their potential. Two current methods to enhance uptake are using external magnetic fields to remotely control particle direction, and functionalising the nanoparticles with a cell penetrating peptide; both of which facilitate cell entry. To date, studies have largely adopted traditional 2D cell monolayers, the results of which cannot reliably be translated to a human body. This study has focused on using 3D collagen gels seeded with human fibroblast cells as a tissue equivalent model for the study of nanoparticle penetration into human tissue. Iron oxide nanoparticles were employed, which have an attached cell penetrating peptide (penetratin); are magnetic (to allow external control via magnetic fields); and are fluorescent (to allow visualisation). Various analytical techniques were used including fluorescence staining, TEM and histology to compare nanoparticle penetration into gel models both with/without penetratin attachment, and with/without the presence of a magnetic field; both of which have previously been shown to increase nanoparticle uptake in monolayer cul-

tures. This study has provided essential insight into the biomedical potential and possible problems of functionalised-nanoparticle tissue penetration.

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Hybrid nanoparticles from cationic lipid and polyelectrolytes as antimicrobial agents

Letícia D. Melo, Elsa M. Mamizuka, Ana M. Carmona-Ribeiro*
Caixa Postal 26077, CEP 05513-970, Universidade de São Paulo, São Paulo SP, Brazil

*Corresponding author.
E-mail: amcr@usp.br (A.M. Carmona-Ribeiro).

Cationic lipids and polyelectrolytes with the quaternary ammonium moiety in their chemical structure are potent antimicrobial agents. In this work, cationic bilayer fragments prepared from dioctadecyldimethylammonium bromide (DODAB), carboxymethylcellulose (CMC) and polydiallyldimethylammonium chloride (PDDA), added in this sequence, produced potent antimicrobial particles that were characterized by dynamic light-scattering and tested against two bacteria species: *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Two different diameters for particles were obtained depending on DODAB concentration. At 0.1 or 0.5 mM DODAB, cationic hybrid particles of DODAB/CMC/PDDA presented final mean diameters of 108 or 500 nm, respectively and zeta-potentials of 30 or 50 mV, respectively. Both particulates yielded the same activity against *P. aeruginosa*: 0% of cell viability at 1–2 μg/mL PDDA as the outermost cationic layer. For *S. aureus*, at 2 μg/mL PDDA, cell viability for larger particles was 0%, while for smaller particles, 12–15% of cell viability was still obtained. The antimicrobial effect was dependent on the amount of positive charge on particles and independent of particle size. PDDA revealed a high potency as antimicrobial agent and *P. aeruginosa* was more sensitive to all cationic assemblies than *S. aureus*.

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Novel formulations for tuberculostatic drugs based on cationic lipid

Lilian Barbassa, Elsa M. Mamizuka, Ana M. Carmona-Ribeiro*
Caixa Postal 26077, CEP 05513-970, Universidade de São Paulo, São Paulo SP, Brazil

*Corresponding author.
E-mail: amcr@usp.br (A.M. Carmona-Ribeiro).

Cationic bilayers in form of bilayer fragments (BF) or large vesicles (LV) provide adequate environment for solubilization and stabilization of antimicrobial drugs with the advantage of being also antimicrobial agents. In this work, BF or LV interaction with two tuberculostatic drugs, rifamicin (RIF) and isoniazide (ISO) is characterized and the assemblies tested against *Mycobacterium smegmatis*. Methods were employed to determine cell viability, minimal bactericidal concentration and entrapment efficiency for both drugs from dialysis experiments. The occurrence of synergism between cationic lipid and rifamicin was a major result of this investigation. The cationic lipid alone killed *M. smegmatis* over a range of low concentrations. Rifamicin drug particles above its solubilization limit could be solubilized by BF at 0.5 mM lipid. LV were leaky to isoniazide whereas Rifamicin could be incorporated in the cationic bilayer at high percentiles. The novel assemblies may become useful in chemotherapy against tuberculosis.

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Antibody targeting of polymeric nanoparticles for cancer therapy

Christopher J. Scott*, Francois Fay
School of Pharmacy, Queens University Belfast, Medical Biology Centre 97 Lisburn Road Belfast BT9 7BL, United Kingdom

*Corresponding author.
E-mail: c.scott@qub.ac.uk (C.J. Scott).

Antibodies are now the most common form of therapeutic compound under preclinical and clinical development. Normally these proteins are clinically employed for their ability to bind to their cognate antigen and elicit biological effects such as receptor antagonism. However, the application of antibodies as drug delivery agents is also an area of keen interest. This strategy has successfully reached the clinic in the form of drugs such as the radioimmunoconjugates ibritumomab tiuxetan (Zevalin®), [131I]-tositumomab (Bexxar®) and the drug conjugate gemtuzumab ozogamicin (Mylotarg®). Despite the clinical application of these drugs, direct drug/radionuclide conjugation has many drawbacks such as the necessity for a linker that does not inactivate the drug compound and possible hapten immunogenicity concerns that may arise from systemic administration. To circumvent these issues we have investigated the development of novel drug-loaded poly(lactic-co-glycolic acid) (PLGA) nanoparticles, coated with a layer of targeting antibodies. This approach avoids direct linkage of the antibody to the drug. We have shown that the conjugation of nanoparticles to antibodies targeting the death receptor Fas can be employed for the specific targeting of colorectal carcinoma cells. Furthermore, we have demonstrated that Fas-targeted nanoparticles encapsulating camptothecin (CPT) elicit an >50-fold improvement in the IC50 of the chemotherapy alone. This improved efficacy is due to several factors including the improved uptake and internalisation of CPT and up-regulation of Fas receptor expression by CPT. The ability to exploit antibodies not only for targeting of drug-loaded nanoparticles, but also to elicit therapeutic effects themselves is an exciting approach to drug delivery. The application of this methodology in cancer and other diseases, where appropriate drug and antibody combinations can be identified, has the potential to synergistically improve their efficacies.

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Cationic PLGA nanoparticles loaded with DNA for gene delivery delivery

Francois Fay*, Christopher J. Scott
School of Pharmacy, Queens University Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL, United Kingdom

*Corresponding author.
E-mail: ffay03@qub.ac.uk (F. Fay).

Nonviral gene delivery vectors such as liposomes, dendrimers and polymeric nanoparticles have recently been developed as alternatives to virus-based vectors in order to reduce immunogenicity and toxicity risks. In most formulations, anionic nucleic acids are bound to the positively charged vector surfaces through charge–charge interactions. However, a recent *in vivo* study has shown that in endosomes the DNA:nanoparticles complexes can disso-