advancements and a unique feature of the CDTM series is the integration of 'Technical Application' sessions. For CDTM2010 Olav Scheimann (St Andrews University, UK) presented a session on EPR spectroscopy and its value in resolving conformations and dynamics of membrane associated proteins. Werner Witke (Leica Microsystems, DE) presented a session on Total Internal Reflection Microscopy (TIRF) and its capacity to study crucial events that occur on and very close to the plasma membrane. Dries Vercauteren (Ghent University, BE) presented a session comparing pharmacological and molecular approaches to inhibit endocytic pathways and the requirements to integrate both to gain a more accurate picture of uptake pathways.

CDTM2010 welcomed over 180 registered delegates from 27 different countries and from five continents. Over 90 delegate posters were presented, with six of these also selected to highlight their work via short talks. The publication of the CDTM2010 abstracts in the journal *Drug Discovery Today* is a major landmark for the symposia series reflecting its international standing in the scientific community, its maturation into an important event in the Drug Delivery calendar and its ability to consistently deliver high quality science both on the podium and through the delegate contributions. It is hoped that all who attended CDTM2010 departed with renewed energy for the scientific challenges they face.

We look forward to CDTM2012.

Mark Gumbleton, Arwyn T. Jones
Co-organisers CDTM2010,
Welsh School of Pharmacy,
Cardiff University, CF10 3NB, UK

e-mails: jonesat@cardiff.ac.uk (A.T. Jones)
gumbleton@cardiff.ac.uk (M. Gumbleton)

DELEGATE ABSTRACTS

A1

Design and development of polymeric nanoparticles for targeted delivery of nucleic acid-based therapeutics to tumor sites

Aude Le Breton[1,2,*], Véronique Préat[1], Olivier Feron[2]

[1] *Louvain Drug Research Institute, Unité de Galénique, Université catholique de Louvain, Avenue Mounier, 73 - bte 7320, 1200 Brussels, Belgium*

[2] *Institut de Recherche Expérimentale et Clinique, Laboratoire 'Angiogenèse et Cancer', Université catholique de Louvain, Avenue Mounier, 52 - bte 5349, 1200 Brussels, Belgium*

*Corresponding author.
E-mail: aude.lebreton@uclouvain.be (A. Le Breton).

Nucleic acids are widely used as potent therapeutics in cancer research. They can either promote gene expression by bringing a gene either not expressed or under-expressed into tumor cells (cDNA), or alternatively silence expression of genes such as oncogenes (RNAi mediators). However, before they can be efficiently translated to the clinic, this technology requires some optimization: nucleic acids and their vehicles need for instance to be protected from rapid elimination from the bloodstream (opsonization, clearance, and nuclease-mediated degradation) and the specificity of tumor addressing has to be validated. Hence a polymeric nanoparticular carrier encapsulating nucleic acids, either plasmid DNA or siRNA, was developed. Nanoparticles are composed of (1) PLGA, a well tolerated and biodegradable polymer, (2) PEG groups to avoid opsonization, (3) PEI moieties to complex nucleic acids and to enhance cytosolic delivery and (4) RGD sequence for active tumor targeting. Nanoparticles were formulated by double emulsion or water-in-oil-in-water method. Physical properties of such nanoparticles were assessed by dynamic light scattering (size and polydispersity index) and laser doppler electrophoresis (zeta potential). The efficiency of nucleic acid encapsulation into the carrier was determined by the Picogreen assay. Cytotoxicity and transfection capacity were assessed in an *in vitro* model of B16F10 melanoma cells. To date, various designs of nanoparticles were successfully formulated with appropriate size, surface charge and encapsulation efficiency. The PLGA nanoparticles did not show cytotoxic effects on cells and, although less efficient than PEI alone, allowed DNA delivery into tumor cells.

doi:10.1016/j.drudis.2010.09.355

A3

Pulmonary delivery of mRNA: *in vitro* and *in vivo* evaluation

Oliwia Andries*, Joanna Rejman, Cindy Peleman, Tony Lahoutte, Stefaan De Smedt, Joseph Demeester, Niek N. Sanders
Ghent University, Laboratory of Gene Therapy, Faculty of Veterinary Medicine, Department of Nutrition, Genetics and Ethology, Heidestraat 19, 9820 Merelbeke, Belgium

*Corresponding author.
E-mail: oliwia.najder@ugent.be (O. Andries).

Gene therapy is a very promising field of research in medicine. The success of gene based therapeutics will depend on a well thought-out and well-designed delivery system, which should guide the nucleic acids into the desired compartment of the selected cells. However, humans and other organisms have developed natural barriers that protect their body against different kinds of pathogens or intruders. During the evolution of the human being, these barriers have become almost perfect and difficult to overcome. The nuclear membrane, one of the final barriers that protect our genes, appears to be the most important and the crucial one to overcome in non-viral gene delivery. In this work we try to avoid the need to overcome this barrier by intracellular delivery of mRNA instead of pDNA. mRNA delivery has many advantages. First, mRNA does not have to overcome the nuclear barrier and therefore mRNA can transfect also non-dividing cells or dividing cells independent of their cell cycle. Second, mRNA cannot integrate in the genome. Consequently, mRNA mediated gene expression is transient and the risk of insertional mutagenesis can be excluded. Third, there is no need to select a promoter [1]. In this work we evaluate whether mRNA complexed with cationic liposomes (composed of e.g. the cationic lipid GL67) are able to transfect the respiratory tissue of mice. The efficacy of the mRNA:liposome complexes and the gene expression kinetics will be studied and compared with pDNA:liposome complexes. In this study we focus in particularly on GL67-based liposomes. GL67 is an amphiphile consisting of a cholesterol anchor lined to a spermine headgroup in a 'T-shape' configuration. It was proven that GL67 based liposomes are the most effective non-viral pulmonary gene delivery systems [2]. Evaluation of the

in vitro luciferase gene expression in A549 lung adenocarcinoma cells and selection of the most optimal mRNA:liposome ratios are the first steps towards this goal. The efficiency of mRNA/GL67 complexes will be compared to its 4th generation plasmid counterparts–pCpG-CMV-Luc/GL67 lipoplexes. This non-viral mRNA delivery system is potentially a more efficient way for delivering therapeutic genes specifically and directly to the respiratory tract. The respiratory tract is a very interesting and important target organ for gene therapy as it is affected by many acute and chronic diseases, such as cancer, cystic fibrosis, asthma, alpha-1-antytrypsin deficiency or respiratory infections. It is quite a special organ with the possibility of non-invasive, topical administration of a drug through the airways.

References

1. Yamamoto A, et al. Current prospects for mRNA gene delivery. *Eur J Pharm Biopharm* 2009;**71**:484–9.
2. Lee ER, et al. Detailed analysis of structures and formulations of cationic lipids for efficient gene transfer to the lung. *Human Gene Therapy* 1996;**7**:1701–17.

doi:10.1016/j.drudis.2010.09.356

A4
siRNA containing nanoparticles: stability of encapsulation and particle size

Kevin Buyens*, Kevin Braeckmans, Joseph Demeester, Stefaan C. De Smedt, Niek N. Sanders
Ghent University, Faculty of Pharmaceutical Sciences, Ghent Research Group on Nanomedicins, Harelbekestraat 72, 9000 Gent, Belgium

*Corresponding author.
E-mail: Kevin.Buyens@ugent.be (K. Buyens).

A large effort is currently put into the development of nano-scaled carrier systems that can guide siRNA molecules to their target cells after intravenous injection. One of the main issues in this research is the integrity of the siRNA containing nanoparticles in the blood stream. The integrity of the nanoparticles comprises both the particle size and the stable encapsulation of siRNA. Techniques currently available for studying the disassembly and size distribution of siRNA containing nanoparticles are time-consuming and incompatible with biological fluids. We initially developed a fluorescence fluctuation spectroscopy (FFS) based method which allows us to monitor the integrity of siRNA-carrier complexes in less than one minute in complex biological media and at very low siRNA concentrations. Second, while the size distribution of the complexes can be easily measured in a clear dispersion by dynamic light scattering or electron microscopy, it cannot be measured in more complex biological media such as plasma or whole blood, which contain several different interfering components. To address this issue, we have developed a novel technique, based on single particle tracking (SPT) microscopy, for studying the size distribution (and aggregation) of nanoscopic drug complexes in biological fluids. For stabilization of the particle size of cationic lipid based nanoparticles, inclusion of lipids conjugated with PEG is widely used to sterically hinder aggregate formation. We have demonstrated that in order to obtain remaining siRNA complexation to the cationic liposomes, effective encapsulation inside the liposome, or in between lipid multilayers is required, since siRNA electrostatically bound to the outer side of the liposomes is quickly pushed away by the ubiquitous albumin molecules in blood which leads to siRNA degradation and loss of effectiveness. Formation of siRNA protecting multilayers is hindered by inclusion of PEG-lipids, a hurdle that needs to be overcome either by post-insertion of the PEG-lipid into multilayer containing siRNA-liposome complexes, or by efficient encapsulation of the siRNA inside the aqueous core of the PEGylated liposome. Size stabilization in buffer can be easily achieved by inclusion of minor percentages (~1%) of PEG-lipids. In whole blood however, we demonstrate that much higher percentages of PEG–lipids (5–10%) are required to achieve size stabilization. This requirement has not been previously considered because of the lack of a suitable technique to study the aggregation phenomena in whole blood. In our work we demonstrate that assaying the physicochemical properties of siRNA encapsulating nanoparticles should always be carried out in the biological media they are designed to be employed in. Two novel microscopy based methods were developed that enable such characterization in biological fluids such as serum, plasma or even whole blood.

doi:10.1016/j.drudis.2010.09.357

A5
Investigating the effects of cationic lipid-mediated toxicity and how to optimize liposomal systems for transfection purposes

S.J. Soenen[1], N. Nuytten[1], S.C. De Smedt[2], M. De Cuyper[1,*]
[1] *Lab of BioNanoColloids, IRC, KULeuven Campus Kortrijk, Kortrijk, Belgium*
[2] *Lab of General Biochemistry and Physical Pharmacy, Department of Pharmaceutical Sciences, University of Ghent, Ghent, Belgium*

*Corresponding author.
E-mail: Marcel.DeCuyper@kuleuven-kortrijk.be (M. De Cuyper).

For magnetic resonance imaging (MRI) of therapeutic cells, these cells are often prelabelled in culture with iron oxide nanoparticles, enabling them to be non-invasively monitored by MRI following transplantation *in vivo*. Magnetoliposomes (MLs) are nanosized Fe3O4-cores (14 nm diameter) each surrounded by a lipid bilayer [1]. Different types of MLs have been utilised for biomedical research applications [2,3], where cationic MLs are more optimally suited for *in vitro* cell labelling [1]. Unfortunately, cationic lipids display several inherent properties which, to date, have not been clearly defined [4]. In the present work, cationic MLs as well as their non-iron oxide-containing vesicular counterparts were used to label NIH 3T3 fibroblasts. Using distearoyltrimethyl ammoniumpropane (DSTAP) as the cationic lipid the effects on cell physiology of the different particles was compared. Different amounts of DSTAP were used, indicating that when the cationic lipids exceed a certain safe threshold (3.33%), this affects cell viability by different mechanisms that are dependent and independent of actual nanoparticle internalization. Internalization-dependent mechanisms are closely linked to the induction of reactive oxygen species and altered Ca^{2+} homeostasis; the indirect mechanisms appear to indicate plasma membrane destabilization by means of transfer of the cationic lipid from the nanoparticles to the plasma membrane. The extent of cationic effects could be modified by: (1) the size of the liposome, (2) the presence of a stabilising iron oxide core, (3) the use of reactive oxygen species or Ca^{2+} channel inhibitors, (4) the nature of the cationic lipid and (5) the nature of the neutral matrix lipids. Based on these results, a novel cationic peptide-lipid conjugate (dipalmitoylphosphatidylethanolamine-