Caco-2 cells through exploitation of the natural receptor governed processes involved in VB12 absorption.

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## A26

## Four-wave mixing imaging to study protein entry and release in mammalian cells

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Optical microscopy is a powerful tool for tracking the binding, internalisation and subcellular trafficking of delivery vectors to mammalian cells. By exploiting multiphoton processes, subcellular structures can be imaged with intrinsic three-dimensional (3D) spatial resolution. Common fluorescent labels in multiphoton microscopy include organic fluorophores, which suffer from photobleaching, and quantum dots which are more photostable but contain cytotoxic elements (such as Cd or In). Gold nanoparticles (GNPs) are ideal optical labels in terms of photostability and bio-compatibility, but emit weak fluorescent signal. We have developed a novel multiphoton microscopy technique that exploits the third-order nonlinearity called four-wave mixing (FWM) of GNPs in resonance with their surface plasmon. In terms of imaging performances, FWM microscopy features a spatial resolution better than the one-photon diffraction limit and optical sectioning capabilities. We show high-contrast background-free imaging of gold-labels (down to 5 nm size) and sensitivity to the single particle level. We are also able to demonstrate a directed dissociation of the GNP from bound proteins at their surface. These results pave the way for active tracking of conjugated nanoparticles, before the controlled release of therapeutically relevant proteins to a localised site of interest.

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## A27

## Efficient gene delivery using acid-responsive lipid envelopes for adenovirus

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Gene therapy involves the delivery of a functional gene by a vector into target cells, resulting in a desired therapeutic effect. Adenovirus (Ad) has shown a great promise in gene therapy [1,2]. However, *in vivo* studies have reported an immunogenic response and an overwhelming accumulation and gene expression in the liver resulting in significant hepatoxicity. These issues currently inhibit the use of this vector for use in clinical therapies. Such limitations have been overcome by engineering artificially enveloped Ad using zwitterionic and cationic lipid bilayers [3,4]. However, this resulted in a significant reduction of gene expression *in vitro*. We observed that this may be due to poor release of the Ad from its lipid envelope. In the present work, we have explored the use of pH-sensitive DOPE:CHEMS lipid-envelopes to stimulate the virus release from the envelope and consequently result in higher levels of gene expression. Artificially enveloped Ad (DOPE:CHEMS:Ad) were prepared by lipid film hydration followed by sonication. The physicochemical characteristics of the resulting hybrid biomaterials were characterised by transmission electron microscopy, atomic force microscopy, dot blot, dynamic light scattering and zeta potential measurements. The enveloped viruses exhibited good stability at physiological pH (7.4) but immediately collapsed and released naked virions at pH 5.5. Furthermore, recombinant Ad encoding for beta-galactosidase (β-gal) enveloped in DOPE:CHEMS showed comparable levels of gene expression to naked Ad in different cell lines. These transfection results were further confirmed by studying the intracellular trafficking of fluorescently labelled, Cy3-Ad using confocal laser scanning microscopy (CLSM). Interestingly, Cy-3 Ad enveloped in DOPE:CHEMS showed a uniform fluorescence distribution within the cytoplasm indicating Ad endosomal release. In addition, pH-sensitive enveloped Ad injected directly into human cervical adenocarinoma (C33a) xenografts grown on the flank of nude mice showed similar levels of gene expression to naked Ad. In conclusion, this type of artificially enveloped Ad offers a promising tool in gene delivery since high level of Ad gene expression can be maintained while one can expect to dramatically improve the innate Ad immunogenicity and hepatotoxicity *in vivo*.

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## A28

## *In vitro* silencing of TGFβ1 in a corneal epithelial cell line using nanoparticles

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*Introduction*: Severe ocular inflammatory disorders constitute a sight-threatening group of diseases that present treatment difficulties due to the intrinsic barriers of the ocular surface. Previous work in our group has demonstrated that epithelial cells from human cornea (HCE cell line) basally secrete TGFβ1 (a commonly detected cytokine in ocular inflammatory diseases). At present, gene therapy (including siRNA-based therapies) holds promise for the treatment of several diseases, including ocular disorders. However, the development of safe and effective delivery vehicles still remains a major challenge for its clinical application. *Purpose*: This work is a proof-of-concept study meant to evaluate the efficacy of the *in vitro* gene silencing technique for different siRNAs targeting relevant pro-inflammatory cytokines

involved in ocular surface inflammation. We also want to determine whether the use of nanoparticulated drug delivery systems, based on cationized gelatine and chondroitin sulfate, as carriers for siRNAs improve the level of gene silencing. *Methods*: HCE cells were transfected with specific siRNAs against TGFβ1 and its Receptor 2 (TGFBR2) or against GAPDH as a negative control. Lipofectamine was used at 1.6 μl/well in 24-well plates and different siRNA concentrations from 20 to 300 nM were assayed. Silencing efficacy was tested, comparing Lipofectamine2000- or Nanoparticle-based transfection, at protein and RNA levels. Potential toxicity was evaluated by means of the XTT test. *Results*: TGFβ1 and TGFBR2 silencing reached 70% at the RNA level (measured by quantitative real-time-PCR) when using Lipofectamine. Lower silencing was detected at the protein level (measured by Western blotting or ELISA). However, the use of nanoparticles did not significantively improve the silencing efficacy of the evaluated siRNAs. siTGFβ1- and siTGFBR2-transfected cells showed viability percentages equivalent to those of control untransfected cells. CONCLUSION: It is possible to silence *in vitro* TGFβ1 and TGFBR2 expression in a corneal epithelial cell line by conventional techniques obtaining acceptable silencing levels while maintaining high cell viability. The use of nanoparticles as siRNA vehicles to improve silencing levels requires further studies

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## A29
## Efficient siRNA delivery and effective gene silencing by lipoplexes

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siRNA is double-stranded RNA typically 21–24 nucleotide base-pairs long. Gene silencing by siRNA has gained wide acceptance in genomics and is already in different phases of clinical trials as a potential therapeutic. Long chain fatty acid conjugates of spermine have previously been synthesized and evaluated in our research group for both gene and siRNA delivery [1,2]. We report the synthesis of two novel unsymmetrical N4,N9-difatty acid conjugates of the naturally occurring polyamine spermine with the aim of developing structure–activity relationships for their potential as non-viral, self-assembly vectors for siRNA delivery. After transfection with lipoplexes of Alexa Fluor® 647-labelled siRNA (a 24-mer from Qiagen), silencing EGFP expression, both the efficiency of delivery and the effectiveness of knock-down (gene silencing) were evaluated in HeLa cells stably expressing EGFP. Analysis was by FACS 48 hours post transfection. All transfection experiments were carried-out in DMEM containing 10% foetal calf serum. The efficiency of intracellular delivery was measured by the (normalized) fluorescence of Alexa Fluor® 647-labelled siRNA; N4,N9-dioleoylspermine (DOS) showed 150% of the delivery efficiency achieved with N4-linoleoyl-N9-oleoylspermine (LOS). However, knock-down results show that LOS is more effective with a reduction of EGFP expression levels from control (100%) to 25 ± 3% at a concentration of 3 μg/well (N/P = 11, $n = 3$ and triplicate replicates). Under the same experimental conditions, DOS reduced EGFP expression to 27 ± 2% at a concentration of 6 μg/well (N/P = 22) and to 32 ± 2% at a concentration of 3 μg/well (N/P = 11). Cell viability was measured as the percentage of viable cells using the Alamar Blue® assay [3]. The results show that at 3 μg/well LOS cell viability is 83 ± 4%, at 6 μg/well LOS cell viability is 46 ± 8%, while at 6 μg/well DOS cell viability is only 32 ± 9%. Transfection of cells with Lipofectamine™2000 resulted in reduction of EGFP expression to 37 ± 3%, with cell viability of 91 ± 6%. We conclude from these results that the unsymmetrical lipopolyamine LOS is an excellent transfecting agent for the delivery of siRNA producing effective gene silencing in the presence of 10% foetal calf serum.

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## A30
## Peptide dendrimer based drug delivery system

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In the past decades, dendrimers have been extensively studied for their unique properties such as spherical nanostructure, monodistributed size and numerous peripheral functional groups. Peptide dendrimers, which were synthesized from amino acids, have been reported as biomaterials for disease diagnosis and treatment due to their excellent biocompatibility and degradability. Herein, we reported the synthesis of peptide dendrimers and their biomedical applications as molecular probes for magnetic resonance imaging (MRI) and carriers for drug/gene delivery. The synthesis of peptide dendrimers was according to a previously reported method [1]. The dendrimers with different generations were synthesized and functionalized. Targeting moieties, mPEG, Ga-DTPA complexes and anti-tumor drugs were immobilized on the peripheral groups of the dendrimers. The dendrimers immobilized Ga-DTPA complexes were used as MRI molecular probes and the relaxivity of contrast was tested on 1.5 T MRI both *in vitro* and *in vivo*in. The generations of dendrimers were 2, 3, 4, and galactosyl moiety was used as targeting ligand for liver imaging. The relaxivity of the contrasts were measured and for G4 dendrimer was 100.8 mM-1•S-1, which was much higher than that of the commercial Ga-DTPA product. The signal intensities were determined by choosing an appropriate region of interest in mouse liver tissue. After 10 minutes injection, the SI increase in liver tissue was observed with an averaged enhancement of 43% for G3T and 37% for G4T, respectively. The non-specific dendritic agents G2, G3 and G4 showed low SI increases. The dendritic probes of G2T, G3T and G4T showed 25%, 35% and 34% relative enhanced SI after 1 hour injection. The peptide dendrimers were fabricated gene vectors and gene transfections of generation 3, 4 and 5 of peptide dendrimers were compared, the