we studied the optimal conditions for attachment and proliferation of the astrocytes and hBECs. Furthermore, we monitored the effect of hBEC growing directly on the surface of an adherent astrocytic monolayer. The tight junctions between the brain endothelial cells forms a diffusion barrier that is responsible for the high paracellular resistance which is a crucial characteristic for any B3-model. In order to test the integrity of this barrier in the B3-model and simultaneously measure the transcellular transport we combined fluorescent compounds and dye labelled large molecules to test the permeability across the barrier. This strategy allows for the discrimination between transcellular and paracellular transport.

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A83
Solid lipid nanoparticles for gene delivery into prostate cancer cells

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Prostate adenocarcinoma is the most common cancer occurring in male. The aim of this study is to develop a gene delivery system based on solid lipid nanoparticles (SLNs) for the transfer of tumor suppressor genes that are able to induce death into prostate cancer cells. Formulations of cationic SLNs, consisting of stearic acid/DOTAP/pluronic, were produced. Additionally, formulations with and without 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) in various molar ratios were tested. The SLNs produced were approximately 100 nm in size and showed a positive surface charge (+40 mV) in water. The SLNs showed excellent stability, as evidenced by size, zeta potential, transfection efficiency over 140 days, and possibility of lyophilization and/or sterilization without loss of efficiency. The SLNs were able to protect genetic material against DNase digestion and showed a transfection capacity comparable to that of Lipofectamine 2000®, a commercially available gene carrier. Interestingly, we found that the transfection efficiency of SLNs in prostate cancer PC3 cells was significantly higher when compared to that in normal human prostate PNT2-C2 cells. Further examination revealed that this is due to enhanced endosomal escape rather than enhanced internalization of SLNs in prostate cancer cells. These results indicate that cationic SLNs are a promising tool for gene delivery into prostate cancer cells.

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A84
Kinases in cationic lipid/polymer-mediated gene delivery

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Cationic lipids/polymers, complexed with DNA (also called lipo/polyplexes), are promising tools for gene delivery or transfection. Lipo/polyplexes have low toxicity, a relative low immunological response and can be synthesized on large scale. Lipo/polyplexes are internalized by cells via endocytosis. The endocytotic pathway that is used by lipo/polyplexes depends on the cell type and the type of lipo/polyplexes, and likely contributes to transfection efficiency. We have recently shown that adhesion receptors are involved in binding and endocytosis of lipoplexes. Cell receptors also have been described for the endocytosis of polyplexes. Receptor occupation can initiate signaling cascades, commonly mediated by kinases, which in turn tightly regulate endocytosis and endocytotic processing. The elucidation of cellular signaling signatures, initiated by lipo/polyplexes and/or those that allow or preclude gene delivery, will be instrumental in understanding the interaction between lipo/polyplexes and cells at the molecular level and contribute to the design of protocols with improved gene delivery efficiency. In this study we have performed a screen with a wide range of validated pharmacological kinase inhibitors, and evaluated their effects on lipo/polyplex transfection efficiency. In this screen a kinase is identified that specifically influences the transfection efficiency of a polyplex. It is further demonstrated that, as a part of the underlying mechanism, this kinase regulates the endocytotic processing of the polyplex and, as a consequence, controls its endosomal escape.

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A85
Peptide-based nano-particle for *in vivo* delivery of siRNA

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The development of short interfering RNA (siRNA), has provided great hope for therapeutic targeting of specific genes responsible of pathological disorders. However their clinical application remains limited by their poor cellular uptake, low bioavailability, and insufficient capability to reach targets *in vivo*. We have designed a novel approach, based on short amphipathic peptides 'CADY' that promotes efficient delivery of siRNA into wide variety of mammalian cell lines and *in vivo* upon systemic and topical administrations. This carrier consisting of a balance between hydrophobic and hydrophilic domains and forms stable discrete 'nanoparticles' with siRNA, through non-covalent interactions. Cellular uptake mechanism of CADY/siRNA nanoparticles is dependent on the size of the particle and involves membrane potential and dynamic, which enables a rapid release of the siRNA into the cytoplasm and promotes a robust down-regulation of target mRNA. CADY-carriers were applied to the delivery of siRNA targeting the cell cycle regulatory protein Cyclin B1 into cancer cells. We demonstrated that when associated with CADY, sub-nanomolar concentrations of siRNA Cyclin B1 significantly knocked down Cyclin B1 protein levels resulting in cell cycle arrest in G2 arrest and blocked cancer cell proliferation. The surface of CADY particles can be functionalized and addition of cholesterol-moiety significantly improves siRNA stability *in vivo*, thereby enhancing the efficiency of this technology for systemic administration following intravenous injection. We have validated the therapeutic potential of this strategy for cancer treatment by targeting cyclin B1 in various mouse tumour models and demonstrate that CADY-mediated delivery of cyclin B1 siRNA prevents tumour growth *in vivo* following systemic intravenous injection. Moreover, we showed that functionalization of CADY particles with other chemical groups or biological moieties can be applied to generate formulations to target specific cell types or tissues which can

be of a major interest for future development. Given the biological response yielded through this approach, we propose that non-covalent, peptide-based delivery technologies hold a strong promise for therapeutic administration of siRNA.

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A86
High-efficient transfection using cationic lipids with programmed biodegradability

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Delivery of nucleic acids into cells has an ever-increasing number of applications with outstanding advances in both gene therapy and biotechnology, highlighting the induction of pluripotency in somatic cells. While the use of viral vectors is currently the most efficient transfection method, their antigenicity along with the risk of potential mutagenesis, among other inconvenients, are important limitations that hinder its application in medicine. Non-viral delivery systems (cationic lipids and polymers) represent an attractive alternative, particularly because of their low-cost, tuneable design and procedural simplicity. However, the *in vivo* efficacy of these carriers needs to be increased for both research purposes and clinical application. As repetitive dosing would be required in any gene therapy treatment, the cytotoxicity due to the use of these chemicals needs to be reduced, ideally by regulating their metabolic fate. To address these issues, a tripodal cationic lipid [1] was specifically designed to undergo complete intracellular metabolisation into naturally occuring compounds aiming to minimise the toxicity associated with its cytoplasmatic residence. Besides the toxicity issue, the incorporation of hydrolysis-prone linkages was addressed to enhance the cationic lipid-DNA dissociation once the lipoplexes have entered the cell by endocytosis. The novel compounds showed remarkable transfection efficiency along with reduced toxicity in a variety of immortalized cells and stem cells. Moreover, preliminary *in vivo* studies underlined the potential applicability of these non-toxic reagents for the delivery of DNA into mouse lung. These reagents, contrary to the most of chemical carriers commercially available, might offer a viable chemical alternative to viral transfection.

Reference

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A87
Immune stimulation following microneedle delivery of influenza virus-like particle (VLP) vaccines to human skin

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Virus-like particles (VLPs) possess a number of features that make them attractive vaccine candidates for immunization against infectious disease. Efficient intra-epidermal delivery of VLP vaccines would exploit the abundance of Langerhans cells (LCs) that reside within the skin epidermis to generate an efficient host immune response. Microneedles (MNs) are currently being developed for the convenient and pain-free delivery of drugs and vaccines across the skin barrier layer. Whilst MN-based vaccines have demonstrated proof-of-concept in mice, it would be extremely valuable to understand how MN targeting of influenza VLP vaccines to the skin epidermis affects activation and migration of LCs in the real human skin environment. MNs with lengths of 700 μm were laser-etched from stainless steel sheets and surface-coated with either influenza H1 (A/PR/8/34) or H5 (A/Viet Nam/1203/04) VLPs. The coated MNs easily and reproducibly penetrated freshly excised human skin, depositing approximately 80% of the vaccine load within 60 s. Experiments conducted in cultured human skin showed that H1 and H5 VLPs, delivered via MNs, stimulated LCs causing morphological changes and a significant decline in total LCs number in epidermal sheets at 24–48 hours compared to untreated skin at the same time points. Histological sections showed that LCs in VLP treated samples were more dispersed throughout the epidermis with substantial numbers in the vicinity of the basement membrane. The response made by LCs was more manifest in human skin treated with H1 VLPs, compared with H5 VLPs. These findings corroborate observations in mouse studies, where H1 VLPs were shown to be significantly more immunogenic than H5 VLPs. Our data provide strong evidence that MN-facilitated delivery of influenza VLP vaccines initiates a stimulatory response in LCs in human skin epidermis. The results complement and support data gained from animal models, suggesting dendritic cells (DCs), including LCs, targeted through intra-epidermal or intra-dermal deposition of the vaccine generates immune response. This study also emphasizes the value of cultured human skin alongside animal studies for informative preclinical testing of intra-dermal vaccines.

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A88
Electrically based transdermal techniques for delivery of therapeutic macromolecules

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Advances in molecular biology have given us a wide range of protein and peptide based drugs that are unsuitable for oral delivery because of their high degree of first-pass metabolism. Though parenteral delivery is successful for developed and commercially available protein and peptide based drugs, chronic and self administration formulations are not the ideal choice through this route. Transdermal delivery is emerging as the biggest application target for these agents, however, the skin is extremely efficient at keeping out such large molecular weight compounds and therapeutic levels are never going to be realistically achieved by passive absorption. Therefore novel transdermal drug delivery systems have been developed with the aim to achieve the objective of systemic medication through topical application to the intact skin surface with benefits of deliver therapeutic macromolecules in desire therapeutic doses to overcome the difficulties associated with the oral route, namely poor bioavailability of drug and the tendency to produce rapid blood