ciate and whilst the nanoparticles can reach the cytoplasm, the cargo DNA is ineffectually retained in endo/lysosomal vesicles and thus unable to perform its therapeutic action. Based on these observations, we have developed a novel poly(lactic-co-glycolic acid (PLGA) nanoparticle formulation to encapsulate and deliver target DNA into the cytoplasm of target cells. Our formulation is based on combining salting out and emulsion-evaporation processes to reduce sonication steps in an attempt to overcome DNA destruction by shearing effect. Using this formulation we have produced a uniform population of 250 nm nanoparticles entrapping plasmid DNA in both supercoiled and open circular structures. Transformation assays using plasmids released from the particles demonstrated retention of DNA functionality in these formulations. As nude anionic nanoparticles particles have previously been shown to preferentially localise in late endosomes, we have also formulated nanoparticles bearing a low cationic charge to provoke their release from the endo/lysosomal pathway. Didodecyl dimethyl ammonium bromide (DMAB) coating results in only a 10% increase in size and no significant alteration of DNA release. Furthermore, study of the localisation of fluorescent DMAB coated NP demonstrated their ability to escape from endosomal compartments into the cytosol. Finally, *in vitro* transfection assays performed on mammalian cells using these positively charged nanoparticles entrapping a GFP coding plasmid have exhibited significantly improved transfection profiles than anionic particles or liposomal reagents.

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A22
Click chemistry for the generation of cell permeable apoptotic peptides

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The use of proteins and peptides as drug molecules has been held back by their proteolytic instability and inability to cross-cellular membranes. Proteins and long peptides are often produced by expression in *E. coli* rather than by solid phase peptide synthesis. A drawback of *in vivo* protein and peptide synthesis is the difficulty to selectively modify the product peptide by the attachment of fluorescent dyes or ligation to other macromolecules like polysaccharides, lipids or peptides. Here we present a facile method to modify an expressed protein or peptide to create a C-terminal alkyne group. This functionality is then used inter alia for conjugation to the cell-penetrating peptide octa-arginine. This will provide a vector for delivery across the plasma membrane of cells. To demonstrate our method, we have produced in *E. coli* a peptide derived from the Bak protein; one of the key regulators of apoptosis in eukaryotic cells. In the cell it is usually found bound to Bcl-xL at the outer mitochondrial membrane. If this interaction is disrupted, Bak oligomerizes and forms pores which trigger mitochondria dependent apoptosis through cytochrome c release. Small peptides derived from the BH3 helix of Bak have been shown to induce apoptosis. We have expressed such a peptide in *E. coli* as a fusion protein. The ketosteroid isomerase fusion protein is insoluble and readily purified from cell extracts. The peptide is then cleaved from the fusion protein by reaction with cyanogen bromide at a strategically inserted methionine residue to generate a homoserine lactone at the C-terminus of the Bak peptide. This lactone is then used for direct amide formation with inexpensive propargylamine. The resulting alkynyl peptide serves as a reagent for highly efficient 'click' reactions to couple to a wide range of azides. Since the Bak peptide is not able to cross the cell membrane, the well-known octa-arginine cell penetrating peptide sequence was added as a delivery vector. Here we discuss the synthesis of this semi-synthetic peptide and its interaction with, and uptake into, cancer cell lines.

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A23
Protein delivery through the intestinal epithelium: a vitamin B12-mediated approach

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The vitamin B12 transport pathway offers potential for enhancing the uptake of orally administered biologicals, including proteins, peptides and immunogens. The oral delivery of these large molecules is often impeded by the epithelial cell barrier and proteolysis occurring at the mucosal surfaces. Research efforts have been made to enhance oral delivery by employing carrier molecules or ligands conjugated to the pharmaceutically active component, capable of exploiting specific receptor-mediated uptake (RME) to provide their co-absorption. One of the few potential ligands available for enabling transcytosis across the epithelium is vitamin B12. There are several sites on vitamin B12 molecule that are suitable for modification to form bioconjugates. The route followed in this work examined the preactivation of the 5′-hydroxyl group on the ribose moiety by the use of carbonyldiimidazole (CDI), followed by attack of a nucleophile to furnish the hexanediamine spacer. The resultant α-ω-aminohexylcarbamate VB12 derivative was conjugated to fluorescent carboxy-functional nanoparticles (<200 nm size), for use as a model for potential therapeutic carriers. These systems were applied to confluent Caco-2 monolayers, which characteristically form tight junctions. Although several cell lines express the IF-B12 receptor responsible for the binding, internalisation and transcytosis of VB12, the Caco-2 cell line was chosen as the preliminary *in vitro* model to study the potential of the VB12 transport system for the delivery of VB12-conjugated nanoparticles. Immunostaining and confocal microscopy were used to verify receptor/transport protein expression by the cells, as an essential prerequisite for ligand-based transcytosis. We demonstrate that the surface modification of nanoparticles with the α-ω-aminohexylcarbamate derivative of vitamin B12 enables their resultant uptake and transport in the apical-basolateral direction of

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