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

doi:10.1016/j.drudis.2010.09.370

A18
Hybrid nanoparticles from cationic lipid and polyelectrolytes as antimicrobial agents

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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*.

Acknowlegements
FAPESP; CNPq.

doi:10.1016/j.drudis.2010.09.371

A19
Novel formulations for tuberculostatic drugs based on cationic lipid

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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.

Acknowlegements
FAPESP and CNPq.

doi:10.1016/j.drudis.2010.09.372

A20
Antibody targeting of polymeric nanoparticles for cancer therapy

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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.

doi:10.1016/j.drudis.2010.09.373

A21
Cationic PLGA nanoparticles loaded with DNA for gene delivery delivery

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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-

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.

doi:10.1016/j.drudis.2010.09.374

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.

doi:10.1016/j.drudis.2010.09.375

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