Poster Session 3 – Drug Delivery

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Use of dendrimer drug carriers to bypass transepithelial efflux transporters

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Dendrimers represent a relatively new class of highly branched polymers (Tomalia 1995) that have found several pharmaceutical applications such as enhancement of the solubility of poorly soluble drugs and the efficient delivery of DNA and oligonucleotides (D'Emanuele et al 2003). Surface engineering has been found to reduce cytotoxicity and enhance the rate of transport of dendrimers across epithelial cells (Jevprasesphant et al 2003). The objectives of this study were to investigate the transport mechanisms of PAMAM dendrimers and surface-modified dendrimer conjugates across Caco-2 cell monolayers, and also the use of dendrimer–drug conjugates to enhance the solubility of poorly soluble drugs and bypass the P-glycoprotein (P-gp) efflux transporter. Dendrimer (G3 PAMAM) and lauroyl-dendrimer conjugates were chemically bound to propranolol base, a model P-gp substrate and low solubility drug, using chloroacyl chloride as a linker. Conjugates were designated G3Px and G3LzPx, where 3 represents the dendrimer generation, z the number of attached lauroyl chains and x represents the number of attached propranolol molecules. Characterisation of conjugates was by HPLC, FT-IR, ^{1}H NMR and ^{13}C NMR. The apparent permeability coefficients (P_{app}) of propranolol, propranolol–dendrimer and propranolol–lauroyl-dendrimer conjugates through Caco-2 cell monolayers were measured in both the apical (A)-to-basolateral (B) and B→A directions at 37°C in the presence and absence of the endocytosis inhibitor, colchicine (Col) (10 μM), and at 4°C and propranolol quantified by HPLC. The effect of a P-gp inhibitor, cyclosporin A (CsA) (10 μM), on the transport of propranolol conjugates was investigated in a similar manner. The effect of dendrimers and conjugates on monolayer integrity was determined from transepithelial electrical resistance (TEER). High-resolution imaging techniques (flow cytometry, confocal laser scanning microscopy (CLSM) and transmission electron microscopy (TEM)) were used to determine the transport mechanism. A significant enhancement of A→B and decrease in B→A P_{app} of propranolol was observed when propranolol was covalently bound to G3 dendrimer. For example, the A→B P_{app} of G3P2 was approximately 3.6 times greater than that of unconjugated propranolol, while no enhancement was observed in simple mixtures of propranolol and G3 dendrimer. A more pronounced increase of A→B transport of propranolol was observed when propranolol was conjugated to G3L6 carriers (14.4 fold increase for G3L6P2). The results suggest that dendrimer carriers bypass the P-gp efflux transporter. The A→B transport of dendrimer and dendrimer–propranolol conjugates was significantly decreased in the presence of Col and at 4°C, suggesting that the mechanism by which dendrimers enhance transport involves endocytosis. No difference in the permeability of propranolol–dendrimer conjugates was found in the presence and absence of CsA, indicating that the conjugates are not P-gp substrates. Flow cytometry, CLSM and TEM studies confirmed a significant level of dendrimer cellular internalisation in Caco-2 cells. The enhancement of propranolol transport by conjugation with dendrimers is due to the bypassing of the P-gp efflux transporter. The mechanism of transepithelial transport, for both dendrimers and conjugates is primarily via an endocytotic transcellular pathway.

D'Emanuele, A., et al (2003) In: Swarbrick, J., Boylan, J. C. (eds) *Dendrimers*. 2nd Edn, Encyc. Pharm. Tech., New York pp 1–21
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Liquid scintillation spectroscopy as an analytical tool for investigating the influence of penetration enhancers on 5-aminolevulinic acid penetration into keratinised tissue

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Aminolevulinic acid (ALA) is widely used in topical photodynamic therapy (PDT). Its zwitterionic nature impairs drug penetration, being ionised at both high and low pH. Topically applied ALA, therefore, penetrates intact *stratum corneum* poorly. Although disordered epithelial barriers presented by many neoplastic skin lesions allow enhanced ALA penetration, its low lipophilicity prevents significant penetration into hyperkeratotic or deep nodular lesions. If topically applied ALA is to be successfully used for PDT, its concentration, and hence that of the photosensitising protoporphyrin IX (PpIX), which it generates in abnormal cells, must exceed a threshold. We have previously used liquid scintillation spectroscopy (LSS) to assess in-vitro penetration of ALA, released from a topically applied cream into lesions of basal cell carcinoma (Ahmadi et al 2004). We have also reported the use of a bioadhesive patch for successful delivery of ALA to lesions of vulval intra-epithelial neoplasia (McCarron et al 2003). The aim of this study was to use liquid scintillation spectroscopy to investigate the influence of penetration enhancers (PE) on the penetration of ALA, released from a bioadhesive patch into an in-vitro skin model. Bioadhesive patches containing 38 mg ALA/cm^2 were prepared as described previously (McCarron et al 2003) and were spiked with known amounts of ^{14}C ALA. Aqueous blends used to prepare patches contained either no PE or 1, 3 or 5% w/w of either dimethyl sulfoxide (DMSO) or oleic acid (OA). Patch segments were applied to equal areas of neonate porcine skin and allowed to remain in place for 4 h at 37°C. Skin samples were then flash frozen in liquid nitrogen and sectioned cryostatically at 90° to the plane of drug diffusion. ALA concentrations at depths down to 2.375 mm were determined by LSS. The normal patch achieved an ALA concentration of 3.01 mg cm^{-3} at 2.375 mm. DMSO had no significant influence on ALA concentration at 2.375 mm ($P = 0.4298$). Even films cast from blends containing 5% w/w DMSO could not significantly increase the ALA concentration at 2.375 mm. Overall analysis of the effect of OA on ALA concentration at 2.375 mm using the Kruskal-Wallis test showed that there was no significant effect ($P = 0.0770$). However, an individual comparison of the mean ALA concentrations, at mean depths of 2.375 mm, achieved using the normal patch, with those achieved using the patches containing OA may be made using the Mann Whitney U test. This test showed that a significant increase in ALA concentration was produced ($P = 0.0495$) using the patch cast from a blend containing 5% w/w OA. Only addition of 5% w/w OA to the blend used to prepare bioadhesive patches significantly increased ALA concentration at 2.375 mm in neonate porcine skin. Addition of DMSO had no significant effect on penetration. Nevertheless, the normal patch, without PE, was capable of generating concentrations of ALA at 2.375 mm in neonate porcine skin that were an order of magnitude greater than those reported as being cytotoxic to neoplastic cell lines in vitro.

Ahmadi, S., McCarron, P. A., Donnelly, R. F., et al (2004) *Exp. Dermatol.* **13**: 1–7
McCarron, P. A., Donnelly, R. F., Woolfson, A. D., et al (2003) *Drug Deliv. Sys. Sci.* **3**: 59–64

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Floating dosage forms to prolong gastro retention: an in-vivo study in the fasted state

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Gastro-retentive dosage forms that float on stomach contents have the potential to improve local therapy and decrease the variation in bioavailability demonstrated by many commercial immediate and modified release preparations. A dosage form has been developed, based on freeze-dried alginate beads, which floats on the surface of the stomach contents thus prolonging the retention time. Prolonged retention has been demonstrated in the fed state (Whitehead 1998). The retention of floating calcium alginate beads was assessed in this study in the fasted state when the beads were swallowed with water or an aqueous solution of citric acid. Floating placebo calcium alginate beads were prepared at an ambient temperature of 25°C by incorporating technetium-99m and stannous chloride (0.1%), into sodium alginate solution to give a final concentration of 2% sodium alginate. The radio-labelling efficiency of the calcium alginate beads was examined by comparing the radioactive counts of a sample of 10 calcium alginate beads and 1 mL of calcium chloride supernatant. The calcium alginate beads were found to take up in excess of 99% of the radiolabel and were deemed suitable for the study. Four healthy males were selected and took part in a two-way crossover study with a washout period of at least a week between study days. The study was approved by the University of Manchester Ethics Committee and ARSAC. After an overnight fast each subject swallowed a sample of calcium alginate beads that contained 4MBq of $^{99m}TcO_4$. The calcium alginate beads were swallowed with either 100 mL of water or 100 mL of citric acid solution 1%. Static images were taken at 10-min intervals using a gamma camera and the data recorded. The data were then assessed by visual examination. A delay in gastric

emptying was observed in three out of four of the subjects when calcium alginate beads were swallowed with the citric acid solution. When compared with the onset of emptying for the calcium alginate beads swallowed with water, the residence time of the calcium alginate beads in the stomach was increased by approximately 50%. The in-vivo study was in direct contrast to a study performed in the fed state by Whitehead (1998). Gastro-retention times achieved in the study by Whitehead were in excess of 5 h for all subjects. The maximum time for which calcium alginate beads were retained in the fasted study was approximately 1.5 h. The gastro-retention of calcium alginate beads in the fasted state has not been prolonged when compared with the administration of calcium alginate beads in the fed state. However, the results indicated that prolonged gastric retention was achieved in the fasted state when the dosage form was administered with the citric acid solution when compared with retention in the absence of citric acid.

Table 1 Gastric emptying times for subjects following administration of calcium alginate beads with 100 mL of water or 100 mL of citric acid solution 1%

Subject no.	Study type	Time to onset of gastric emptying (min)	Further time to completion of gastric emptying (min)
1	Fasted + water	30	55
	Fasted + citric acid	48	15
2	Fasted + water	54	15
	Fasted + citric acid	21	15
3	Fasted + water	20	5
	Fasted + citric acid	42	35
4	Fasted + water	40	50
	Fasted + citric acid	57	25

Whitehead, L. (1998) *An investigation into a gastro-retentive dosage form*. PhD Thesis, University of Manchester

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Design and assessment of drug-dendrimer conjugates for oral drug delivery

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Dendrimers based on polyamidoamine (PAMAM) units possess a well-defined structure that allows a precise control of size, shape and terminal group functionality (Tomalia et al 1985). Dendrimers have several pharmaceutical applications (D'Emanuele et al 2003), such as enhancement of drug solubility and permeability. PAMAM dendrimers have been shown to cross cell barriers at sufficient rates to act as potential carrier/delivery systems, and surface engineering has been found to reduce cytotoxicity and enhance transport (Jevprasesphant et al 2003). In this study the design, synthesis, and characterisation of a series of zero generation (G0) PAMAM dendrimer-based prodrugs for the enhancement of drug solubility and permeability is described. Naproxen was selected as a model low solubility drug and conjugated to dendrimers either directly by an amide bond or by an ester bond (via lactic acid as a linker). The in-vitro release of drug from the dendrimer–naproxen conjugates was examined over a range of pH and in 80% human plasma. Prodrugs were synthesized using an equimolar ratio of dendrimer and naproxen. The carboxylic acid groups of both naproxen and naproxen–lactic acid complex were conjugated to the amine groups of PAMAM G0 dendimer using N,N′-carbonyldiimidazole (CDI) as a coupling agent. The resulting water-soluble prodrugs were characterized by ^{1}H and ^{13}C NMR and RP-HPLC. Hydrolyses of the prodrugs were studied at 37°C in hydrochloric acid buffer (pH = 1.2), phosphate buffer (pH = 7.4) and borate buffer (pH = 8.5) and in 80% human plasma. The results indicate a high chemical and enzymatic stability of the amide-linked conjugate over ten days at each pH and also in 80% human plasma. The stability of the ester conjugates under physiological conditions (pH = 7.4 and 37°C) and at pH = 8.5 was high, with at least 90% of the conjugate remaining intact after ten days; at pH = 1.2, only 2% of the ester conjugate was hydrolysed in 24 h. In 80% human plasma the ester conjugate was slowly hydrolysed and after 24 h 38% of naproxen was released. The high chemical stability of the ester conjugate at = pH 1.2 and the slow release of the parent drug in 80% human plasma make these conjugates suitable for further study as potential prodrugs. Their enzymatic stability in rat liver extract and permeability through Caco-2 cell monolayers are under investigation.

D'Emanuele, A., et al (2003) In: Swarbrick, J., Boylan, J. C. (eds), *Encyclopedia of pharmaceutical technology*. 2nd Edn, Marcel Dekker, New York pp 1–21
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Tomalia, D. A., et al (1985) *Poly. J*. (Tokyo, Japan) **17**: 117–132

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Incorporation of crystalline tetracycline hydrochloride in Witepsol-based microparticles

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Aminolevulinic acid (5-ALA) is a commonly used photosensitizer in PDT, but is hydrophilic and fails to penetrate through intact skin readily. Penetration enhancers have been used to improve distribution of 5-ALA into the skin and iron chelators have been added to enhance production of the naturally occurring photosensitizer, protoporphyrin IX, the end cellular product of ALA administration. Nevertheless, long application times are needed, which exacerbates the degradation of ALA within the dosage form. This is particularly so, given that most cutaneous dosage forms have an aqueous phase at physiological pH, factors that have been shown to accelerate ALA degradation (Bunke et al 2000). Placing the drug in its crystalline form within a water-insoluble particulate matrix may be a strategy that could enhance stability within a topical dosage form. The aim of this study was to evaluate the incorporation and release of a model water-soluble drug (tetracycline HCl) after encapsulation of the crystalline form within a temperature responsive lipid matrix, such as Whitepsol. The effect of temperature on drug release was investigated. Three methods were used to load drug inside the lipid microparticles. Both a hot (solvent-free) and cold homogenisation procedure (solvent assisted) were used to produce O/W and W/O/W double emulsions, using different surfactants and solvents and the effect of each was studied. Several modifications were made (pH, addition of NaCl, addition of excess tetracycline HCl to aqueous and the oil phases) were investigated. These procedures were compared with a novel spraying method, requiring only molten lipid and drug. The formed particles were evaluated using scanning electron microscope (SEM) and sized by photon correlation spectroscopy (PCS). Drug loadings and release were measured spectrophotometerically at 360 nm. Where appropriate, results were analysed using a one-way analysis of variance. $P < 0.05$ was taken to indicate a statistically significant difference. Cold homogenisation produced low mean drug loadings (3% of theoretical) with Witepsol H15. Hot homogenisation produced higher mean drug loadings with Witepsol H15 (11% of theoretical) and significantly lower ($P = 0.035$) mean loadings with stearic acid (4% of theoretical). The spaying method produced mean drug loadings (99% of theoretical), which were significantly greater than both the cold homogenisation ($P < 0.0001$) and the hot homogenisation ($P < 0.0001$) methods. Drug release from particles prepared using the spraying method increased with increasing release medium temperature. SEM showed a narrow size distribution of spherical shaped particles produced by the three procedures. Span 80 (239 nm)- and poloxamer 188 (281 nm)-stabilised emulsions showed the lowest mean particle sizes, while lauryl sulfate (3280 nm) and cetrimide (3449 nm) showed the largest mean particle sizes. A spray procedure using Witepsol forms temperature responsive drug loaded particles that could be used to enhance the stability of water-soluble drugs, such as ALA, when incorporated in a dosage form for application to skin.

Bunke, A., Zerbe, O., Schmid, H., et al (2000) *J. Pharm. Sci*. **89**: 1335–1341

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The in-vitro percutaneous absorption of aluminium from topical products

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Aluminium is widely used as an antiperspirant in cosmetic and pharmaceutical products. Recent interest has focused on the alleged link between aluminium absorption and both breast cancer and Alzheimer's disease (Lindsay et al 2002; Mirick et al 2002; Darbre 2003). It is the aim of this study to measure the percutaneous absorption of aluminium across skin in-vitro. Several shop-bought

products were examined (Driclor and Vaseline Intensive Care Roll-ons, Arrid Ultra Dry and Sure for Men Sticks). As it may be argued that the infinite dose experiment has little relevance to the clinical/consumer use of these products, both finite and infinite dose experiments were conducted to gain relevant "in-use" and theoretical estimates of percutaneous absorption. Experiments were conducted over a 24-h duration using porcine skin in Franz-type diffusion cells, with the donor phase occluded. Total ion analysis was by atomic absorption spectroscopy (AAS). Two trends were apparent. In both finite and infinite dose experiments, aluminium penetration into the receptor compartment was observed. However, this was highly dependent upon the nature of the formulation. Comparing infinite dose and finite dose experiments indicated that both the rate of diffusion and the total amount of aluminium penetrating the skin were substantially lower for finite dose systems. In addition, while absorption continued to rise linearly throughout the duration of the infinite dose experiment, finite dose experiments demonstrated that absorption began to plateau during the experiment. This was most likely due to donor phase depletion of the penetrant. Further, stick products showed little absorption in finite dose experiments. This is most likely due to the applied dose and the nature of the formulation. These results are summarised in Table 1. Further, deposition of aluminium in the stratum corneum was observed from tape stripping experiments. Penetration of aluminium appears to be dependent upon both the method of application to the skin and the nature of the formulation. The issue of formulation may explain the failure of quantitative models of percutaneous absorption (Moss et al 2003) to accurately predict the results presented herein. However, the levels of aluminium observed penetrating across the skin from these products are well below safety limits, and the levels ingested via other routes, as discussed previously (i.e. Flarend et al 2001). This is an emotive field of research, and as such it is important to interpret these results in the context of the experimental method used. Further research in this field is underway in our laboratory.

Table 1 Aluminium absorption from topical products across porcine skin

Product name	Total Absorbed (infinite dose) (ppm)	Total absorbed (finite dose) (ppm)
Driclor Roll-on	403	193
Sure for Men Roll-on	116	13.6
Arrid Ultra Dry Stick	22.6	None detected
Sure for Men Stick	50.8	2.46

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Flarend, R., et al (2001) *Food Chem. Tox.* **39**: 163–168
Lindsay, J., et al. (2002) *Am. J. Epidemiol.* **156**: 445–453
Mirick, D. K., et al (2002) *J. Natl Cancer. Inst.* **94**: 1578–1580
Moss, G. P., et al (2003) *J. Pharm. Pharmacol.* **55**: S17

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Delivery of non-viral gene delivery vectors into the desmoplastic region of spheroid co-cultures of fibroblasts and tumour cells

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Non-viral gene delivery may allow safe and targeted treatment of cancer in the clinic in the future. Current research focuses on the characterisation of delivery vectors in-vitro and in-vivo to understand how physicochemical properties may be manipulated to maximise delivery. Non-viral delivery systems do not achieve the high level of delivery seen with viral delivery constructs, but the relative ease of synthesis and lack of toxicity make them a 'going concern' for investors in pharmaceutical research. We report the characterisation of two peptide-based delivery vectors and the study of penetration in tumour cell and fibroblast/tumour cell co-culture spheroids. Upon interaction with tumour cells, fibroblasts elicit a desmoplastic response resulting in the deposition of dense extracellular matrix (ECM). The ECM in solid tumour presents a steric and electrostatic obstacle to macromolecular constructs and one of the objectives of this work is to achieve enhanced delivery by overcoming this barrier. Two peptides reported to have DNA-binding ability 15mer LK15 (KLLKLLLKLLLKLLK) and 26mer LAH4 (KKALLALALHHLAHLALHLALALKKA) were complexed with plasmid DNA encoding the fusion gene enhanced green fluorescent protein and firefly luciferase *Photinus pyralis* (pEGFPLuc). Complexes of peptide and DNA were characterised by dynamic light scattering, agarose gel retardation, electrophoretic mobility as zeta potential and complex formation assessed by ethidium bromide exclusion or quenching of DNA-bound YOYO-1 fluorescence. Penetration of complexes was characterised in spheroids composed of NIH3T6 fibroblasts and either RIF-1 or SCC VII tumour cells, or SCC VII alone. Peptides were compared with standards poly-lysine 14 ('PLL' MW 1000–4000) and polyethylenimine ('PEI' average MW 2000) and found to be either equally efficient as (LAH4) or more efficient than (LK15) PLL but less efficient than PEI in DNA condensation. Peptide/DNA complexes were formed over a range of cation excesses to study the effect of the size/charge ratio on the depth of penetration into the spheroids. Additional work in progress includes the comparison of YOYO-3 to YOYO-1 as a marker for DNA condensation, the study of transfection ability of the complexes in spheroids and whether penetration and/or transfection efficiency can be improved by pre-incubating the spheroids in a solution of bovine testicular hyaluronidase.

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Physicochemical investigations into thermoresponsive synthetic polymer–DNA complexes

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Synthetic cationic polymers are of increasing interest as potential gene therapy vectors but still show reduced transfection efficiency compared to modified viruses. This is partly because the factors affecting intracellular uptake of DNA complexed with cationic polymers are not yet fully understood. We have been developing thermo- and pH-responsive polymers to address key questions relevant to the binding and release of DNA in-vitro and in cell culture. In particular, we are interested in determining whether thermoresponsive polymers can improve transfection activity via changes in plasmid compaction around the phase transition temperatures of the polymers rather than through changes in binding affinity brought about by phase transitions. (Hinrichs et al 1999; Takeda et al 2004; Twaites et al 2004). Synthesis of poly(N-isopropylacrylamide) (PNIPAm) co-polymers with cationic side-chains was carried out by conventional free-radical and partially controlled (ATRP) routes. Polymer complexes with pX 61 plasmid DNA (6144 base pairs) were then prepared in aqueous solutions at physiological ionic strengths. The binding of plasmid DNA to these polymers and the subsequent release of DNA from the complexes were probed by gel retardation assays, fluorescence spectroscopy and dynamic light scattering in solution/suspension. Confocal microscopy enabled us to monitor the fate of complexes in cell culture, and atomic force microscopy in aqueous solution was used to probe the structures of polymer DNA aggregates immobilized at surfaces. The experimental data show that linear and branched co-polymers containing PNIPAm can bind DNA with high affinity, and that the detailed structures of the thermoresponsive polymer-DNA complexes are temperature dependent. Temperature-dependent particle sizes were exhibited by thermoresponsive polymers, whereas non-responsive polymers aggregated over the same temperature ranges (Table 1). Preliminary studies indicate rapid transport of DNA to cell nuclei and we are currently investigating transfection efficiency of these polymers when complexes are prepared under varying conditions of pH and temperature.

Table 1

Polymer–DNA complex[a]	LCST (°C)	Mean R (nm)	
		20°C	45°C
PEI	none	45	aggregation
PEI-octanamide	none	43	aggregation
PEI-PNIPAm (8%)	30–40	58	52
PNDHA 6:3:1 mw 234 kDa	22–30	66	48
PNDHA 6:3:1 mw 124 kDa	32–40	57	45
PNDAAU (3:1.6:1)	28–44	66	50

[a]Particle sizes for most stable complexes: N:P 4 for PEI, and N:P 2 for PNIPAm co-polymers. Standard error omitted because the radius is a mean of different species, including free polymer, polymer-DNA complexes and some aggregates. Abbreviations: PEI = poly(ethyleneimine), PNDHA = poly((NIPAm)-co-(N, N'-dimethylaminoethyl) methacrylate-co-hexylacrylate), PNDAAU = poly((NIPAm)-co-(N,N'-dimethylaminoethyl)methacrylate-co-11-acrylamidoundecanoic acid).

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A molecular motor controlled by a polymeric switch – a potential DNA packaging and delivery device

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The packaging and transport of DNA across cellular barriers is central to emerging gene therapies and may also play a key role in novel diagnostic/analytical devices for clinical applications. In work funded by the Wellcome Trust SHOWCASE grant, we have developed a nano-scale motor able to translocate DNA and to control the motor activity we have equipped it with a thermoresponsive polymeric switch. This molecular motor system is based on an engineered restriction-modification enzyme, EcoR124I, that binds and then translocates DNA, a process powered by ATP (Firman et al 2000). Initially we have focused on developing synthetic switching devices that alter the activity of the motor, using, as the switch, the well-known thermoresponsive polymer, poly(N-isopropylacrylamide) (PNIPAm). Changes of temperature cause this polymer to undergo a coil-globule transition, changing its conformation and thereby blocking the enzyme active site, inhibiting DNA translocation. Such nano-scale devices comprising of proteins conjugated with polymers, especially those that change their properties with temperature or pH, are of growing interest in the biomedical sciences (Dautzenberg et al 2000; Shimoboji et al 2002). Synthesis of thermoresponsive polymers was achieved by conventional free radical co-polymerization of N-isopropylacrylamide with substituted acrylamides of varying hydrophobicity/hydrophilicity to fine-tune the polymer phase transition, or Lower Critical Solution Temperatures (LCST). Aminoethanethiol was used as a chain transfer agent during polymerization to control molecular weight and to provide amine termini for further reaction, while dansyl-substituted monomers were incorporated for fluorescence detection. End derivatisation of the amines with conventional coupling reagents yielded maleimide-tipped polymers that exhibited coil-globule phase transitions at temperatures of 26–42°C, and with varying molecular weights of 5–14 kDa. Polymer conjugation with the EcoR124I protein subunits was achieved in aqueous solutions at physiological ionic strength via reaction at an engineered cysteine residue close to the N-terminus with the polymer maleimide termini. The polymer enzyme conjugates were further characterized via gel retardation assays, dynamic light scattering and AFM. Enzymatic assays confirmed that the polymer switch was capable of regulating the properties of the DNA binding protein (switching the motor ON and OFF), with marked changes in enzymatic activity above and below polymer phase transition temperature. A mixture of the engineered EcoR124I enzyme active units with PNIPAm exhibited 50% DNA methylation below the phase transition temperature of the polymer, whereas DNA methylation by the same enzyme but with a covalently bound PNIPAm chain was inhibited by 90% below the polymer LCST. The same methylation assay carried out above polymer LCST showed less than 10% difference between the enzyme-polymer mixture and the enzyme-polymer conjugate. Preliminary data suggest that this was due to steric shielding of the enzyme site by the chain extended polymer (i.e. the motor was switched OFF below LCST), but 'open access' to the active site following coil-globule polymer collapse (the motor was switched back ON following polymer collapse). The successful regulation of a very complex enzyme by conjugated smart polymers opens up numerous possibilities for development of nano-scale devices with properties that can be regulated under biologically relevant conditions.

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Investigation into the effect of HPMC concentration and solvent system on the properties of HPMC gels

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Hydroxypropyl methylcellulose (HPMC) is a water-soluble polymer, which is frequently used in the formulation of pharmaceutical systems. It is understood that the rheological properties of gels directly influence their clinical performance (Jones et al 1997). However, while the rheological properties of aqueous HPMC gels have been studied, there is comparatively little information concerning the effects of co-solvents on the rheological properties of these systems. The objective of this study was therefore to investigate the effect of N-methyl pyrrolidone, a model co-solvent that is known to enhance drug permeability across skin, on the mechanical and rheological properties of HPMC gels. Gels containing HPMC (3–10% w/w) were prepared using three molecular weights of HPMC (80–120, 15 K and 100 K) in 5 different solvent systems (100:0, 80:20, 50:50, 20:80 and 0:100 water:N-methyl pyrrolidone (NMP)), as previously reported (Jones et al 1997). Texture profile analysis was performed using 10 mm diameter probe, a 15 mm compression depth and a compression rate of 10 mm s^{-1}. Oscillatory rheometry was performed at 20°C using a CSL^2 100 Carri-Med rheometer in association with parallel plate geometry and a plate gap of 1 mm. Frequency sweeps were performed from 0.01 to 10 Hz at 20°C, with strains varying from 6.5×10^{-3} up to 3.5 rad (Jones et al 1997). The effect of polymer concentration, molecular weight and solvent composition were statistically analysed using a three-way analysis of variance ($P < 0.05$ denoted significance). In oscillatory rheometry, the dynamic viscosity and tan δ of all formulations decreased whereas the storage (G') and loss (G'') modulus increased with increasing frequency. Increasing the polymer concentration and molecular weight increased the mechanical and elastic nature of the formulations due to increased polymer chain entanglement. All systems were gels with the exception of 3% w/w HPMC 15 K in defined solvent systems. Solvent composition affected the resultant mechanical and rheological properties. Increasing the concentration of NMP increased the rheological properties of the various gel systems up to a maximum solvent ratio, dependent on the molecular weight of HPMC. For example the maximum mechanical properties and storage/loss modulus values were observed for 80–120, 15 K and 100 K molecular weight blends in 20:80, 50:50 and 80:20 water:NMP, respectively. The effect of solvent composition on the rheological/mechanical properties may be accredited to increased polymer chain entanglement and swelling, leading to an elastic expanded structure. However in the absence of water there was a marked decrease of rheological properties due to an insufficient ability by NMP to maintain solubility of HPMC (Table 1). This study has shown that manipulation of HPMC concentration and molecular weight can produce a wide variety of mechanical and rheological properties. This information has direct implications for the formulation of non-aqueous gels composed of HPMC.

Table 1 The effect of solvent blend (water:NMP) on the hardness, compressibility, G' (@ 1 Hz) of formulations containing 5% HPMC 100 K

Sol.	Hardness (KN)	Compressibility (KN mm)	G' (KPa)
100:0	1.02 ± 0.08	0.87 ± 0.07	0.23 ± 0.02
80:20	2.18 ± 0.19	1.88 ± 0.15	0.82 ± 0.06

Jones, D. S., et al (1997) *Int. J. Pharm.* **151**: 223–233

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Phosphorylcholine (PC) based polymers as potential vectors for gene delivery

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Phosphorylcholine-based polymers have been demonstrated to be highly biocompatible and capable of producing a protein-resistant surface on implantable medical products. Here we introduce a novel PC-based material: a 2-(dimethylamino)ethyl methacrylate–block-2-(methacryloyloxyethyl phosphorylcholine) (DMA-MPC) diblock copolymer as a candidate vector for gene delivery. DMA homopolymer has been previously confirmed to condense DNA efficiently into condensates able to transfect a number of different cell lines. However, steric stabilization of the complexes, to improve colloidal stability, reduce opsonisation and possibly prolong systemic circulation is desirable. The new DMA–PC copolymers have a potential to create this steric barrier, and are investigated in this study. A family of well-defined DMA-MPC diblock copolymers was synthesized by Atom Transfer Radical Polymerisation (ATRP). Particle size analysis and morphology studies were carried out using photon correlation spectroscopy (PCS) and transmission electron microscopy (TEM), respectively. The ability of the copolymers to protect DNA from enzymatic degradation, was assessed by gel electrophoresis following incubation with DNase I and displacement with poly(aspartic acid). The effect of

varying the lengths of MPC in DMA-based polymers on DNA condensation and protection from enzymatic degradation were studied. The length of DMA block was kept constant at 40 monomer units while the MPC moiety length was varied to be 10, 20, 30, 40 and 50 units. The results clearly showed that the presence of MPC block prevents aggregation of the DNA condensates by providing steric stabilization, creating condensates with sub-200 nm average diameter. However, the steric stabilization effect of the MPC block has to be balanced with the decreased ability of the copolymers to provide efficient DNA condensation as the length of the MPC moiety increases. The reduced level of DNA condensation was found to be directly related with the decreased level of DNA protection from enzymatic degradation in-vitro. This work shows that the presence of the PC block prevents aggregation of the DNA complexes with DMA-MPC copolymers by creating a steric stabilizing layer, but that careful design of the copolymer architecture is required to balance the stabilizing and DNA condensing properties of the copolymers.