siRNA and also silencing of the BCR-ABL protein in K562 CML cells. Tat-LK15 peptide [1], a fusion of Tat and membrane lytic peptide LK15, was used to non-covalently complex siRNA targeting the BCR-ABL mRNA (b3a2 isoform). Complexation of siRNA by Tat-LK15 was studied using fluorescence correlation spectroscopy (FCS) in the presence of the intercalating dye YOPRO-1. Cy5 labelled siRNA was used to study uptake in K562 cells using flow cytometry and confocal microscopy. The reduction in BCR-ABL protein levels was observed by Western blot. Results were compared with K562 cells transfected with lipofectamine/siRNA complexes. MTT assay was performed to study the cytotoxicity of the Tat-LK15/siRNA complexes. The YOPRO-1 competitive binding assay revealed efficient condensation of siRNA by Tat-LK15 and Lipofectamine™ at charge ratios higher than 3:1 (less than 10% of YOPRO-1 labelled siRNA). Flow cytometry studies using varying amounts of siRNA showed an increase in intracellular existence of Cy5-siRNA also leading to an increase in percentage positive transfected cells. Confocal microscopy confirmed the increase in intracellular localization upon transfection with higher amount of siRNA 4 hours and 24 hours post-transfection. Finally RNAi was observed using siRNA, which resulted in 70–80% reduction in BCR-ABL protein levels at lower concentrations. However, silencing observed using siRNA did not last longer than 48 hours. Cytotoxicity studies show that Tat-LK15/siRNA complexes are not toxic when lower concentrations of siRNA are used. Here, we show that Tat-LK15 can be a potential vector in delivering siRNA targeting genes of clinical significance.

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A56

Carbon nanotube-dendron series for siRNA delivery: mechanisms of cellular internalisation

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Carbon nanotubes have been attracting attention as tools for various biomedical applications. Chemical surface functionalization of multi-walled carbon nanotubes (MWNT) has shown remarkably increased aqueous solubility and debundling of nanotube aggregates that makes this material a promising candidate for biological applications. In this work, a series of dendron-MWNT derivatives were synthesized as potential vectors for siRNA delivery [1]. To elucidate the mechanism of cellular internalization characteristics of the dentron-MWNT:siRNA complexes, a fluorescence probed, non-coding siRNA sequence was used and its nanotube-mediated cytoplasmic delivery was studied in comparison to that by cationic liposomes. siRNA delivered by the dendron-MWNT was found throughout the cytoplasm including the nucleus. The siRNA delivered by cationic (DOTAP:cholesterol) liposomes was co-localized with endosomal markers indicating primarily an endocytosis pathway for internalization as previously described in the literature. The cellular transport of the siRNA was significantly increased with higher dendron generations conjugated on the nanotube surface at physiological conditions (37 °C) as well as under endocytosis-inhibiting conditions (4 °C). This work demonstrated that clathrin-coated endocytosis is a contributing but not the major pathway for the cellular internalization of the dentron-MWNT:siRNA complexes and could offer a great advantage via direct cytoplasmic delivery of siRNA for effective gene silencing.

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A57

Cellular internalisation of humanized IgG antibody changes by functionalization onto multi-walled carbon nanotubes

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Antibodies have been extensively used as anti-neoplastic therapeutics clinically and preclinically as they allow for therapeutic and specific targeting to specific cell receptors. The humanized CTMO1 IgG antibody was raised against the membrane-associated antigen of human milk fat globules (HMFG) derived from the anti-HMFG mouse monoclonal antibody CTMO1, but with similar affinity to the polymorphic epithelial mucin-1 (MUC-1). Anticancer drugs derived from murine HMFG1 have been under development in phase III clinical trial [1]. Carbon nanotubes have remarkable physicochemical properties offering an array of interesting features. In the context of this study, their large surface area offered a template for conjugation with a variety of monoclonal antibodies. Multi-walled carbon nanotubes (MWNT) were chemically functionalized with humanized CTMO1 IgG. The MWNT-IgG constructs were observed to target MUC-1 positive cells, but were retained at the plasma membrane with limited internalization. In contrast, a time-dependent cell surface binding and internalization was observed for the humanized CTMO1 IgG alone. The co-localization of the fluorescently labeled IgG with markers of specific cellular compartments was also studied using confocal laser scanning microscopy, to determine its mechanism of cellular uptake and trafficking pathway. The results here indicated that the size and aggregation state of the MWNT-IgG constructs played a determinant role in their interaction with cells. The design and development of CNT-antibody con-

structs needs further optimization in order to constitute a viable novel platform for cancer treatment with the purpose of combinatory therapeutic/diagnostic functionality.

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A58

Role of cell-surface carbohydrates and plasma membrane components in the internalization of cell-penetrating peptides

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Among cell-penetrating peptides, penetratin is widely used as a molecular device to cross membranes and transport biologically active molecules inside cells [1,2]. But, the underlying internalization mechanisms for such behaviour is still studied and discussed [3]. The idea is now well accepted that the physico-chemical properties of the cargo [4], the cell-penetrating peptide [5], and the disulfide-bridge in the conjugate [6], have an impact in the intracellular delivery pathways of the conjugate. Therefore, it is obvious that the internalization pathways and the final localization of conjugates within cells can hardly be anticipated. We have previously reported that penetratin internalizes in cells at 37 °C and 4 °C, thus through translocation and endocytosis pathways [7]. The translocation process occurs at low micromolar penetratin, while endocytosis is activated at higher concentrations. We have now studied the impact of cell-surface (GAG, sialic acid) and plasma membrane (cholesterol) components in the temperature-dependent cell internalization efficiency [8] and pathways [7] of penetratin and other well-studied cell-penetrating peptides. These results will be presented and discussed.

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A59

Development of a microwell device for correlative light and electron microscopy

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New innovations and techniques are constantly being developed within the field of microscopy with the aim of generating higher through-put analysis and/or gaining the maximum data out of a single sample. Correlative light electron microscopy or CLEM involves bringing together the two most common aspects of microscopy, fluorescence and electron microscopy. The weaknesses in fluorescence microscopy, low resolution, can be counteracted by the highly detailed electron microscopy images. On the other hand, the weakness of electron microscopy for live intracellular tracking, can be counteracted using fluorescence microscopy. This project involves the development of a microwell array technique to allow a user to correlatively image the same cell under both fluorescence microscopy and scanning electron microscopy (SEM). Microwell arrays were ablated into borosilicate glass and PDMS (silicone elastomer) cover slips using 193 nm and 157 nm excimer lasers (MetaFAB, Cardiff University). The surface of the substrate is first coated with a sacrificial layer before ablation thus providing an important step in helping to remove ablation debris during sonication. PDMS surfaces were further modified to optimise cell adhesion by oxidizing the surface using UV/ozone treatment and reacting with APTES (aminopropyltriethoxysilnae) to create an amine modified surface. Initially, for proof of concept, KG1a (acute myelogenous leukaemia) cells were allowed to settle into the microwells before being exposed to transferrin as an endocytic marker or a pro-apoptotic peptide linked to the cell penetrating peptide R8 to determine whether apoptosis can be monitored. The cells were then imaged by confocal microscopy then fixed, dehydrated, dried and splutter coated for imaging by SEM. We have successfully imaged uptake of transferrin and the effects of a pro-apoptotic peptide whilst cells were resting within the microwells. We have also obtained correlative images of KG1a cells imaged before fixation under light microscopy and the same cells under SEM. By comparing cell number and their position within the microwells before and after fixation we are confident of achieving correlative microscopy. For adherent cells we are able to create microwell arrays of varying sizes in both glass and PDMS. Post-ablation processing increased microwell quality whilst the auto-fluorescence in glass was reduced by various cleaning steps. However, switching to PDMS provided a much lower auto-fluorescent substrate on which to work. PDMS is naturally very hydrophobic (contact angle ~105°); using UV/ozone we were able to reduce the hydophobicity of the surface (contact angle ~40°). This formation of hydroxyl groups on the surface allowed for further modification using APTES, which improved cell adhesion. We can now obtain correlative images using confocal microscopy and SEM of the same cells and are developing further methods for TEM correlative light electron microscopy studies.

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