Coupling of these ligands showed a highly beneficial effect for transfection efficacy to TNF-α activated endothelial cells compared to non-targeted SAINT-O-Somes. The intracellular delivery of anti VE-cadherin siRNA SAINT-O-Somes to activated endothelial cells resulted in a specific, 70% down-regulation of VE-cadherin gene expression. In conclusion, we demonstrated that SAINT-O-Somes are stable, high capacity carriers for effective siRNA delivery into endothelial cells that present the requirements for *in vivo* application.

See reference below for additional reading

1. Adrian JE, et al. Targeted SAINT-O-Somes for improved intracellular delivery of siRNA and cytotoxic drugs into endothelial cells. *J Control Release* 2010;(March).

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A53
Toxin assisted intracellular delivery of gold nanoparticles

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Targeted intracellular delivery of biomolecules using nanoparticles has attracted many of the science disciplines. Nanoparticles because of their tuneable size and unique optical properties are emerging not only as imaging probes but also serving as intracellular cargo delivery carriers. Gold nanoparticles are best candidate for all these applications because of their not particularly reported cytotoxicity and ease of biofunctionalization. For intracellular cargo delivery application, it is necessary that a carrier is not only has the capacity to carry the biomolecule efficiently but also able to deliver it to the cytosol which is the main site for all physiological and chemical activities inside the cell. It is well documented that on intracellular delivery, bioconjugated gold nanoparticles are trapped by endolysosomes where their biomolecular coating degrades eventually. For avoiding this fate and for gaining access into the cytosol, we used a new approach, that is, toxin assisted delivery for gold nanoparticles. A bacterial toxin streptolysin-O is a secreted protein of 61 kDa which forms pores in plasma membrane of host cell for gaining access into the cytosol. It has been used as a simple and rapid mean of transfection for intracellular delivery of oligonucleotides and siRNA. Our results confirm that SLO treated cells showed an increased cellular uptake of gold nanoparticles then untreated cells. We also studied the effect of poly ethylene glycol (PEG) on SLO assisted cellular uptake by increasing the PEG amount gradually and found that PEG affects the cellular uptake adversely. We are currently combining fluorescence microscopy, photothermal microscopy and transmission electron microscopy to fully understand the mechanism, localization and fate of gold nanoparticles during SLO assisted uptake.

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A54
Quantifying uptake and distribution of arginine rich peptides at therapeutic concentrations using fluorescence correlation spectroscopy and image correlation spectroscopy techniques

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Due to an apparent ability to enter cells in an energy independent manner, cell-penetrating peptides (CPPs) are increasingly being used as vectors for the delivery of macromolecules into cells. But 20 years on, their uptake and intracellular distribution are still debated [1] especially as most studies have been carried out at relatively high concentrations (micromolar), while therapeutic doses more likely to be in the nanomolar range. Thus, we hypothesised that taking advantage of fluorescence correlation spectroscopy (FCS) and image correlation spectroscopy (ICS) should help to understand the delivery mechanisms (especially the intracellular distribution) of arginine rich peptides TAMRA-Tat and TAMRA-nona-arginine (R9) at therapeutic doses. TAMRA-Tat and TAMRA-R9 peptides were incubated for one hour with both Caco-2 and HeLa cells. Initial observation of uptake was carried with a Zeiss LSM510 Meta Confocor 2. FCS and ICS were then used to measure peptide concentrations (density of particles per beam waist area) in distinctive areas and in the whole cell (cartography). ICS, implemented in parallel to FCS, was developed in house based on the work of P. Wiseman's group [2,3]. Subcellular distribution was analysed with confocal microscopy revealing two main areas – punctate and cytoplasmic regions – sampled initially with FCS to obtain diffusion times and concentration. Diffusion times in the punctate areas are very long (300 ± 50 μs) compared to the cytoplasm (26 ± 8 μs) at 500 nM, suggesting a bound component compared to free peptide. As FCS cannot sample the whole cell, ICS provided a more complete view of the distribution of TAMRA-Tat and TAMRA-R9 in which large areas of the cells behave as the 'cytoplasmic' area used in FCS. Our results indicate that arginine rich peptides are observed at nanomolar concentrations in all areas sampled. At concentrations below 500 nM, punctate and discrete areas are clearly labelled suggesting a possible entry via an endocytosis only mechanism. Finally, as the bulk concentration increases the fraction detected in the cytoplasm increases suggesting the simultaneous presence of a non-endocytotic mechanism of entry. Overall, FCS and ICS demonstrate that they provide invaluable information on the cellular delivery of peptides at therapeutic levels.

See reference below for additional reading

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2. Kolin DL, Wiseman PW. *Cell Biochem Biophys* 2007;**49**:141–64.
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A55
Tat-LK15, a Tat-fusion peptide, to deliver therapeutic siRNA in chronic myeloid leukemic cells

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Chronic myeloid leukaemia (CML) is caused by the reciprocal translocation of chromosomes 9 and 22 resulting in the formation of the BCR-ABL fusion protein, which exhibits deregulated tyrosine kinase activity. Hence, BCR-ABL would be a key target for developing a therapy for CML. We have used the potential of RNA interference to study the silencing of this oncoprotein. siRNA has been used to target wide range of genes in various cell types using cell penetraing peptides (CPPs). In this study we have evaluated the ability of the Tat fusion peptide, Tat-LK15 [1] to study uptake of

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.

Reference

1. Saleh AF, et al. Improved Tat-mediated plasmid DNA transfer by fusion to LK15 peptide. *J Control Release* 2010;**143**:233–42.

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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.

Reference

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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-