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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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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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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