Cationic polyhedral oligomeric silsesquioxane (POSS) units as carriers for drug delivery processes†

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Quaternary ammonium functionalized polyhedral oligomeric silsesquioxane (OctaAmmonium–POSS®) units, widely employed as additives in ceramic and polymeric systems, possess many attributes which make them attractive as biocompatible drug carriers: nanoscale size, three-dimensional functionality, efficient cellular uptake, low toxicity, and high solubility.

Silica-based materials are widely used in the field of biomedicine because of their chemical inertness and biocompatibility.[1–3] These materials include diverse applications such as coatings on surgical instruments[4] and catheter tubing[5] to contact lens production.[6,7] Silica nanoparticles have been developed for use in magnetic resonance imaging,[8] the delivery of antisense oligonucleotides,[9] and the delivery of drugs such as dexmedetomidine[10] and cefradine.[11] Recent developments in silicone hydrogels are providing utility as a matrix for transdermal drug delivery,[12] while silicone microspheres are being developed for pH-controlled drug delivery in the gastrointestinal tract.[13] Furthermore, advancements in the field of nanotechnology have provided us with a valuable insight into both the dynamics of membrane transport and the intracellular mechanisms involved upon the introduction of a drug.[14–16] In this communication, we report the ability of polycationic amine-functionalized polyhedral oligomeric silsesquioxanes (OctaAmmonium–POSS®)‡ to serve as carriers and potential drug delivery agents.

POSS units are three-dimensional, cubic shaped, building blocks that contain an inorganic inner siloxane core (6 Å^3) with the possibility of chemical modification at each of the eight corners of the POSS unit (Fig. 1).[17] POSS derivatives have been shown to withstand a variety of thermal and chemical conditions[18] and therefore should display high *in vivo* stability. In addition to ease of chemical functionalization and chemical stability, POSS units exhibit a variety of other attributes that make them attractive as drug delivery agents. Suitably functionalized POSS units are readily soluble in water, suggesting that a POSS-based drug delivery system could be taken orally. The small size and high charge density of the POSS units should also make these systems easily transferrable through vascular pores, resulting in increased tissue uptake. Finally, POSS units are readily synthesized discrete units, eliminating issues that arise from the polydispersity of linear polymers and the synthetic challenges associated with dendrimers.

To probe the utility of silsesquioxanes as drug delivery agents, OctaAmmonium–POSS® units were labeled with a fluorescent dye by neutralization of the ammonium sites on the POSS units with triethylamine and subsequent substitution with a succinimidyl ester derivative of the BODIPY dye (POSS–BODIPY,‡ Fig. 1a). BODIPY is a commonly employed fluorescent cellular membrane marker, which can be readily conjugated to various systems in order to track cellular migration patterns. The remaining ammonium groups on the POSS–BODIPY conjugate provide an overall positive charge, allowing for solubility in aqueous environments and increased cellular uptake. A similar amine terminated BODIPY dye was used as a control (Fig. 1b). Fluorescence confocal microscopy studies were then conducted tracing the cellular migration of POSS–BODIPY in Cos-1 cells (Fig. 2).

Cos-1 cell cultures were approximately 10% confluent, and exhibited normal morphologies before POSS–BODIPY and control amine terminated BODIPY addition. Following four hours of incubation at 37 °C and 5.7% CO_2 with POSS–BODIPY (10 μg mL^{-1}) or control amine terminated BODIPY (10 μg mL^{-1}), cells were washed with phosphate-buffered saline and fresh media was added. Live fluorescence confocal images effectively demonstrate that the POSS–BODIPY units become localized in the intracellular regions, mainly in the cytosol (Fig. 2a).

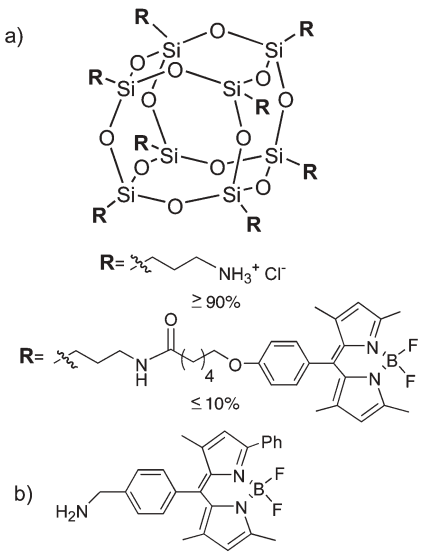

Fig. 1 (a) OctaAmmonium–POSS® units functionalized with fluorescent labeled BODIPY (POSS–BODIPY) and (b) control amine terminated BODIPY.

† Electronic supplementary information (ESI) available: experimental details and IR and ^{1}H NMR spectra of OctaAmmonium–POSS® and POSS–BODIPY. See http://www.rsc.org/suppdata/cc/b4/b416266h/

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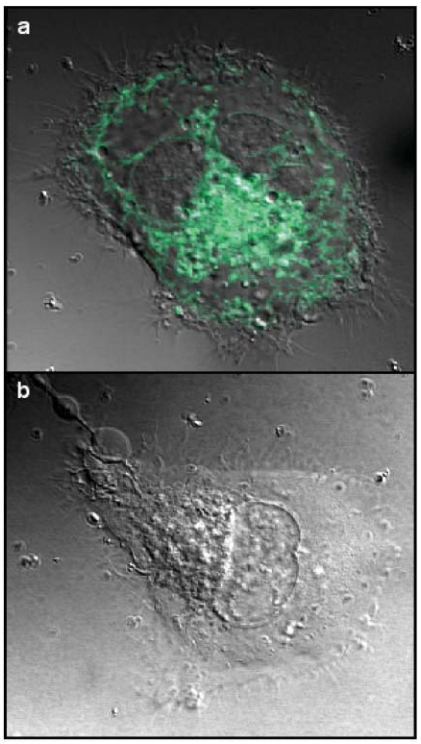

Fig. 2 (a) Fluorescence confocal microscopy images with POSS–BODIPY show efficient uptake in the cytosol. (b) Control microscopy images with amine terminated BODIPY show no cellular uptake of the dye in the absence of POSS.

Control microscopy images with the amine terminated BODIPY show no cellular uptake of the dye in the absence of POSS (Fig. 2b). Three dimensional z-stacks of these cells confirm the above observations (data not shown).

These images also indicate that the cells are unaffected in the presence of POSS–BODIPY, as their cellular morphology remains intact. MTT viability assays run on this system confirm this observation, as activity levels in POSS–BODIPY exposed cells were no different to untouched controls (Fig. 3). These levels were consistent after 36 hours of incubation at 1 mM, demonstrating extremely low toxicity levels.

In conclusion, we have demonstrated that amine-functionalized POSS units exhibit very low toxicity and efficient uptake in the cytoplasm of Cos-1 cells. The migration pattern of POSS–BODIPY is drastically different from the non-conjugated control, demonstrating that the POSS unit is directly responsible for this behavior. The POSS–BODIPY conjugate was evenly dispersed in the cytosol indicating that the conjugates enter the cell *via* diffusion, and not through endocytosis. Interestingly, there was a distinct lack of nuclear uptake, demonstrating a level of specificity in cellular localization. Conjugation of a small molecule either directly to OctaAmmonium–POSS®, or through a cleavable linker should allow for the delivery of drugs which are insoluble in water or exhibit low cellular uptake; research that we are currently undertaking.

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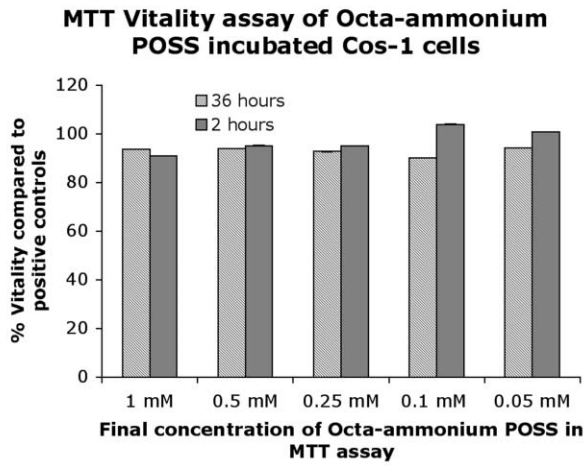

Fig. 3 Vitality of Cos-1 cells incubated with OctaAmmonium–POSS® at 37 °C at 5.7% CO_2 for 2 and 36 hours. Percentage vitality was calculated for each sample by comparing absorbance readings of POSS incubated samples to positive controls.

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Notes and references

‡ OctaAmmonium–POSS® is a registered trademark of Hybrid Plastics, Inc. Conjugation of BODIPY to the POSS units was shown not to alter the dye's fluorescent properties.

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