Target-Specific Stimuli-Responsive Protoporphyrin IX Polysilsesquioxane Nanoparticles to Improve Photodynamic Therapy for Cancer Treatment

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Statement of Purpose: Photodynamic therapy (PDT) has emerged as an alternative approach to chemotherapy and radiotherapy for cancer treatment. PDT is based on the photochemical reactions between a light-activated chemical (photosensitizer, PS) and light of an appropriate wavelength to afford cytotoxicity via the generation of reactive oxygen species (ROS) [1].The PS is perhaps the most critical component of PDT. Traditionally, porphyrins molecules have dominated the field. Nevertheless, these PS agents have several disadvantages, with low water solubility, poor light absorption and reduced selectivity for targeted tissues being some of the main drawbacks. Polysilsesquioxane (PSilQ) nanoparticles are crosslinked homopolymers formed by the condensation of functionalized trialkoxysilanes or bis(trialkoxysilanes). We believe that PSilQ particles provide an interesting platform for developing PS nanocarriers. Several advantages can be foreseen by using this platform such as carrying a large payload of PS molecules; their surface and composition can be tailored to develop multifunctional systems, and due to their small size, nanoparticles can penetrate deep into tissues and be readily internalized by cells [2]. In this work, target-specific PSilQ nanoparticles with a high payload of PSs that are degraded upon intracellular reducing environment were synthesized, characterized, and applied *in vitro*. The network of this nanomaterial is formed by modified protoporphyrin IX (PpIX) molecules chemically connected via a redox-responsive linker. These nanoparticles were further functionalized with polyethylene glycol (PEG) and folic acid to improve their colloidal stability and target capability. The phototoxicity of this PpIX-based PSilQ nanomaterial was successfully demonstrated *in vitro* using cancer cells.

Methods: *Synthesis of redox-responsive silyl-functionalized protoporphyrin IX ligand.*The synthesis the PpIX silyl ligand was carried out through a three steps approach. First, the carboxylic acid groups on PpIX were converted to their corresponding succinimide ester forms using coupling chemistry. The next step involved the nucleophilic acyl substitution reaction in the presence of cysteine. The final step comprised the disulfide exchange reaction of the activated PpIX-cysteine derivative with mercaptopropyl triethoxysilane. A control ligand, which is not redox-responsive, was also synthesized following a similar procedure. All the ligands synthesized in this project were analyzed by ^{1}H NMR, IR, UV-vis and MALDI-MS. *Synthesis and functionalization of redox-responsive PpIX-PSilQ nanoparticles (RR-PpIX-PSilQ NPs).* The synthesis of RR-PSilQ NPs was carried out by following the reverse microemulsion method using a quaternary microemulsion system. The components of this emulsion are cyclohexane/water, hexanol and Triton X-100. The RR-PpIX-PSilQ NPs were further functionalized with PEG and folic acid-PEG polymers. A similar protocol was followed for the control PpIX-PSilQ NPs. The nanoparticles synthesized in this project were characterized by dynamic light scattering (DLS), Z-potential, thermogravimetric analysis (TGA), and scanning electron microscopy (SEM). *Phototoxicity and Cytotoxicity.* HeLa cells were seeded at a density of 1 x 10^4 cells/mL in a 96-well cell plates and incubated for 24 h at 37 °C. Cells were then inoculated with PpIX-PSilQ NPs and RR-PpIX-PSilQ NPs (1, 5, and 10 μg/mL) for 24 h. Samples were exposed to a light source (400-700 nm; 170 ± 3 mW/cm^2) for 20 min. After irradiation, the cells were incubated in cell media for 24 h and the cell survival was tested by the MTS assay. A similar procedure, but in the absence of light, was followed to determine the "dark" toxicity of the PSilQ NPs.

Results and Discussion: SEM pictures display the size of RR-PpIX-PSilQ NPs, which is 50-100 nm in diameter (**Figure 1**). DLS and the neutral Z–potential (-2.5 mV) corroborate that the surface of the particles has been successfully functionalized with PEG chains. The RR-PpIX-PSilQ NPs have a remarkably high content of PpIX molecules of 54.4 % wt according to TGA. The RR-PpIX-PSilQ NPs are biocompatible in the absence of light in a concentration up to 10 μg/mL as was determined by MTS assay (**Figure 2**). The phototoxicity of RR-PpIX-PSilQ NPs was evaluated under light exposure for 20 min using the MTS assay after 24 h of incubation. The redox-responsive system showed a higher phototoxic effect than the control sample (**Figure 2**). Presumably, because of the intracellular release of PpIX molecules avoids the aggregation and self-quenching of PS agents.

Figure 1. SEM micrograph of RR-PpIX-PSilQ-NPs.

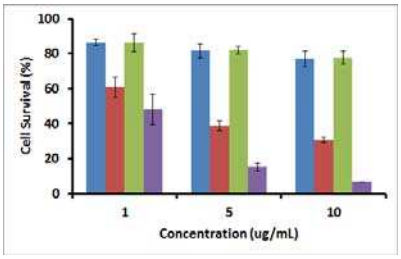

Figure 2. Dark toxicity of PpIX-PSilQ (blue) and RR-PpIX-PSilQ (green) NPs. Phototoxicity of PpIX-PSilQ (red) and RR-PpIX-PSilQ (purple) NPs.

Conclusions: Overall, our data prove that the PDT efficacy can be improved by developing redox-responsive PSilQ materials which selectively release PS agents as individual units inside the cells.

References: [1] O'Connor, A. E.;et. al. Photochem. Photobiol., 2009;85:1053–1074. [2] Vivero-Escoto, J.L., et. al. Proc. of SPIE 2014;8931:89310Z1-10