Optical hyperthermia conducted by Silica/gold nanoshells to induce transgenic expression

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Introduction and Objective

Nanoshells (NS) consist of a spherical dielectric nanoparticulated core surrounded by a thin metal shell [1]. By varying the relative thickness of the nanoparticle (NP) core and its metallic shell, it is possible to generate NPs that can either absorb or scatter light at a desired wavelength. We generated NS with a dense silica core of 160-170 nm in diameter and a gold shell of 12-15 nm thick optimized to absorb near-infrared (NIR) light, particularly in a spectral range known as the "water window" (700-1200 nm). This window represents a region of the electromagnetic spectrum characterized for a minimal absorption by water and biological chromophores [2]. Laser irradiation of NS at their peak extinction coefficient results in the conversion of light energy into heat, which raises temperature in the NPs surroundings. In this study we explored the capability of NS to induce transgene expression in human cells activated by optical hyperthermia.

Materials and methods

The preparation of NS followed the general procedure outlined by Oldenburg *et al.* [3]. The dense silica cores were prepared using a modified Stöber process. The surface of the silica NPs was functionalized with amine groups via silane coupling. Separately, a suspension of Au seeds (from 1-2 nm to 3-4 nm) was prepared following the method reported by Duff *et al.* [4]. The gold seeds were anchored on the amino groups of the functionalized silica cores. The gold shell was then completed by adding additional gold solution and potassium carbonate. NS were characterized by transmission electron microscopy (TEM). Laser irradiation was carried out with an IR Laser system consisting in a 808 nm-wavelength laser diode coupled to an optic fiber of 400 µm and a power controller, placed in a thermostatized chamber. Absorption measurements of the NP-based dispersions were obtained using a UV-Vis-NIR spectrometer. A HeLa-pDsRed-Monomer clonal cell line harboring the encoding sequence of the fluorescent fusion protein DsRed-Monomer under the control of the extremely heat-sensitive human hsp70 promoter was generated. Hela-pDsRed-Monomer cells respond to subtle increases of culture temperature by increasing the expression of the transgene. Cell viability was assessed in cultures treated for 24 h in the presence or absence of NS, using the alamarBlue assay. Cell internalization of NS was visualized using the reflection mode of the spectral confocal microscope (CLSM). For *in vitro* photothermal studies, HeLa-pDsRed-Monomer cells were exposed to NS and irradiated from the top surface with the 808 nm infrared diode laser. After irradiation, the fluorescence signal derived from the DsRed-Monomer protein was visualized using CLSM.

Results and conclusions

TEM micrograph in Figure 1A showed the NS structure. NS presented a clear dense silica-core coated with a high density layer corresponding to the gold shell. It is worth noting the homogeneity in the size distribution of synthesized NS. These NPs showed a strong absorption of the light radiation in the 500-900 nm range of the NIR region (Figure 1B). After addition of NS to the culture medium, the material was exposed to a 808 nm-wavelength laser system integrated in a thermostated chamber to establish

the environmental temperature at physiological conditions. The heat dissipated from NS significantly increased, in a dose-dependent manner, the temperature of the culture medium where they were immersed (Figure 1C). Prior to the development of the optical hyperthermia protocols, cell viability and NS internalization were tested in cells exposed to the material for 24 h. NS treatment did not impaired metabolic activities of the cells, indicating a good biocompatibility of the material. Cells were able to internalize NS, which appeared located as clusters inside the cells. Application of NIR light at 808 nm on cultures treated with NS generated an increase of intracellular temperature that efficiently activated the expression of fluorescent DsRed-Monomer protein controlled by the thermo-sensitive promoter (Figure 2A). In the absence of NIR laser treatment, cells were not able to activate the expression of the fusion protein (Figure 2B). In summary, we demonstrated the suitability of NS as photothermal agents to induce transgene expression after sub-lethal heat treatment conducted by NIR radiation. This finding points to the great potential of NS in multimodal therapeutic protocols based in the combination of optical hyperthermia and gene therapy.

References

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Figures

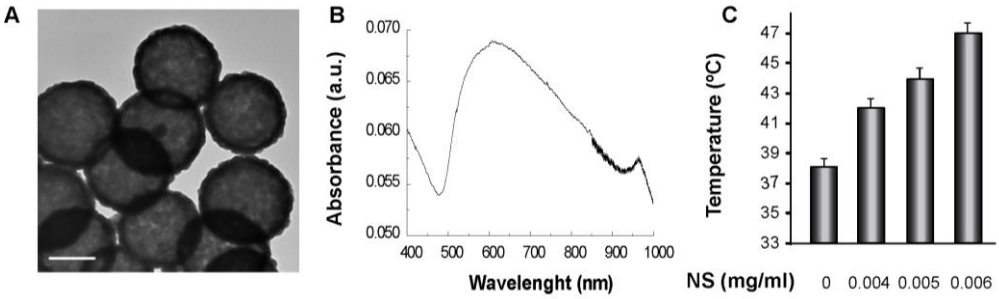

Figure 1. (A) TEM image of NS. Bar = 50 nm. (B) Absorbance spectra of NS. The peak absorbance wavelength corresponds to the NS surface plasmon resonance. (C) Temperature measurements of culture media containing increasing concentrations of NS, after 10 min of exposure to NIR laser.

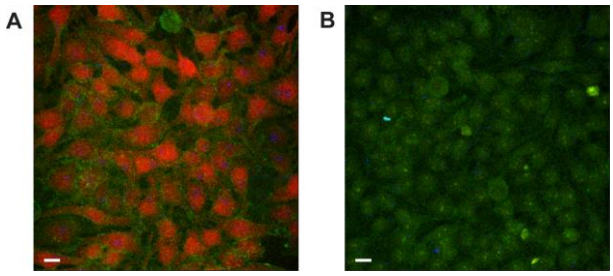

Figure 2. Transgene expression after sub-lethal optical hyperthermia. Hela-pDsRed-Monomer cells were exposed to NS for 24 h, washed to remove non-internalized NS and exposed (A) or not (B) to NIR laser at 808 nm for 30 min. The fluorescent signal derived from the expression of DsRed-Monomer protein was observed using CLSM after 24 h. Confocal maximum projections show DsRed-Monomer (red) and actin (green) stained cells. NS (blue) were detected by reflection. Bars = 10 µm.