Photocytotoxicity effect of polyelectrolyte microcapsules with chlorin E6

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Introduction

Photodynamic therapy (PDT) is a photochemistry-involved treatment process that uses different photosensitizers to generate cytotoxic reactive oxygen species (ROS) under light activation, thus causing cell apoptosis and necrosis and tissue destruction. PDT has some advantages with other methods of treatment of oncology such minimal toxicity to normal tissues or organs because the generation of ROS is a light-triggered process and photosensitizer usually are not toxic in dark. Some agents for PDT have poor cellular uptake that limit the current applications of PDT in cancer. Therefore, the development of new PDT agents with effective tumor homing ability, and are excitable by near-infrared (NIR) light with much better tissue penetration, is still needed.

Materials and methods

Polyelectrolyte microcapsules were made by method layer-by-layer with magnetic nanoparticles (caps-ClE6) and chlorine E6 (ClE6).We used concentration of capsule 10-20 per cell and the same concentration of free ClE6 56 and 112 pg/cell (2.8 and 5.6 μg/mL). The dark cytotoxicity and phototoxic effect of caps-ClE6 and free ClE6 were evaluated using the MTT and NRU colometric tests on mouse hepatoma cells Mh22a. After 24-h incubation, the cells were irradiated with red light (660 nm, 15 min). The cell viability was determined after 24 hours. The targeting in vitro was determined in Petri dish using permanent magnet during 24 hours. The cell death assessed using fluorescent dyes (acridine orange and ethidium bromide) after irradiation with red light.

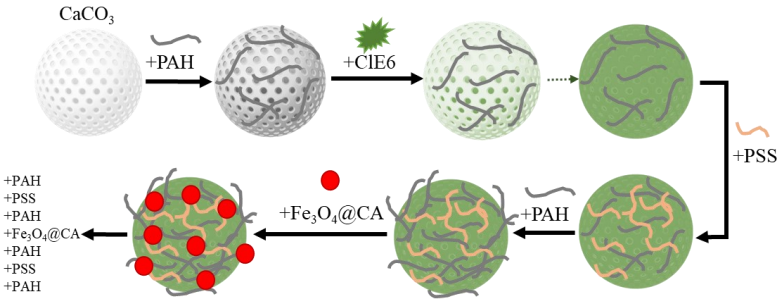

Figure 1. Scheme for the synthesis of microcapsules with the following composition: CaCO3/PAH: Ce6/ PAH / PSS / (PAH NpFe3O4@CA)2 / PAH / PSS.

Results

One microcapsule contains 4 pg MNP and 5.6 pg ClE6. MTT and NRU assay results showed that the viability of Mh22a at concentrations of 10-20 capsules per cell was more 70%. In case of free ClE6 in concentration 112 pg/cell (5.6 μg/mL) the viability was 26% by MTT test and 40% by NRU test. After exposure of red light the cell death was 92% at a concentration of 20 microcapsules per cell and 95% for free ClE6. For caps-ClE6 by NRU test the dark viability was 87%, after red light – 34%. After incubation of the cells with caps-ClE6 in Petri dish with the permanent magnet and irradiation with red light fluorescence staining showed almost complete cell death by necrosis and apoptosis on the magnet and no cell death outside the magnet.

Conclusions

Chlorin E6 containing microcapsules had less dark cytotoxicity and the same phototoxicity effect when exposed to red light compared with free chlorine E6. The incubation the cells with caps-ClE6 on magnet and irradiation with red light could to kill the cells in area of the magnet without damage surrounding cells.

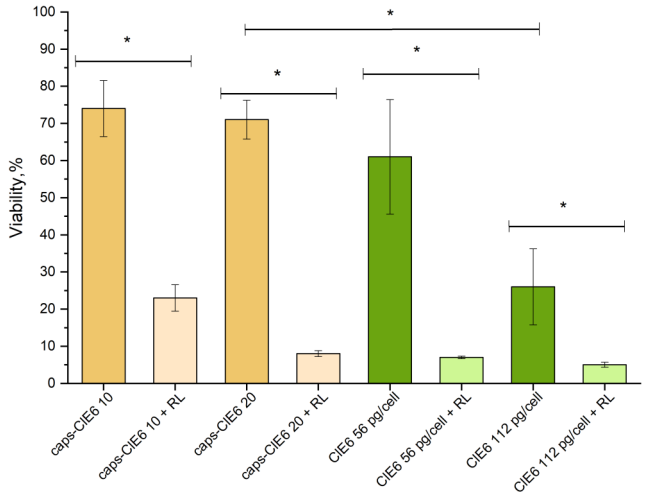

Figure 2. MTT test on Mh22a with caps-ClE6 and free ClE6 without Red light (RL) and after RL 660 nm 15 min

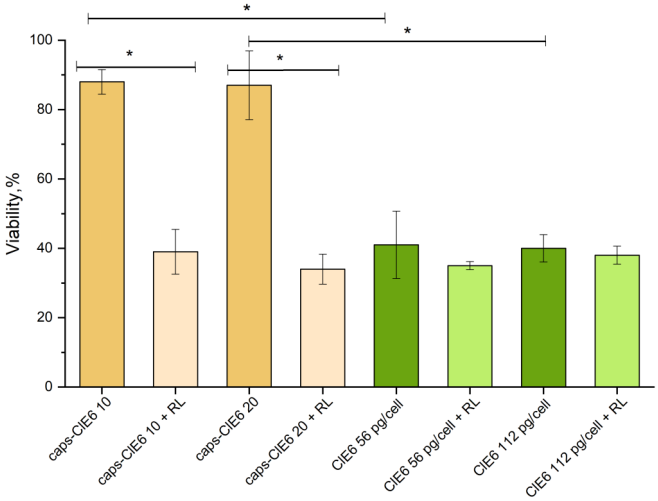

Figure 3. NRU test on Mh22a with caps-ClE6 and free ClE6 without Red light (RL) and after RL 660 nm 15 min

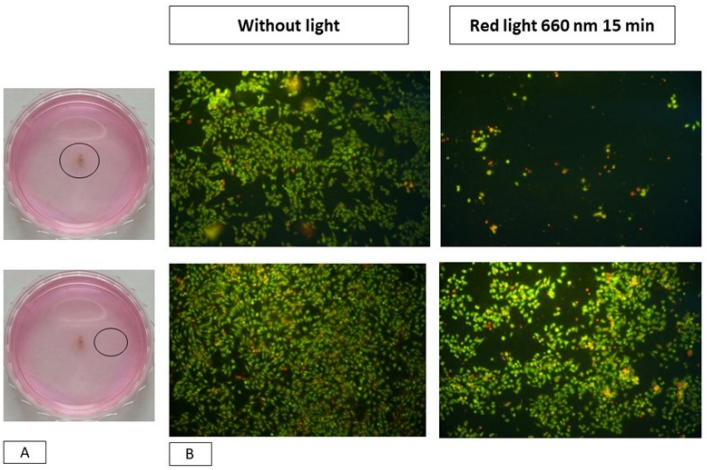

Figure 4. Magnetic targeting in vitro. (a) Photographs of a Petri dish with Mh22a after exposure to an external magnetic field for 4 hours. Fluorescence images of cells co-stained with AO/EB after light irradiation (660 nm, 15 min) taken at different locations in the Petri dish. Green – live cells, yellow and orange – apoptotic cells, red – dead cells.