Zwitterionic phosphorylcholine as a better ligand for gold nanorods cell uptake and selective photothermal ablation of cancer cells†

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The conjugation of zwitterionic phosphorylcholine onto gold nanorods leads to enhanced and selective uptake within cancer cells.

The great promise for nanotechnology in medical applications has focused on the development of different forms of nanoparticles for cancer therapy.1 A novel technology named near-infrared (NIR) photothermal therapy has recently attracted much attention due to the ability of NIR laser to penetrate into deep tissues with high spatial precision.2 Gold nanorods (GNRs) have been regarded as one of the most efficient exogenous agents for NIR photothermal cancer therapy.3 However, for further biomedical applications, these GNRs should not only present high photothermal conversion efficiency, but also colloidal stability4 and biocompatibility5 in the physiological environment. Surface modification is a good choice to solve these problems and polyethylene glycol (PEG) is the first choice to enhance the dispersion stability and biocompatibility of nanoparticles.4 Tumour selective targeting to depress heat in the surrounding normal cells is another key issue for safe and effective photothermal therapy. Although the PEG modified nanoparticles will benefit from their passive accumulation in the cancer tissues via the enhanced permeability and retention (EPR) effect, they can not selectively target the cancer cells and will finally exhibit poor cell uptake abilities.6 The specific targeting on cancer cells is usually achieved by immobilization of antibodies or other ligands that recognize tumour-associated antigens.8

Phosphorylcholine (PC) is a zwitterionic molecular segment present at the end of some lipids and the external surfaces of cell membranes. The presence of phosphorylcholine on polymeric micelles and nanoparticles has been shown to increase their dispersion stability and decrease cytotoxicity.2,6,7 We have previously reported that zwitterionic PC showed a much better ability to stabilize big gold nanoparticles than the neutral PEG.8,9 Chen et al.10 have demonstrated that PC conjugated silver nanoparticles kept the cell uptake ability, which can be another good reason for further exploring the potentials of zwitterionic ligands in biomedical applications. Here we demonstrate that nasopharyngeal cancer cells (CNE-1) internalized much more GNRs@PC than GNRs@PEG. More interestingly, the amount of GNRs@PC in CNE-1 cancer cells was much more than in rhinal epithelia normal ones. For the first time, to our knowledge, the selective cell uptake between cancer cells and related normal ones was achieved via simple surface chemical modification without adding active ligands for specific recognition of membrane biomarkers. Consequently, the in vitro NIR laser irradiation assay verified the effective ablation of cancer cells at low laser energy without lethal harm to the normal ones. All GNRs used here were synthesized according to the wet chemistry method invented by Murphy et al.3 The gold surface was conjugated with 11-mercaptoundecyl phosphorylcholine (HS-PC) or 11-mercaptoundecyl polyethylene glycol (HS-PEG) by ligand exchange with CTAB on the as-prepared GNRs (see ESI† Scheme S1 and Fig. S1). The molecules on the surfaces were verified by FT-IR spectroscopy and δ-potential analysis (see ESI† Fig. S2). The dispersion stability of GNRs@PC was investigated in PBS solution, blood plasma and cell cultivation media according to their UV-Vis absorbance changes12 (see ESI† Fig. S3). It was found in our case that neither the absorption peak wavelength nor the peak half-width was greatly altered with the time up to 24 h. After one day, the peak wavelength changes and the peak half-width changed in PBS solution, blood plasma and cell cultivation media were all much smaller than would be expected if aggregation happened, as reported in the literature (see ESI† Fig. S3).a,b The MTT assay demonstrated that both the PC and PEG modifications brought low cytotoxicity within a relatively higher concentration range of GNRs compared with the as-prepared GNRs@CTAB (see ESI† Fig. 4).

The cell uptake abilities of GNRs@PC and GNRs@PEG were investigated to understand the effect of surface ligands on cell internalization. CNE-1 cells were cultivated in 96-plate wells to nearly 100% confluence. Either GNRs@PC or GNRs@PEG was then added and incubated with cells for certain times. The cell internalization was investigated by inductively coupled plasma atomic emission spectroscopy (ICP-AES), TEM and UV-Vis spectroscopy. After 12 h, the contents of GNRs inside cells were investigated quantitatively by ICP-AES analysis.7,11 The ICP-AES results (Fig. 1(D)) reflected that the number of GNRs@PC within each cell largely surpassed the number of GNRs@PEG. The phenomena were supported by the TEM images of cellular ultrathin sections. A considerable amount of GNRs@PC was dispersed in the cytoplasm (Fig. 1(A)), whereas there was almost no GNRs@PEG inside cells (Fig. 1(B)). The UV-Vis absorbance changes which represented the GNRs concentration

† Electronic supplementary information (ESI) available: Preparation of GNRs@PC and GNRs@PEG, biostability assay, cytotoxicity MTT assay, cell uptake assay, and NIR photothermal assay. See DOI: 10.1039/b915125g
alterations in the media (see details in ESI†) were used to investigate cell uptake kinetics. When cultivated with CNE-1 cells, the GNRs@PC UV-Vis absorbance in the cultivation medium dropped faster and lower than the GNRs@PEG UV-Vis absorbance (Fig. 2(A)). After 12 h, the decreased UV-Vis absorbance amount of GNRs@PC was four times more than that of GNRs@PEG. All of the data above gave strong support for the higher capability of GNRs@PC to be internalized by CNE-1 cancer cells.

The cell uptake difference of GNRs@PC by CNE-1 cancer cells and rhinal epithelia normal ones was further studied. The ICP-AES results in Fig. 1(D) indicated that the gold content in normal cells was much less than in the cancer ones. Similarly, the decreased UV-Vis absorbance amount of GNRs@PC within rhinal epithelia normal cells only reached one third of that in CNE-1 cancer cells after 12 h (Fig. 2(B)). This trend could also been seen from TEM images where a scarcity of GNRs@PC can be found in rhinal epithelia normal cells (Fig. 1(C)). The PC modification seemed to cause a difference in cell uptake of nanoparticles between cancer and normal cells derived from the same parts of tissues or organs, without further active targeting design. It was surprising yet exciting to find, for the first time as far as we know, that the selective cell uptake of nanoparticles between cancer cells and normal ones could be achieved via simple surface PC modification.

Although cell uptake studies of nanoparticles into the cytoplasm or even into the nucleus have already been reported, the detailed mechanisms are still in hot debate. It is well known that the passivation of PEG will depress cell uptake and show low cytotoxicity. However, other surface modifications, including phospholipids and zwitterionics, have also been reported to reduce cytotoxicity while retaining the cell uptake ability. This ability has been ascribed to the same possibility of fusion with cell membranes demonstrated by liposomes. The existence of a large number of nanorods outside endosomes in our results indicated that the cell uptake of GNRs@PC might bypass the normal endosomal route. Compared with normal
systems and should be continued to be investigated.

In the field of biomedical nanotechnology, zwitterionic phosphorylcholine can be suggested as one of the next potential candidates for selective uptake of GNRs@PC by cancer cells. This is because PC not only brings good dispersion stability and low cytotoxicity, but also leads to enhanced and selective uptake within cancer cells. The selective accumulation of GNRs@PC makes them able to kill cancer cells at a low laser energy which will not harm the neighboring normal ones.

In conclusion, we demonstrate that the covalent conjugation of PC onto GNRs not only brings good dispersion stability and low cytotoxicity, but also leads to enhanced and selective cell uptake within cancer cells. The selective accumulation of GNRs@PC makes them able to kill cancer cells at a low laser energy which will not harm the neighboring normal ones. This journal is © The Royal Society of Chemistry 2010

Notes and references


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