Silica coating improves the efficacy of Pd nanosheets for photothermal therapy of cancer cells using near infrared laser†

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Thickenss does matter! The ultrathin nature of 1.8 nm-thick Pd nanosheets prevents them entering cells effectively. A 13-times enhancement in the cells’ uptake of the Pd nanosheets has now been achieved by silica coating together with surface functionalization, therefore significantly improving their NIR photothermal cell-killing efficacy.

Owing to the deep tissue penetrating nature of near infrared (NIR) radiation, photothermal therapy using NIR laser has recently emerged as a new candidate technique for cancer treatment. In this technique, NIR absorbers are delivered to target cells or tissue and irradiated with a NIR laser. The absorbed irradiation is then converted into heat to kill the surrounding cancer cells. Thus, there is no doubt that the advancement of NIR photothermal cancer therapy is linked to the development of hyperthermia agents that can effectively convert NIR to heat.

Over the past decade, metal nanostructures have appeared as the most studied agents for NIR photothermal therapy due to their tunable surface plasmon resonance (SPR) properties. Nanorods, nanoprisms, nanoplates, nanoshells, and nanocages/nanoboxes of Au/Ag are the most common metal nanostructures that display NIR SPR features. However, studies have demonstrated that many anisotropic and hollow Ag/Au nanostructures exhibiting NIR SPR were lacking good photothermal stability upon irradiation of NIR laser. For example, the heat generated by continuous-wave NIR irradiation could melt Au/Ag nanoparticles into solid particles, leading to the loss of their NIR SPR and thereby inevitably imposing limitations in their practical therapeutic applications.

In a typical modification experiment, uniform ultrathin hexagonal Pd nanosheets (NS) with the average edge length of 41 nm were first prepared according to our method described previously (Fig. 1a). The silica coating was then performed by dispersing and stirring the Pd nanosheets in an ethanolic solution of tetraethoxysilane (TEOS) using...
methylamine as the catalyst.\textsuperscript{21} As illustrated in Fig. 1b, the silica coating on the Pd nanosheets resulted in the formation of uniform silica coated Pd nanosheets (SPNS). The overall thickness of the core-shell SPNS nanodisks was measured to be \( \sim 32 \) nm, significantly larger than the original thickness of the Pd nanosheets. It is worth pointing out that using methylamine, as an alternative to ammonia, to catalyze the hydrolysis of TEOS is critical to avoid the etching and aggregation of Pd nanosheets during the silica coating process (Fig. S1).

Most importantly, similar to the parental Pd nanosheets, the as-prepared SPNS exhibit the strong surface plasmon resonance (SPR) absorption in the NIR region (Fig. 1c). The photothermal effect of SPNS induced by the NIR SPR absorption was investigated by monitoring the temperature of 1 mL aqueous solutions of various concentration of SPNS (i.e., 0, 7, 15 and 30 ppm Pd) irradiated by a NIR laser (808 nm, 1 W). As shown in Fig. 1d, the temperature of the solution SPNS containing 30 ppm Pd rose from 27.6 to 48.4 °C after 10 min irradiation.

In order to study how charge and morphology influence the uptake of Pd nanosheets by living cells, comparison studies were performed among the four Pd samples: original Pd NS, polyethyleneimine (PEI)-modified Pd NS, SPNS and SPNS functionalized with amino groups (SPNS-NH\(_2\)). As revealed by zeta potential measurements (Fig. 2a), both the unmodified and silica-coated Pd NS were negatively charged in the PBS buffer (pH = 7.4). The charge of the Pd NS nanosheets was changed to positive after modified with PEI. Similarly, when the surface of SPNS was functionalized with amino groups by reacting with aminopropyltriethoxysilane (APTES),\textsuperscript{22,23} the resulting SPNS-NH\(_2\) particles became positively charged.

To qualitatively determine the Pd uptake by living cells, inductively coupled plasma mass spectrometry (ICP-MS) was used. The human hepatoma cells (QGY-7703) were incubated for 12 h separately with media containing the above four different Pd NS samples in the same concentration (107 \( \mu \)g mL\(^{-1}\) Pd, total volume 0.5 mL). After incubation, the media were removed and the cells were washed carefully with PBS solution to remove extracellular nanoparticles. The cells were then detached from the plate by treatment with trypsin-EDTA. After being washed, the cells were digested with \textit{aqua regia} and diluted for ICP measurements. As illustrated in Fig. 2b, without any surface modification, only 0.1% of the Pd NS (0.11 ppm) were taken up by the cells. The modification of the Pd nanosheets with PEI increased the uptake rate of the Pd nanosheets to 0.2% (0.21 ppm). This increment can be attributed to the enhanced attraction between the positively charged nanosheets and the negatively charged cell membranes. A much more dramatic increment in the uptake was observed for the silica-coated Pd nanosheets. After the 12 h incubation, 0.5% (0.52 ppm Pd) of SPNS entered the cells. As compared to that of the original Pd nanosheets, the four-time increment in

![Fig. 2](image-url)  
**Fig. 2** (a) Zeta potential distributions of original Pd NS, PEI-modified Pd NS, SPNS and SPNS-NH\(_2\) in the PBS buffer (pH = 7.4). (b) The Pd uptake rate of Pd NS, PEI-modified Pd NS, SPNS and SPNS-NH\(_2\) as measured by ICP. (c) The fluorescence spectra of SPNS and FSPNS labeled with FITC. (d,e) Representative fluorescence microscopic images of QGY-7703 cells after 12 h incubation with FSPNS and FSPNS-NH\(_2\), respectively. The scale bars in d and e are 100 \( \mu \)m. (f) Flow cytometric profiles of QSG-7703 cells after being incubated with FSPNS and FSPNS-NH\(_2\).

![Fig. 3](image-url)  
**Fig. 3** (a) Viability of healthy liver cells incubated for 48 h with different-concentrations of original Pd NS and SPNS-NH\(_2\). The cell viabilities were measured by standard MTT assay. For Pd NS, the dose amounts are the corresponding Pd contents. The dose amounts of SPNS-NH\(_2\) are the total content of Pd and SiO\(_2\). The SiO\(_2\)/Pd mass ratio in SPNS-NH\(_2\) was 18.6. (b,c) Micrographs of QGY-7703 cells after 12 h incubation with SPNS-NH\(_2\) and original Pd NS, respectively, and also 2 min irradiation of NIR laser (1.4 W cm\(^{-2}\), 808 nm). The scale bars in b and c are 100 \( \mu \)m. (d) Viability of QGY-7703 cells incubated with original Pd NS, PEI-modified Pd NS, SPNS and SPNS-NH\(_2\) upon irradiation of NIR laser (1.4 W cm\(^{-2}\), 808 nm) for different periods. Before irradiation, the incubated cells were washed twice with PBS solutions.
the Pd uptake implied the importance of the increased thickness due to the silica coating. The uptake rate of the incubated Pd nanosheets was further observed to climb to 1.3% (1.43 ppm Pd) when the surface of SPNS was functionalized with amino groups. Such an uptake was 13 times greater than that of the original Pd NS, indicating that SPNS-NH₂ nanodisks could be a better candidate for photothermal killing of cancer cells.

One advantage of silica coating on Pd nanosheets is that fluorescent probes can be easily incorporated for straightforward imaging of the Pd nanosheets in living cells. In our studies, FITC was selected as the fluorescent probe and covalently doped into the silica shell of SPNS. The resulting fluorescent SPNS, denoted as FSPNS, are highly fluorescent (Fig. 2c), facilitating the direct detection of intracellular uptake of the Pd nanosheets by living cells using fluorescence microscopy or flow cytometry. After 12 h incubation in the medium containing FSPNS (14 μg mL⁻¹ Pd), as revealed by fluorescence micrographs (Fig. 2d), fluorescence signals were readily observed inside the cells (QGY-7703). In comparison, much stronger fluorescence signals were detected in the cells that were incubated with amino-functionalized FSPNS (FSPNS-NH₂) (14 μg mL⁻¹ Pd) for the same period (i.e., 12 h) (Fig. 2e). Flow cytometry experiments were also carried out to quantitatively compare the uptake difference between FSPNS and FSPNS-NH₂. As clearly revealed in Fig. 2f, the mean fluorescence intensity of the cells incubated with FSPNS-NH₂ was much higher than those with FSPNS. All these results further verified that positively charged FSPNS-NH₂ were taken up by the cells more easily than FSPNS, consistent with our ICP measurements.

While promoting the uptake by increasing the thickness and facilitating the surface charge modification, the silica coating on the ultrathin Pd nanosheets retained the excellent biocompatibility of the Pd nanosheets (Fig. S2†). The viable cell count for healthy liver cells was only reduced by 13.4% after 48 h exposure to a SPNS-NH₂ solution containing 1000 μg (Pd + SiO₂) mL⁻¹ (Fig. 3a). Therefore, without laser irradiation, SPNS-NH₂ alone barely killed the cancer cells (Fig. S3†). Upon NIR laser irradiation, however, the incubated cells were killed effectively. In our photothermal studies, the cells were incubated with various Pd nanosheets (55 μg mL⁻¹ Pd) for 12 h before the irradiation of an 808 nm laser providing 1.4 W cm⁻². As illustrated in Fig. 3b, 100% of the cells incubated with SPNS-NH₂ were killed after 2 min irradiation. In comparison, the same irradiation killed only half of the cells incubated with the unmodified Pd nanosheets (Fig. 3c). The improved photothermal cell-killing efficacy by SPNS-NH₂ was also confirmed by MTT assay (Fig. 3d).

In conclusion, coating silica on ultrathin Pd nanosheets has been demonstrated as an effective approach to promote the uptake of Pd nanosheets by cancers cells. Together with the increased thickness of the nanosheets by silica coating, the positive charge induced by surface functionalization increased the Pd uptake by over ten times. The enhanced uptake significantly promoted the photothermal cell-killing efficacy of the functionalized silica-coated Pd nanosheets, making them promising for in vivo applications.

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Notes and references