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A novel magnetic drug delivery nanocomplex with a cisplatin-conjugated Fe_3O_4 core and a PEG-functionalized mesoporous silica shell for enhancing cancer drug delivery efficiency†

Chandrababu Rejeeth,‡*[a] Raju Vivek‡[a] and Soundarapandian Kannan[ab]

Towards the rapid synthesis and efficient action of a smart drug delivery nanosystem based on coating a layer of PEG functionalized mesoporous silica onto a cisplatin (CDDP) conjugated Fe_3O_4 nanocomposite. We achieve a number of nanocomplex enhancements of the biomagnetic carriers' therapeutic functionality. Such a novel drug-delivery nanosystem has potential applications in cancer treatment.

In recent years the development of nanosystems for drug delivery has attracted much attention, since nanosystems are an active and secure way to administer therapeutic levels of drugs for targeting specific tissues, organs, or cellular systems.[1] Cisplatin has been widely employed in medicine to treat a variety of cancers such as small cell lung, breast, ovarian, bladder, head and neck due to its potent ability to cross-link with DNA upon entering cells.[2] In particular, magnetic micro- and nanoparticles are presently recognized as some of the most promising examples of such carriers.[3] Magnetic tagging of drugs enables transport with the use of an external magnetic field, leading to tissue-specific release of drugs and the alleviation of side effects. Nanocomplexes of magnetic particle based drug carrier systems have physicochemical properties that can be significantly affected, such as colloidal stability, drug release behavior, and magnetic intensity of the carrier, which are critical to their clinical applications.[4] To date, only two structural models have been developed: (i) the drug is conjugated or physisorbed onto the surface of polymer coated magnetic particles; (ii) a mixture of drugs and magnetic particles are embedded into a polymer.[5] Although simple, this approach for the direct attachment of drug molecules onto the surface of magnetic particles, either by physical adsorption or chemical bonding, can cause serious problems since the drug can be dissociated from the system and released in non-target areas. Specifically, biocompatible magnetite (Fe_3O_4) nanoparticles have been heavily pursued as versatile carriers for diagnostic and therapeutic applications with super paramagnetism and they are also excellent contrast agents for magnetic resonance imaging (MRI).[6] Moreover, the drug release rates for these reported systems are dependent on one factor alone, either the breaking of the bonding of the drug to the carrier or the swelling and degradation of the polymer. The resultant relatively fast drug release will increase drug leakage during transport and thus will decrease the effective concentration of the therapeutic agent at the target site.

Herein, we synthesize a novel magnetic drug delivery nanosystem, composed of cisplatin conjugated Fe_3O_4 nanoparticles embedded in a polyethylene glycol (PEG) functionalized mesoporous silica ($mpSiO_2$) shell (Fe_3O_4–CDDP/$mpSiO_2$-PEG). The detailed synthesis procedure for the nanosystem, as illustrated in Scheme 1, is described in the ESI.† The presence of a mesoporous silica shell is demonstrated to lead to a number of advantages with respect to the magnetic carrier's therapeutic functionality for treating cancers. It not only provides a protective layer of drug molecules and magnetite nanoparticles, but also imposes a further obstacle for CDDP release from the carrier in addition to the cleavage of chemical bonds, and consequently it exhibits a slower release behavior than that of CDDP-conjugated Fe_3O_4 nanoparticles alone. The mesoporous silica shell can also be easily coupled with targeting ligands. In addition, the surface modified hydrophilic and biocompatible polymer PEG may prevent recognition of endothelial reticulum stress, therefore allowing drugs to be administered over prolonged periods of time.[5] The impartial nature of the PEG layer could also facilitate internalization of the carrier by target cells.[7] By the thermal decomposition of $Fe(acac)_3$ with oleylamine as a capping agent,[8] we first prepared monodisperse Fe_3O_4 nanoparticles (Fig. S1, ESI†), with a size of 50 nm as estimated from the SEM image in Fig. 1a. To facilitate subsequent conjugation

[a]*Proteomics & Molecular Cell Physiology Laboratory, Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore-641 046, TN, India. E-mail: crejee@gmail.com; crejee@sjtu.edu.cn; Tel: +91 9486138085*

[b]*Department of Zoology, Periyar University, Salem-636 001, TN, India*

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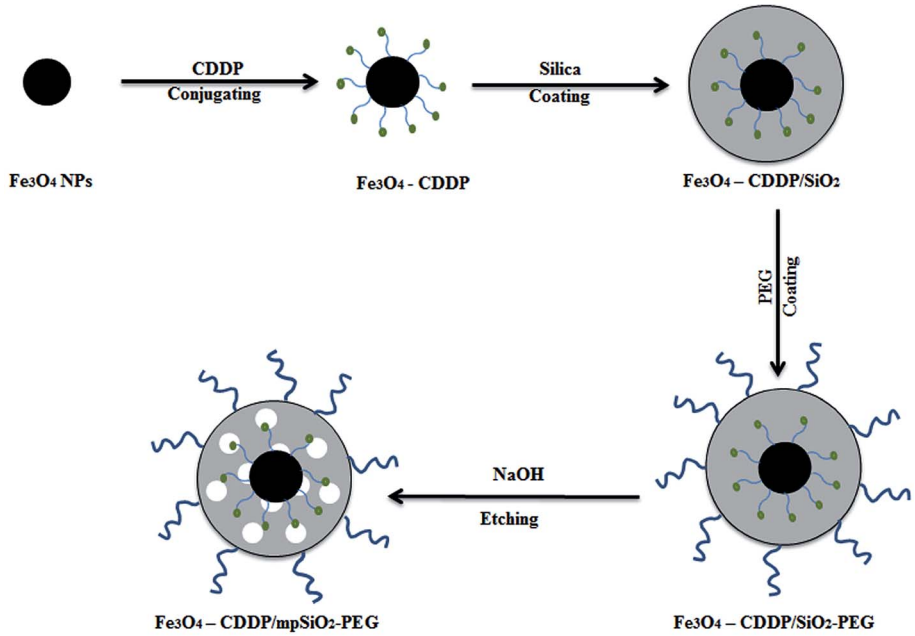
‡ Current address: Department of Biomedical Engineering, Shanghai Jiao Tong University, Shanghai, China.



Scheme 1 Schematic representation of the synthesis of the magnetic drug delivery nanosystem composed of a Fe_3O_4–CDDP conjugated core and a PEG-functionalized porous silica shell.

between CDDP and the magnetic nanoparticles, the as-prepared Fe_3O_4 nanoparticles were functionalized with methyl 3-mercaptopropionate *via* the formation of Fe–S bonds.[9]

The $-OCH_3$ group was then converted into a $-NHNH_2$ group by a hydrazinolysis reaction. Hydrazinolysis is required because the hydrazide end-groups ($-NHNH_2$) that provide the amide linkage with CDDP are acid-labile linkers with the ability to release the conjugated drug in a weakly acidic environment (pH 5–6), which is present in the endosomes of the cancer cells.[7] FT-IR spectra were collected to monitor the changes in surface ligand molecules in each of the aforementioned steps (Fig. S2, ESI†). Coating a mesoporous silica shell onto the Fe_3O_4–CDDP nanoparticles was carried out *via* a revived Stober method.[9] The density of the mesoporous silica shell can be readily tuned by controlling the amount of tetraethylorthosilicate (TEOS).[10] The TEM images of the Fe_3O_4–CDDP/mpSiO$_2$ with 250 ml of TEOS show rather monodisperse core/shell structures with an average size of 80 nm and a slightly elliptical shape. In this work, the dense silica shell is readily converted into a mesoporous one using a "surface-protected etching" strategy as described in the literature.[11] PEG was employed to protect the outmost silica layer before etching of the dense silica shell by NaOH to endow the nanoparticles with the best hydrophilicity. In this process, the drug would not disengage from the surface of the magnetic nanoparticles as the cleavage of the amide linkage only occurs easily in acidic medium. Under controlled etching conditions, the dense silica shell Fe_3O_4–CDDP nanoparticles are converted into mesoporous shell nanoparticles with a final particle size of 100 nm and perfect elliptical shape. Fig. 1b shows the TEM image of the silica coated CDDP-loaded magnetic nanoparticles, with a size of 100 nm, after etching for 60 min. The elemental composition was also confirmed from the EDS-mapping of a single nanocomposite, shown in Fig. 1c, where the red spots (Fe_3O_4) correspond to the black, central areas and

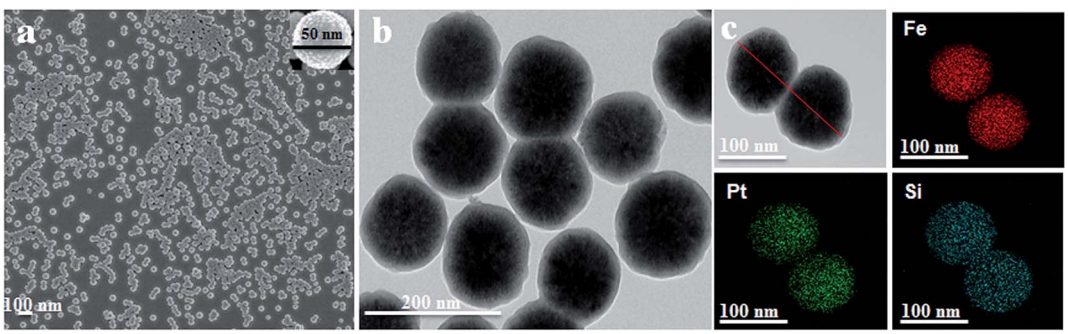


Fig. 1 shows Fe_3O_4–CDDP/mpSiO$_2$-PEG nanocomposite. (a) Fe_3O_4–CDDP SEM, (b) Fe_3O_4–CDDP/mpSiO$_2$-PEG TEM, and (c) elemental identified EDS mapping.

the lighter areas are composed of Pt (green) and Si (blue), which all confirm the localization of the Fe_3O_4 nanoparticles in the core and Pt and Si atoms in the shell of the Fe_3O_4–CDDP/$mpSiO_2$-PEG nanocomposite.

The final step is to etch the dense silica shell into a mesoporous shell, allowing the requisite chemicals or biological species to reach the Fe_3O_4–CDDP core nanoparticles to participate in cleavage of the drug-conjugated bond, and for the dissociated CDDP to cross the silica shell. The mesoporous silica shell still acts as a physical barrier preventing aggregation of the magnetic particles and the direct exposure of the drugs, and can also be further coupled with other functional molecules such as antibodies or organic dyes. In addition, the presence of a mesoporous silica shell brings higher sensitivity to an external magnetic field (Fig. S3, ESI†). The profile of the N_2 adsorption–desorption isotherms, determined by the BJH model on the Fe_3O_4–CDDP/$mpSiO_2$-PEG nanocomposite at various relative pressures (P/P_o), shows a gradual change over a wide range of P/P_o (Fig. S4, ESI†). In this case, the pores exhibit a very wide size distribution (5–30 nm), characteristic of a disordered sample.[12] This structural feature is favorable for releasing the drug molecules at the target sites while serving as an inhibitor against leaking of the Fe_3O_4–CDDP nanoparticles.

The CDDP loading capacity of the mesoporous drug carrier system, estimated using UV-vis spectroscopy, is 16.5 μg mg^{-1}, which is lower than that of the core Fe_3O_4–CDDP particles (65.5 μg mg^{-1}), due to the presence of the mesoporous silica shell functionalized with PEG. Fig. 2 depicts the CDDP release profiles of the Fe_3O_4–CDDP nanoparticles at a pH value of 5, and the Fe_3O_4–CDDP/$mpSiO_2$-PEG nanocomposite at pH values of 5 and 7.4, respectively. Considering the acidic environment of cancer cells, the pH value of a phosphate buffer solution for *in vitro* drug release is chosen to be 5.0 in this experiment. Just as we expected, the CDDP release rate of the Fe_3O_4–CDDP/$mpSiO_2$-PEG nanocomposite is slower than that of the Fe_3O_4–CDDP nanoparticles, due to the presence of the mesoporous silica shell, through which CDDP is released from the carrier. This process is a typical diffusion-controlled process,[13] with the CDDP release behavior of the nanocomposite being further influenced by the pore channels of the silica shell as well as the cleavage of the amide bonds. In addition, due to the pH dependence of the cleavage of the amide bonds and the presence of the mesoporous silica shell,[10] the CDDP release rate of the synthesized Fe_3O_4–CDDP/$mpSiO_2$-PEG is much slower under physiological conditions (Fig. 2). Thus the prepared drug delivery nanoparticles are expected to act as an intracellular depot and to promote sustained drug retention. Rapid dissociation of the drug from the nanoparticles may result in its premature release into the blood stream, significantly reducing the efficient delivery of the drug molecules to the desired tissue/organ, and it being retained there for a sustained period of time.

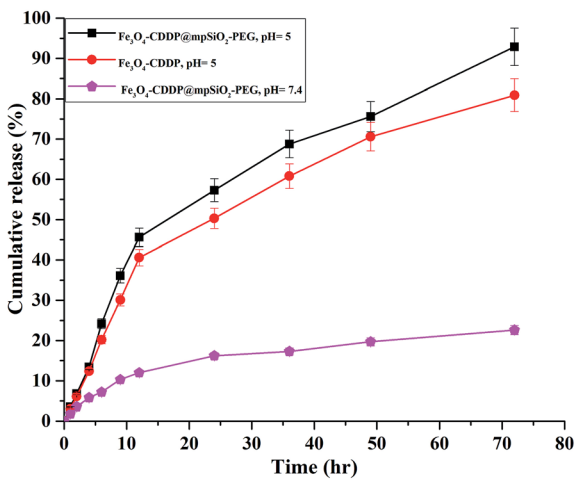


Fig. 2 CDDP release profiles from Fe_3O_4–CDDP nanoparticles and Fe_3O_4–CDDP/$mpSiO_2$-PEG core/shell nanocomposite at 37 °C.

The Fe_3O_4–CDDP/$mpSiO_2$-PEG nanocomposite can be internalized by the cells through an endocytosis process, evidenced from the obvious fluorescence of FITC observed in the cytoplasm around the nuclei (blue, stained with DAPI) after the FITC-labeled nanoparticles were incubated with HeLa and MCF-7 cells for 3 hours (Fig. S5, ESI†). To improve target specificity, as discussed above, the drug carrier system can also be easily functionalized with a targeting ligand *via* a silicone coupling agent. Here folic acid (FA) was selected to modify the porous drug carrier system *via* 3-aminopropyltriethoxysilane (APTES) for increased and specific uptake of the drug carrier in tumor cells overexpressing the folate receptor, such as MCF-7 and HeLa cells. Fig. 3 shows bright field, dark field and merges of MCF-7 (low level of FA expression) and HeLa (high level of FA expression) cells, incubated with the Fe_3O_4–CDDP/$mpSiO_2$-PEG nanocomposite functionalized with FA for 3 hours (Fig. 3a) and 12 hours (Fig. 3b). Much stronger fluorescence of CDDP was seen in HeLa cells than in MCF-7 cells after 3 hours (Fig. 3c) and 12 hours (Fig. 3d); suggesting a specific uptake of the FA modified mesoporous magnetic drug carrier system by the cells with FA receptors. These results indicate that the Fe_3O_4–CDDP/$mpSiO_2$-PEG nanocomposite functionalized with FA is preferentially internalized by the cells *via* a receptor-mediated endocytosis process. The endosomes then fuse with low pH lysosomes to cleave the amide bonds between CDDP and the particle, allowing CDDP to be released first from the Fe_3O_4 particle surface and then to enter *via* the pore channels into the nucleus of the target cell where it exhibits antitumor activity. We performed the experiments with a low level of FA expression using MCF-7 cells and the cytotoxicity of the FA modified nanocomposite was slightly higher than that of the corresponding nanocomposite without FA modification due to non-specific targeting of folate receptors. In the case of HeLa cells the higher levels of FA expression on the cell surface allow the FA modified nanocomposite to easily bind with the folate receptors leading to endocytosis and a significantly higher cytotoxicity in HeLa cells compared to the nanocomposite without FA modification (Fig. S6, ESI†). It is noted that the Fe_3O_4/$mpSiO_2$-PEG core/shell nanocomposite without CDDP has a very low cytotoxicity with respectable biocompatibility at a wide range of concentrations (25 μg ml^{-1} to 125 μg ml^{-1}), as shown in both cells (Fig. S7, ESI†).[14] All these facts strongly suggest that the effective antitumor activity can be attributed to the CDDP released from the carriers into the cells. Finally, the

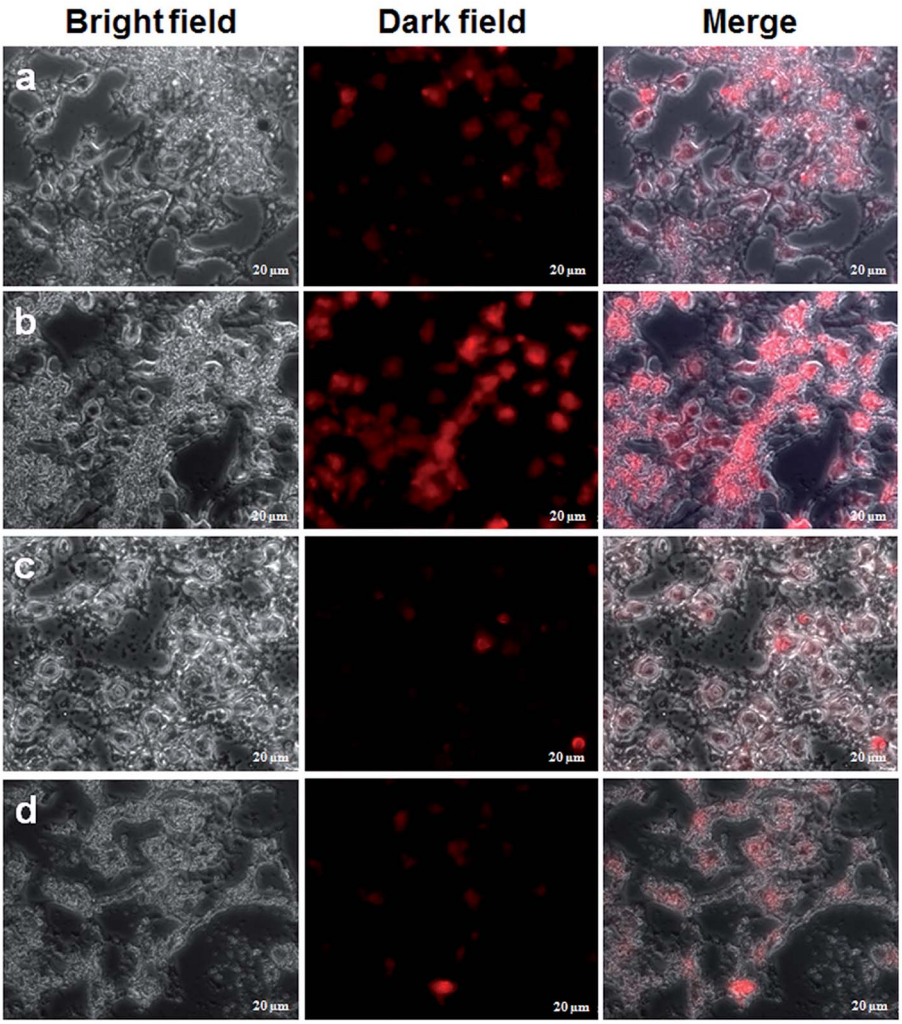


Fig. 3 Bright field, dark field and merge images of HeLa cells incubated with the Fe_3O_4–CDDP/mpSiO$_2$-PEG core/shell nanocomposite modified with FA for 3 h (a) and 12 h (b) and MCF-7 cells incubated with the Fe_3O_4–CDDP/mpSiO$_2$-PEG core/shell nanocomposite modified with FA 3 h (c) and 12 h (d).

cellular inhibitory effect of the Fe_3O_4–CDDP/mpSiO$_2$-PEG nanocomposite in HeLa (high level of FA expression) cells was studied using nuclear staining with DAPI. The complex at a concentration of 10 μM showed characteristic apoptotic structures such as reduced and fragmented chromatin after 12 hours of treatment (Fig. S8 ESI†).

In summary, we have developed a novel magnetic drug delivery nanosystem, in which a layer of PEG functioned mesoporous silica is coated onto a CDDP-conjugated Fe_3O_4 nanocomposite. This drug delivery nanosystem provides a number of advantages with respect to the magnetic carrier's therapeutic functionality, such as good biocompatibility, ease of functionalization with targeting ligands, high sensitivity to an external magnetic field, and a further "barrier" to drug release (besides the need for the cleavage of the drug conjugated bonds), which decreases the amount of CDDP dissociated from the carrier and released in non-target spots during transportation. Such a novel drug-delivery nanosystem has potential applications in cancer treatment, particularly in magnetic hyperthermia cancer targeting drug delivery technologies.

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