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A novel magnetic drug delivery nanocomplex of cisplatin-conjugated Fe_3O_4 core and a PEG-functionalized mesoporous silica shell nanocomposites for enhancing drug delivery efficiency of cancers

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Towards the rapid synthesis and efficient action of a smart delivery nanosystem based on coating a layer of PEG functionalized mesoporous silica shell upon cisplatin (CDDP) conjugated Fe_3O_4 integrated nanocomposites. We achieve affords a number of nanocomplex enhancements in the discovery of the biomagnetic carriers' therapeutic manner to treat cancer.

In the recent years the development of nanosystem drug delivery is much attention, since it is an active and secure, administer in therapeutic level for specific tissues, organs, or cellular system. [1] Cisplatin, has been widely employed in the clinic to treat a variety of cancers such as small cell lung cancer, breast, ovarian, bladder, head and neck due to the potent activity to cross-link with DNA upon entering the cells. [2] In particular, magnetic micro and nanoparticles are presently recognized as one of the most promising modalities of such carriers. [3] Magnetic tagging is drug edge of accessible to transport, the range of substandard treatment with the guide of an external magnetic field, dominant to a tissue-specific release of drugs and so abbreviating the side effects. The nanocomplex of magnetic particle based drug carrier systems, significantly affecting physicochemical properties, such as colloidal stability, drug release behavior, and magnetic intensity of the carrier, is critical to its clinical applications. [4] To date, only two structural models have been developed: (i) the drug is conjugated or physisorbed to the surface of polymer coated magnetic particles; (ii) a mixture of drugs and magnetic particles is embedded in polymer. [5] However, simple, approach to direct attachment of drug molecules to 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 to non-target areas. Specifically biocompatible magnetite (Fe_3O_4) nanoparticles have been heavily pursued as a versatile carrier for diagnostic and therapeutic applications with super paramagnetic and are excellent contrast agents for magnetic resonance imaging (MRI). [6] Moreover, the drug release rate of reporting systems is 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 the transport and thus decrease of the effective concentration of the therapeutic agent at the target site.

Herein, we synthesis an integrated design of a novel magnetic drug delivery nanosystem, conjugated cisplatin with Fe_3O_4 nanoparticles, was 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 in view of the magnetic carrier's therapeutic functionality to treat cancers. It is not only provides a protective layer of drug molecules and magnetite nanoparticles, but also imposes a further obstacle for the CDDP release from the carrier in addition to the cleavage of chemical bond, 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 the endothelial reticulum stress, therefore allowing drugs to be administered over prolonged periods of time. [5] The impartial nature of the PEG circle could also facilitate internalization of the carrier by target cells. [7] By thermal decomposition of Fe (acac) $_3$ with oleylamine as capping agent, [8] we first prepared monodispersed Fe_3O_4 nanoparticles (Fig. S1, ESI) and conjugated with CDDP in the size of 50 nm as estimated from the SEM image in Fig. 1a. To facilitate subsequent conjugation between CDDP and the magnetic nanoparticles, the as - prepared Fe_3O_4 nanoparticles were functionalized with methyl 3-mercaptopropionate *via* Fe-S bonds. [9]

The $-OCH_3$ group was then converted to $-NHNH_2$ group by hydrazinolysis reaction. Hydrazinolysis is required because 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 the weak acidic environment (pH 5-6) and present in the endosome of the cancer cells. [7] FT-IR spectra were collected to monitor the changes of 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 TEOS show rather monodisperse core/shell structure with an average size of 80 nm, and are slightly elliptical in shape. In this work, the dense silica shell is readily converted to a mesoporous one using a "surface-protected etching" strategy as described. [11] PEG was employed to protect the outmost silica layer before etching of the dense silica shell by NaOH to endow the nanoparticles with 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 easily occurs in acidic medium. Under controlled etching conditions, the dense silica shell Fe_3O_4-CDDP nanoparticles are converted to mesoporous shell nanoparticles with final particles size and perfect shape. Fig. 1b shows the TEM image of the silica coated CDDP-loaded magnetic nanoparticles of 100 nm after etching for 60 min. The elemental composition was also confirmed from the EDS-mapping of a single nanocomposite in Fig. 1 (c) where the red spots (Fe_3O_4) correspond to the black and the central areas and lighter areas are composed of Pt (green) and Si (blue), which are all confirmed the localization of Fe_3O_4 atoms in the core and Pt, Si atoms in the shell of the Fe_3O_4-CDDP/mpSiO_2-PEG nanocombosite.

The final step is to etch the dense silica shell into mesoporous shell, allowing requisite chemicals or biological species to reach the Fe_3O_4-CDDP core nanoparticles to participate in cleavage of the drug-conjugated bond, and 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 drugs, and can also 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 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/Po), shows a gradual change over a wide range of P/Po (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 of 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 by UV-*vis* spectroscopy, is 16. 5 μg mg^{-1}, 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 functioned 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 with a pH value 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] CDDP release behavior of the nanocomposite is further influenced by the pore channels of the silica shell besides the cleavage of the amide bond. In addition, due to the pH dependence of cleavage of the amide bond 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 and higher 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 nanoparticles may result in its premature release in the blood stream and significantly reduce the efficient delivery of the drug molecules to the desired tissue/organ, and being retained there for a sustained period of time.

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 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 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 show bright

field, dark field and merges of MCF-7 (Low level of FA expression) and HeLa (High level of FA expression) respectively, incubated with the Fe_3O_4-CDDP/mpSiO_2-PEG nanocomposite functioned with FA for 3 hours Fig 3a 12 hours Fig 3b. Much stronger fluorescence of CDDP was seen in HeLa cells than in MCF-7 cells for 3 hours Fig 3c 12 hours Fig 3d; suggesting 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 functioned 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 bond 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 performs antitumor activity. We performed the experiment of low level FA expression in MCF-7 cell cytotoxicity of the FA modified corresponding nanocomposite is slightly higher than that of the without FA modification due to non-specific targeting of folate receptor. But, in the case of HeLa cells higher level of FA expression on the cell surface targeting with FA modified nanocomposite easily bind with the folate receptor mediated endocytosis and significantly higher cytotoxicity in HeLa cells compared to without FA modified nanocomposites (Fig. S6, ESI) It is noted that the Fe_3O_4/mpSiO_2-PEG core/shell nanocomposite without loading of CDDP have very low cytotoxicity in respectable biocompatibility a wide range of concentrations (25µg/ml to125µg/ml), as shown in both cells. (Fig. S7, ESI) [14] All these facts strongly suggest the effective antitumor activity of CDDP released from the carriers in the cells. Finally, the cellular inhibitory effect of the Fe_3O_4-CDDP/mpSiO_2-PEG nanocomposite in HeLa (High level of FA expression) cells has been studied using nuclear staining with DAPI. The complex at a concentration of 10 µM showed characteristic apoptotic structures such as reduced and fragmented chromatin 12 hour treatments (Fig. S8 ESI)

In summary, we have developed a novel magnetic drug delivery nanosystem, in which a layer of PEG functioned mesoporous silica shell is coated onto the CDDP-conjugated Fe_3O_4 nanocomposite. This drug delivery nanosystem provides a number of advantages in view of the magnetic carrier's therapeutic functionality, such as good biocompatibility, ease of functionalization with targeting ligands, high sensitivity to external magnetic field, and a further "barrier" on the drug release, besides the cleavage of the drug conjugated bond, so decreasing the amount of CDDP dissociated from the carrier prior to release in non-target spots during transportation. Such a novel drug-delivery nanosystem promoted to a find potential applications in cancer treatment for further using magnetic hyperthermia cancer targeting drug delivery technology.

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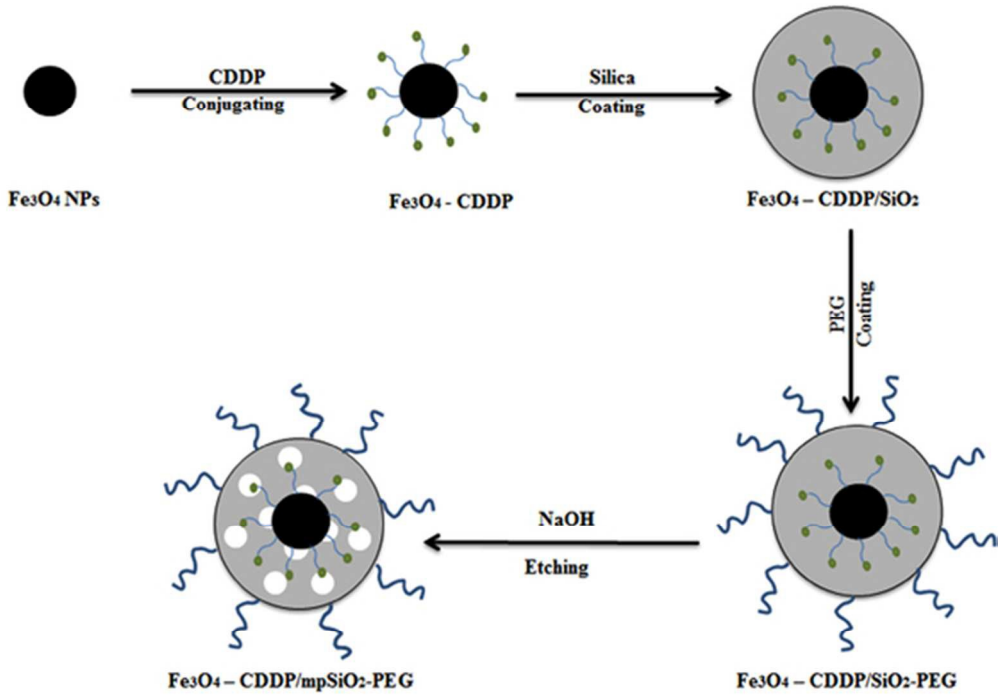

Scheme 1 Schematic representation of the synthesis of the magnetic drug delivery nanosystem composed of Fe3O4-CDDP conjugation cores and a PEG-functionalized porous silica shell.

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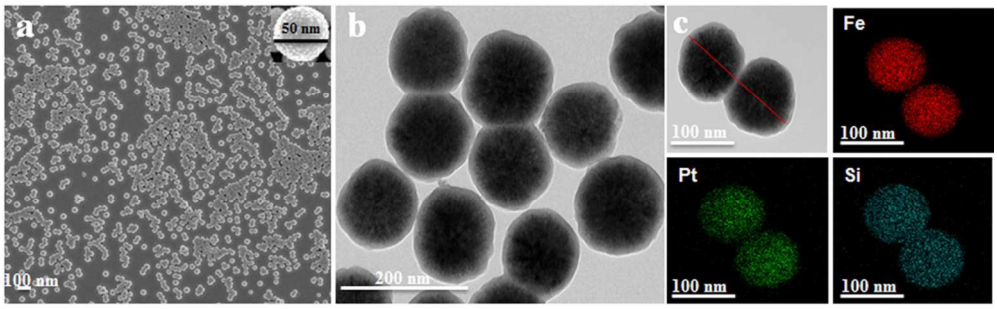

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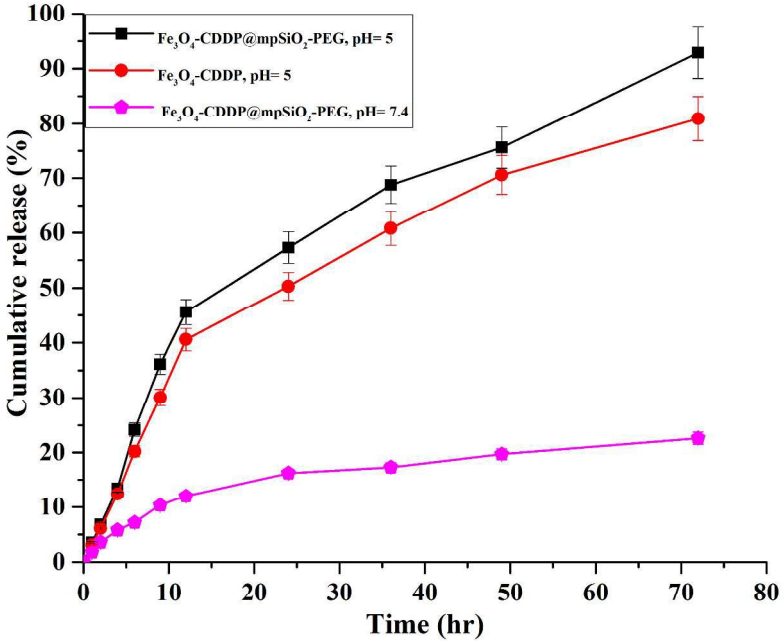

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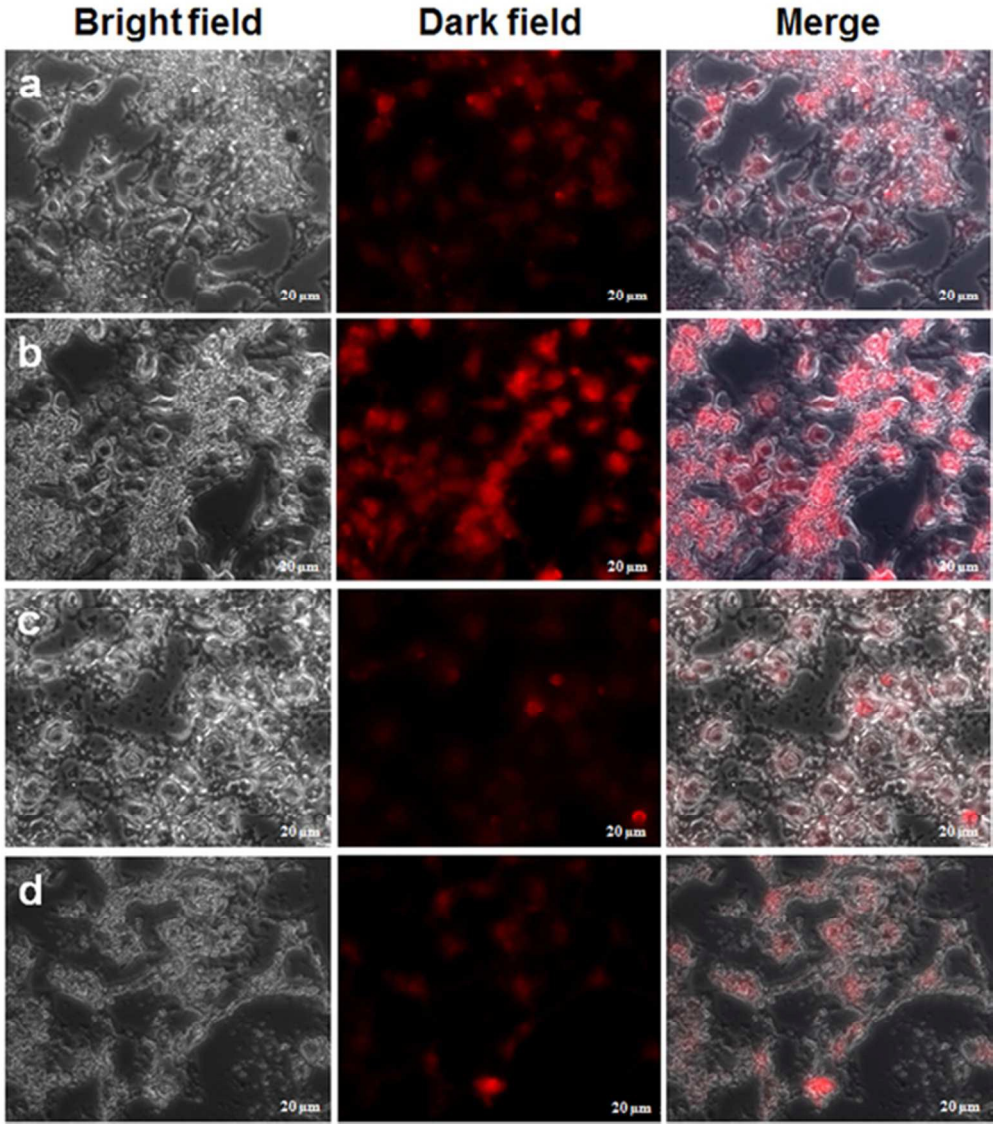

Fig. 3 Bright field, dark field and merge images of HeLa cells incubated with the Fe3O4-CDDP /mpSiO2-PEG core/shell nanocomposite modified with FA for 3 h (a), 12 h (b) and MCF-7 cells incubatedwith the Fe3O4-CDDP /mpSiO2-PEG core/shell nanocomposite modified with FA 3 h (c)12 h (d).
49x56mm (300 x 300 DPI)