# Efficient drug delivery for cancer treatment using $SiO_2$-LDH nanocomposites

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## ABSTRACT

Well-dispersed $SiO_2$ nanodot-coated LDH nanohybrids have been prepared via a nanodot-coating strategy. $SiO_2$@LDH nanohybrids show good dispersion in aqueous solution and cell culture medium. The anticancer delivery test demonstrates significant inhibition of cancer cell proliferation using $SiO_2$@LDH nanohybrids to deliver methotrexate (MTX).

***Keywords***: layered double hydroxide (LDH), functionalization, drug delivery, self-assembly

## 1. INTRODUCTION

Target-specific delivery with sustained release of anticancer agents has attracted considerable research interest in cancer chemotherapy.[1, 2] For this purpose, layered double hydroxides (LDHs), a family of anionic clay materials, have been considered as a promising candidate for drug and gene delivery due to their unique properties, such as high anion exchange capacity, low cytotoxicity, pH-controlled release, good biocompatibility, tunable particle size, and protection of drugs and genes in the interlayer.[3-8] Many studies have shown that various biofunctional molecules, including DNA, siRNA, drugs, and vitamins, have been successfully stabilized and preserved by incorporating into LDHs, showing high delivery efficiency and bioactivity in in-vitro tests.[3, 6, 9-14] However, LDH nanoparticles easily form a random agglomeration in phosphate buffer solution (PBS) and cell culture medium. This issue severely affects the bioavailability, circulation, and target delivery of LDHs in animal tests.

To circumvent this issue, surface functionalization of LDH is highly desirable. Recently, Oh et al[15] functionalized LDH nanoparticles through condensation of aminopropyltriethoxy-silane (APTES) on the LDH nanoparticle surface and then conjugated with the cancer-cell-specific ligand, folic acid (FA) to amine-functionalized $SiO_2$. They observed that coupling FA to MTX/LDH hybrids enhanced cellular uptake and inhibited cancer-cell proliferation in FR overexpressed cells compared to MTX/LDH. However, the ionic nature of the LDH material makes surface functionalization time-consuming and unstable, which limits the successful application in drug and gene delivery. Therefore, cost-effective rational design of mutilfunctional LDH nanocarriers with high effiency, good bioavailability, low cytotoxicity and long circulation time is necessary.

As is well known, silica nanoparticles ($SiO_2$ NPs) with defined size, morphology and surface properties can be easily functionalized with various functional groups and cancer-cell targeting moieites. Thus, coating LDH nanoparticles with $SiO_2$ nanodots can circumvent the complicate and time-comsuming modification process,[15] which would render the possibility of designing a new generation of drug/gene delivery system.

In this work, uniform $SiO_2$ nanodots with a negative zeta potential (~10 nm, zeta potential of -38.8 mV, **Fig. 1**) were coated onto the surface of LDH nanoparticles (80-150 nm, zeta potential of +42.5 mV, **Fig.** 1) to form $SiO_2$@LDH nanohybrids. These nanohybrids are monodispersed in PBS and cell culture medium, in sharp contract with LDH nanoparticles that are readily aggregated. These nanohybrids can be further conjugated with target moieties for targeting delivery. To study the drug delivery efficiency of the nanohybrid systems, methotrexate (MTX), a widely used anti-cancer agent, was used in this work as a model drug, and thus MTX-LDH suspension and nearly monodispersed $SiO_2$@MTX-LDH nanohybrid suspension were prepared in the similar way and further testes in cancer cell treatment.

## 2. EXPERIMENTAL

### 2.1 Synthesis of LDH and MTX-LDH suspension

Layered double hydroxide (LDH) and MTX-LDH suspensions were synthesised via coprecipitation-hydrothermal method, a procedure published elsewhere [1]. Briefly, $Mg(NO_3)_2$ (3.0 mmol) and $Al(NO_3)_3$ (1.0 mmol) were dissolved in 10 mL distilled water and quickly added to 40 mL NaOH solution (6.0 mmol) under vigorous stirring, following by 10 min stirring. The LDH slurry was collected via centrifuge separation. After washing with distilled water twice via centrifugation, LDH slurry was resuspended in 40 mL distilled water. The resulting inhomogeneous suspension was transferred to an autoclave

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(stainless steel with a Teflon lining) and heated to 100 $^{o}$C for 16 h. After hydrothermal treatment, a transparent, homogenous suspension $Mg_2Al$-LDH was obtained.

Preparation of MTX-LDH suspension was similar. After collecting the LDH slurry via centrifuge separation, the slurry was exchanged with 0.1 mmol MTX in 40 mL solution (neutralized with dilute NaOH solution, with pH of 8-9) for 1 h. After washing and separation via centrifugation, the MTX-LDH slurry was dispersed in 40.0 mL distilled water and transferred to an autoclave for hydrothermal treatment at 100 $^{o}$C for 16 h.

## 2.2 Synthesis of $SiO_2$@LDH and $SiO_2$@MTX-LDH colloidal suspension

To prepare $SiO_2$@LDH and $SiO_2$@MTX-LDH suspension, $SiO_2$ nanodots were pre-prepared via a microemulsion method. Briefly, 0.73 g octane and 0.014 g L-arginine were dissolved in 14 mL distilled water at 60 $^{o}$C and 0.2 g TEOS was added to the solution under vigorous stirring at 60 $^{o}$C for 4 h. Then, 14 ml of as-synthesised LDH and MTX-LDH was added to the above solution at 60 $^{o}$C under stirring for 20 h, respectively. After washing with distilled water for 4 times, the as-obtained $SiO_2$@LDH and $SiO_2$@MTX-LDH nanohybrids were resuspended in distilled water, respectively.

## 2.3 Characterization

Photon correlation spectroscopy (PCS, Nanosizer Nano ZS, MALVERN Instruments) was used to analyse the particle size distribution of LDH suspensions. The same instrument was also used to measure the Zeta potential of LDH nanoparticles in as-prepared suspensions. Powder X-ray diffraction (XRD) patterns were recorded on a Rigaku Miniflex X-ray diffractometer with variable slit width at a scanning rate of 2°/min with 2θ ranging from 2.5° to 80° using Co Kα radiation (λ=0.17902 nm). Mg and Al concentrations in all samples were determined by inductive coupled plasma atomic emission spectroscopy (ICP-AES) on a Varian Vista Pro instrument. Transmission electron microscopy (TEM) images were obtained on a JEOL 1010A transmission electron microscope at an acceleration voltage of 100 kV. Element analysis of C, H and N was performed by Flash EA 1112 CHNS-O analyser (Thermo Electron Corp., US)

# 3. RESULTS AND DISCUSSION

$SiO_2$ nanodots coated LDH nanohybrids were synthezised via self-assemmbly by electrostatic interactions. As shown in **Fig. 2A** and **2B**, LDH and MTX-LDH nanoparticles have hexagonal morphology with the particle size in the range of 80-150 nm (**Fig. 1A**). After coated with $SiO_2$ nanodots, $SiO_2$@LDH and $SiO_2$@MTX-LDH nanohybrids (**Fig. 2C** and **2D**) retained the hexagonal morphology. The average size of silica nanodots was around 10-12 nm (**Fig. 1A, 2E** and **2F**). Note that uniform $SiO_2$ nanodots were evenly attached on the surface of LDH nanosheets (**Fig. 2C and 2D**). XRD patterns of LDH, $SiO_2$@LDH, MTX-LDH and $SiO_2$@MTX-LDH (**Fig. 3**) exhibit the characteristics of LDH structure with a rhombohedral symmetry, as reflected by (003), and (006) peaks. After being coated with $SiO_2$ nanodots, the (003) and (006) peaks of the LDH phase became weaker and broader, suggesting the crystallinity of LDH nanoparticles was lowered during $SiO_2$ dot-coating. An additional peak at 5.9$^{o}$ corresponding to an interlayer spacing of approx. 1.75 nm was obseved in the MTX-LDH and $SiO_2$@MTX-LDH samples (**Fig. 3B** and **3D**), suggesting MTX was intercalated into the LDH layers.[14, 16, 17]

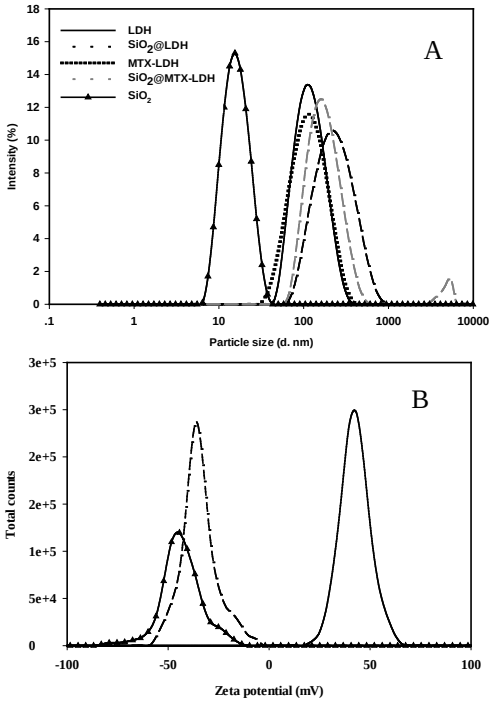


**Fig. 1** (A) Particle size distribution of LDH (line), $SiO_2$ (line with triangle), $SiO_2$@LDH (dash), MTX-LDH (dot) and $SiO_2$@MTX-LDH (grey dash), (B) zeta potential of LDH (line), $SiO_2$ (line with triangle) and $SiO_2$@LDH (dash)

Element analysis using CHN analyzer and ICP-MS measurement revealed the composition of as-obtained samples, as summarized in Table 1. Note that the Mg/Al atomic ratio was 2.1, close to the nominal ratio (2.0). The MTX loading amount was 9.7 wt%, close to the designed MTX loading, i.e. 10% of the total anion capacity in LDH. The molar ratio of Al/Si was about 0.28, approx. equivalent to a mass ratio of 1:1 between the two constituents (LDH:$SiO_2$).

As shown in **Fig. 2B**, LDH nanoparticles in suspension possess a positive zeta potential of +42.5 mV and $SiO_2$ nanodots in suspension a negative zeta potential of -43.5 mV.Therefore, when mixing LDH nanoparticle suspension with silica nanodot suspension, LDH acts as a substrate to attract the negatively charged silica nanodots via electrostatic attractions. Meanwhile, the electrostatic repulsion between silica nanodots makes themsleves to repel one another so as to evenly distribute on the LDH surface,

as seen in **Fig. 2C** and **2D**. These $SiO_2$@LDH nanohybrids exhibit a negative zeta potential of -31.1 and -32.5 mV (**Fig. 2A and Table 1**), respectively. We noted that $SiO_2$@LDH and $SiO_2$@MTX-LDH nanohybrids were able to form stable and well-dispersed suspension in water, PBS and culture medium after ultrasonication.

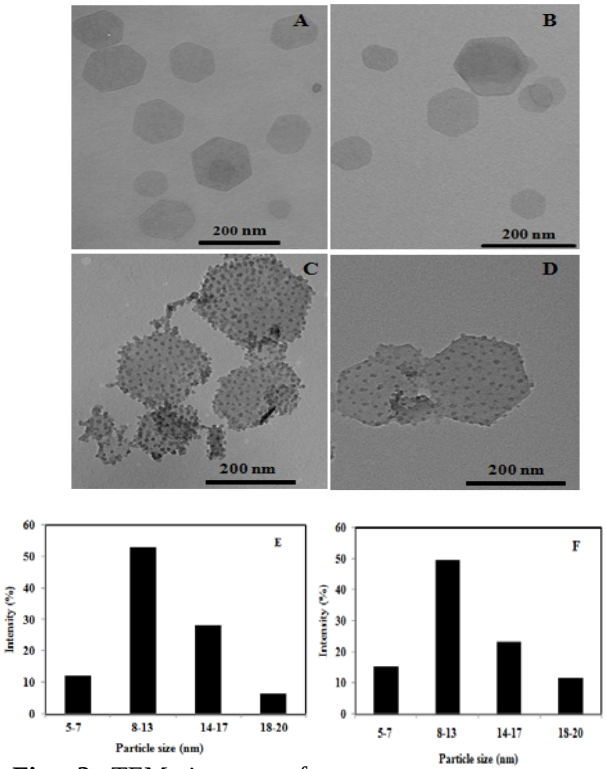


**Fig. 2** TEM images of LDH (A), MTX-LDH (B), $SiO_2$@LDH (C) and $SiO_2$@MTX-LDH (D); and particle size distribution of $SiO_2$ nanodots (E and F) on the LDH and MTX-LDH surface.

In summary, $SiO_2$ dot-coated LDH nanohybrids were prepared following our proposed strategy, which can be nearly monodispersed in PBS and culture medium. It should be mentioned that using this method, the size of silica nanodots and their coverage on the LDH surface can be easily tuned by varying TEOS amount. Moreover, by virtue of diverse surface properties of $SiO_2$ nanodots, $SiO_2$@LDH nanohybrids can be further functionalised with various biofunctional molecules, which would allow the fabrication for advanced multifuntional drug and gene delivery system.

To test if the nanohybrids can be effectively taken up by cancer cells, we use human cervical cancer cell line HeLa as a model. The LDH and $SiO_2$@LDH nanoparticles were loaded with 21-bp dsDNA tagged with Cy3 (red flourescence) to signal the uptake.[12, 19] As shown in **Fig. 4**, both LDH and $SiO_2$@LDH nanoparticles can be effectively taken up by HeLa cells at the LDH:dsDNA mass ratio of 40:1, demonstrating that short gene segments can be efficiently delivered to cells by both systems. Note that the cellular uptake of LDH nanoparticles has been reflected by bright red spots in the cells (**Fig. 4A**), suggesting the internalized LDH particles were aggregated to some degree in the cytoplsma. In contrast, only faintly reddish scattered dots were observed within the cells in the case of $SiO_2$@LDH nanohybrids (**Fig. 4B**), indicating less aggregation of $SiO_2$@LDH particles in the cytoplasma. This clearly shows that $SiO_2$@LDH nanohybrids exhibit better dispersion within the cells. In addition, comapred to LDH alone, $SiO_2$@LDH nanoparticles show the weaker red signal, which may be caused by the slower cellular uptake due to the negative charges of $SiO_2$@LDH nanoparticles.

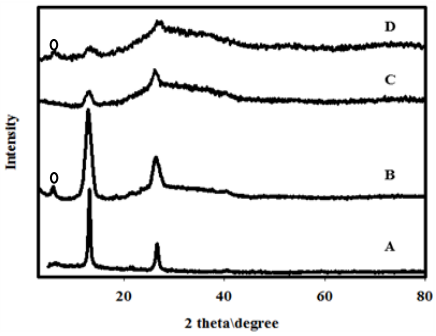


**Fig. 3** XRD patterns of LDH (A), MTX-LDH (B), $SiO_2$@LDH (C) and $SiO_2$@MTX-LDH (D) nanohybrids. '0' indicates the new basal diffraction peak of the MTX-intercalated LDH phase.

**Table 1** The element analysis results of LDH and $SiO_2$@LDH nanoparticles

| samples | Mg/Al ratio | N (wt%) | Particle Size (nm) | Zeta potential (mV) |
|---|---|---|---|---|
| LDH-Cl | 2.1 | - | 109.4 | +42.5 |
| $SiO_2$ | - | - | 15.0 | -43.5 |
| $SiO_2$@LDH | 2.1 | - | 207.7 | -31.1 |
| MTX-LDH | 2.1 | 1.91 | 102.2 | - |
| $SiO_2$@MTX-LDH | 2.1 | 1.08 | 174.3 | -32.5 |

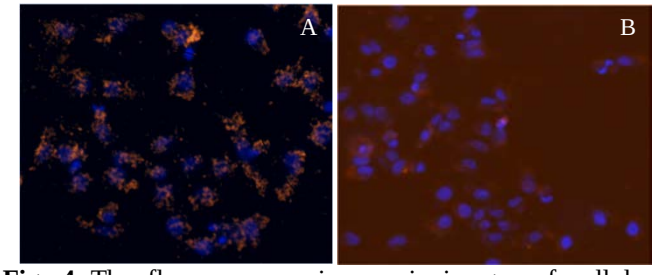


**Fig. 4** The fluorescence microscopic images of cellular uptake of LDH (A) and $SiO_2$@LDH (B) in HeLa cells. using oligo DNA-Cy3 as the marker with the mass ratio of LDH:DNA = 40:1.

We further studied the MTX delivery efficiency in the chemotherapeuic treatment of human osteosarcoma cell line 143B PMLBK TK (ATCC Cat No CRL-8304) using the nanohybrids as the delivery vehicle. As shown in **Fig. 5**,

both MTX-LDH and $SiO_2$@MTX-LDH nanohybrids exhibited the effective suppression of cancer cell growth in a does-dependent manner, which can be attributed to quick uptake of LDH and $SiO_2$@LDH nanoparticles. In comparison, MTX-LDH hybrids with or without $SiO_2$ modification showed a similar tumor suppression efficiency. Relatively, MTX-LDH caused more cancer cell death (90-93%) than $SiO_2$@MTX-LDH (85-90%) at the MTX dose of 0.064-1.02 μg/ml, and the supression efficiency in both cases was higher than MTX itself. This observation reveals that $SiO_2$ modification on LDH has slightly slowed down the internalisation of LDH nanohybrids due to the negative charges of $SiO_2$@LDH nanohybrids. As $SiO_2$ nanodots in $SiO_2$@LDH nanohybrids are further functionalized with PEG and targeting moieties, the surface negative charges of $SiO_2$@LDH nanohybrids would be much less and thus quick cellular uptake and targeting would be both achieved. Therefore, $SiO_2$@LDH nanohybrids can be the promising efficient delivery vehicles.

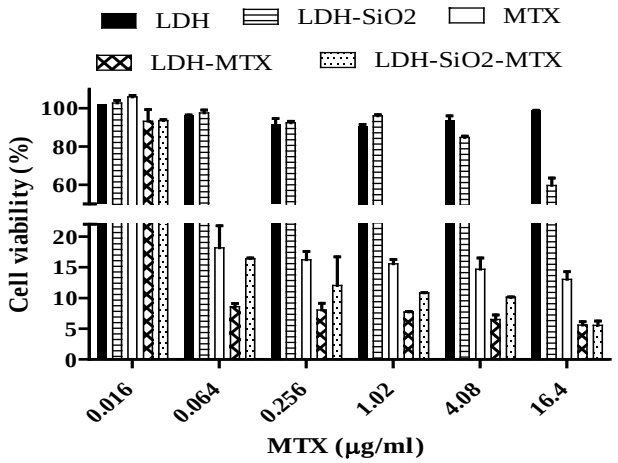


**Fig. 5** The cell viability of osteosarcoma cells exposed to different amounts of MTX associated with or without LDH/ $SiO_2$@LDH. Cells without any treatment were used as the positive control (100% viability). The concentration of the particle control group (LDH or $SiO_2$@LDH) was the same as that in LDH-MTX or $SiO_2$@LDH-MTX hybrids, approx. 10 times as the MTX concentration.

In addition, both LDH and $SiO_2$@LDH nanoparticles showed a low cytotoxicity (cell viability >85%) when the concentration was lower than 40 μg/ml (10 times as the MTX amount), but $SiO_2$@LDH nanohybrids at higher concentration (200 μg/ml) reduced the cell viability to 60% (**Fig. 5**), which could be attributed to the trace amount of surfactants associated with $SiO_2$.

## 4. CONCULSIONS

In summary, $SiO_2$@LDH nanohybrids were successfully synthesized through a facile, versatile and reproducible route. The negatively charged $SiO_2$ nanodots were uniformly distributed on the positively charged LDH nanoparticles. As-synthesised $SiO_2$@LDH nanohybrids retained the LDH structure and formed stable suspension in aqueous solution, PBS and culture medium. The cellular uptake tests showed that the $SiO_2$@LDH nanohybrids exhibited better dispersion within the cells after internalisation, with effective inhibition of cancer cell prolification even less uptake amount of $SiO_2$@LDH-MTX nanohybrids compared with LDH-MTX. Therefore, monodispersed $SiO_2$@LDH nanohybrids can be an excellent nonviral delivery carrier for drug and gene deivery.

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