Efficient drug delivery for cancer treatment using SiO₂-LDH nanocomposites

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ABSTRACT

Well-dispersed SiO₂ nanodot-coated LDH nanohybrids have been prepared via a nanodot-coating strategy. SiO₂@LDH nanohybrids show good dispersion in aqueous solution and cell culture medium. The anticancer delivery test demonstrates significant inhibition of cancer cell proliferation using SiO₂@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 SiO₂. 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 multifunctional LDH nanocarriers with high efficiency, good bioavailability, low cytotoxicity and long circulation time is necessary.

As is well known, silica nanoparticles (SiO₂ NPs) with defined size, morphology and surface properties can be easily functionalized with various functional groups and cancer-cell targeting moieties. Thus, coating LDH nanoparticles with SiO₂ nanodots can circumvent the complicated and time-consuming modification process,¹⁵ which would render the possibility of designing a new generation of drug/gene delivery system.

In this work, uniform SiO₂ 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 SiO₂@LDH nanohybrids. These nanohybrids are monodispersed in PBS and cell culture medium, in sharp contrast 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 SiO₂@MTX-LDH nanohybrid suspension were prepared in the similar way and further tested 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(NO₃)₂ (3.0 mmol) and Al(NO₃)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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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 °C for 16 h.

2.2 Synthesis of SiO₂@LDH and SiO₂@MTX-LDH colloidal suspension

To prepare SiO₂@LDH and SiO₂@MTX-LDH suspension, SiO₂ 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 °C and 0.2 g TEOS was added to the solution under vigorous stirring at 60 °C for 4 h. Then, 14 mL of as-synthesised LDH and MTX-LDH was added to the above solution at 60 °C under stirring for 20 h, respectively. After washing with distilled water for 4 times, the as-obtained SiO₂@LDH and SiO₂@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 20 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

SiO₂ nanodots coated LDH nanohybrids were synthesised via self-assembly 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 SiO₂ nanodots, SiO₂@LDH and SiO₂@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 SiO₂ nanodots were evenly attached on the surface of LDH nanosheets (Fig. 2C and 2D). XRD patterns of LDH, SiO₂@LDH, MTX-LDH and SiO₂@MTX-LDH (Fig. 3) exhibit the characteristics of LDH structure with a rhombohedral symmetry, as reflected by (003), (006) peaks. After being coated with SiO₂ nanodots, the (003) and (006) peaks of the LDH phase became weaker and broader, suggesting the crystallinity of LDH nanoparticles was lowered during SiO₂ dot-coating. An additional peak at 5.9° corresponding to an interlayer spacing of approx. 1.75 nm was observed in the MTX-LDH and SiO₂@MTX-LDH samples (Fig. 3B and 3D), suggesting MTX was intercalated into the LDH layers.14, 16, 17
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.

![TEM images](image)

**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 multifunctional 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 fluorescence) to signal the uptake. 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 cytoplasma. 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, compared 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.

![XRD patterns](image)

**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>
<thead>
<tr>
<th>samples</th>
<th>Mg/Al ratio</th>
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<th>Zeta potential (mV)</th>
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<td>-</td>
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<tr>
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<td>174.3</td>
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</tr>
</tbody>
</table>

![Fluorescence images](image)

**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 chemotherapeutic 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 dose-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 suppression efficiency in both cases was higher than MTX itself. This observation reveals that SiO$_2$ modification on LDH has an advantage in both cases.

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 nanoparticles 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. CONCLUSIONS

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 proliferation 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 delivery.

REFERENCES