Layered double hydroxide nanostructures and nanocomposites for biomedical applications

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Layered double hydroxide (LDH) nanostructures and related nanocomposites have attracted significant interest in biomedical applications including cancer therapy, bioimaging and antibacterial treatment. These materials hold great advantages including low cost and facile preparation, convenient drug loading, high drug incorporation capacity, good biocompatibility, efficient intracellular uptake and endosome/lysosome escape, and natural biodegradability in an acidic environment. In this review, we summarize the development of three types of LDH nanostructures including pristine LDH, surface modified LDH, and LDH nanocomposites for a range of biomedical applications. The advantages and disadvantages of LDH nanostructures and insights into the future development are also discussed.

1. Introduction

Layered double hydroxides (LDH) are a type of anionic clay with the formula generally expressed as $[\text{M}_{1-x}^{2+}\text{M}_x^{3+}(\text{OH})_2]^{x-}\left[A^{n-}\right]_{3n-2}\text{H}_2\text{O}$, where divalent cations $\text{M}^{2+}$ are $\text{Mg}^{2+}$, $\text{Fe}^{2+}$, or $\text{Co}^{2+}$; trivalent cations $\text{M}^{3+}$ are $\text{Al}^{3+}$, $\text{Fe}^{3+}$, or $\text{Gd}^{3+}$; and non-framework and exchangeable anions $A^{n-}$ are $\text{Cl}^-$, $\text{CO}_3^{2-}$, $\text{NO}_3^-$, etc.\(^1\)–\(^5\) (Fig. 1). LDH are positively charged because $\text{M}^{2+}$ anions are partially substituted by $\text{M}^{3+}$. LDH nanostructures have been widely utilized for many applications, including energy, healthcare, catalysis and optics. This review will focus on the biomedical applications of LDH nanostructures and nanocomposites. Herein, we classify LDH nanostructures into 3 categories including pristine LDH nanostructures, surface modified LDH nanostructures including nanoparticles and recently reported nanosheets, and LDH nanocomposites. These LDH nanomaterials are useful for a range of biomedical applications (Fig. 2). For example: (1) $\cdot\text{OH}$ groups make LDH sensitive to an acid environment and degrade easily in the biological environment to release loaded therapeutic molecules for bio-responsive drug delivery and (2) the positive charge of LDH allows the material to effectively interact with negatively charged cell membranes for efficient intracellular delivery. Due to the broad applications, LDH nanostructures are increasingly attractive and the synthesis, characterization and applications of LDH have undergone rapid development during the last decade.\(^6\)–\(^11\) The number of
publications on LDH in both general and specific biomedical applications is increasing every year.

Pristine LDH nanoparticles were initially introduced for drug and gene delivery for cancer chemotherapy and then LDH nanostructures with different morphologies and geometries were developed for a wider range of applications. During this journey, to satisfy the requirements of advanced drug delivery systems and different biomedical applications, surface modified LDH nanostructures and LDH based nanocomposites have also been explored. In this review, we summarize and highlight the most important development of these three types of LDH nanostructures for biomedical applications, discuss about their advantages and disadvantages, and also provide our insights into the challenges and future applications of LDH nanomaterials.

2. Pristine LDH nanostructures for drug and gene delivery

Biological barriers in the human beings can effectively restrict hazardous agents and thus protect them from harmful diseases. As one of the most common biological barriers, the cell membrane can isolate, protect and regulate cells from the external environment.\(^{12-16}\) However, the fine and sophisticated structure of the cell membrane unavoidably and strictly prohibits the transport of drugs and genes into the cytoplasm and nucleus, resulting in minimal or even negligible delivery efficiency.\(^{17}\) To address this problem, methods including biological,\(^ {18-22}\) chemical,\(^ {23-27}\) and physical approaches\(^ {28-33}\) have been extensively developed. Among these technologies, nanomaterials show great potential to permeate the cell membrane and greatly increase drug delivery efficiency with low toxicity.\(^ {34-41}\) Besides drug delivery application, nanostructures are also broadly used for other biomedical applications such as bioimaging, sensing, and separating protein/harmful agents.\(^ {42-46}\)

2.1 Cancer therapy and drug delivery

Pristine LDH nanoparticles were first introduced in the 2000s as a nonviral vector for biomolecule and deoxyribonucleic acid (DNA) delivery by Choy and co-workers.\(^ {47}\) In 2004, Choy et al. reported that pristine LDH nanoparticles could load negatively charged drug molecules for cancer therapy.\(^ {48}\) Methotrexate (MTX) was loaded into the interlayer space of LDH nanoparticles by mixing LDH with MTX at room temperature for 3 days through ion-exchange by replacing the NO\(_3\) ions. After drug loading, the interlayer space of LDH nanoparticles expanded from 8.4 to 19.9 Å, but the structure and function of MTX and LDH nanoparticles were maintained without detectable change. The in vitro cell line test results showed that MTX-LDH nanoparticles were effective sooner than MTX alone. With 24 hours co-incubation of cell lines and drugs, the viability of
the cancer cells treated with MTX remained over 90%; in comparison, the viability of cells in the MTX-LDH treated group dropped to below 80%. Attractively, MTX-LDH nanoparticles effectively suppressed the proliferation of SaOS-2 cancer cells (osteosarcoma, human), but showed a negligible effect on normal cells (fibroblasts, human tendon). This work first demonstrated that LDH nanoparticles could act as a biocompatible non-viral vector for enhanced anti-cancer drug delivery.

The effectiveness of using LDH nanoparticles for drug delivery and the attractive cancer cell killing specificity were later demonstrated also in cisplatin prodrug delivery.\(^9\) In terms of drug delivery effectiveness, disuccinatoisoplatin (DSCP)-loaded LDH nanoparticles exhibited dramatically higher cytotoxicity than the free prodrug: the IC\(_{50}\) value of DSCP against A2780 cancer cells was \(1.75 \pm 0.20\) \(\mu\)M, while the value was \(0.34 \pm 0.17\) \(\mu\)M for LDH–Pt(IV) (LDH nanoparticles loaded with DSCP), indicating a 5-fold reduction of the IC\(_{50}\) value (Table 1). With regard to cell specificity, LDH-Pt(IV) nanoparticles were significantly less effective towards the tested normal cells (NIH3T3, HS27 and BEAS-2B). For example, the IC\(_{50}\) values of cisplatin and DSCP were \(5.1 \pm 0.8\) and \(30 \pm 2\) \(\mu\)M, respectively, while the value of LDH–Pt(IV) was over 50 \(\mu\)M. These comparisons indicate that DSCP-loaded LDH nanoparticles are very effective in killing cancer cells, but relatively safe to healthy cells, which is ideal for anticancer drug design.

To explain the cancer cell targeting specificity, the platinum content of cells was investigated after incubation with DSCP and DSCP-LDH. It was found that LDH-Pt(IV) could deliver 10-fold more drug to cells compared to DSCP alone.

Following the test in cell lines, Choi et al. studied MTX-LDH nanoparticles with an average diameter of 130 nm for in vivo cancer therapy.\(^9\) Free MTX and MTX-LDH nanoparticles were intraperitoneally injected into two groups of mice bearing orthotopic human breast tumors. In the biodistribution analysis, it was discovered that the tumor–liver drug accumulation ratio of the MTX-LDH nanoparticle treated group was 6 times higher than that of the MTX-treated group. In line with this LDH nanoparticle enabled targeted delivery of MTX to the tumor, the antitumor efficacy of the nanoparticle treated group was significantly improved, indicated by 81.4% and 74.3% reductions of tumor volumes compared with that in the phosphate buffered saline (PBS) and free MTX group, respectively. This also shows that the efficacy of free drug is very low.

Besides cancer chemotherapy, LDH nanoparticles were also utilized as a photosensitizer vector for photodynamic therapy. Duan and co-workers reported that the incorporation of zinc phthalocyanine (ZnPc) into the gallery of LDH nanoparticles resulted in high singlet oxygen production efficiency, improved photostability, good biocompatibility and low cytotoxicity.\(^{31}\) The \textit{in vitro} test showed that a low dosage of ZnPc (10 \(\mu\)g mL\(^{-1}\)) caused potent damage to HepG2 cells with less than 15% viability. The \textit{in vivo} investigation demonstrated that LDH nanoparticles effectively inhibited tumor growth with an ultra-low dose of 0.3 mg kg\(^{-1}\) and a low optical fluence rate of 34 J cm\(^{-2}\). Recently, Mei et al. reported two-dimensional LDH monolayers with a diameter of 50 nm and a thickness of 0.8 nm for controlled loading and targeted delivery for cancer therapy.\(^{52}\) In this research, monolayered LDH nanosheets were loaded with the anticancer drug doxorubicin (DOX) with a high loading capacity of 3.6 mg mg\(^{-1}\) (DOX/LDH). The fabricated nanovectors, which could be further conjugated with the targeting agent folic acid, exhibited selective high toxicity towards cancer cells (KB cancer cells) and reduced toxicity to normal cells.\(^{52}\) Gao et al. developed a supramolecular photosensitizer (isophthalic acid/LDH nano-hybrids) that could be activated by a near-infrared laser for efficient two-photon photodynamic therapy.\(^{53}\) Upon 808 nm laser irradiation, this LDH based hybrid demonstrated significantly increased tissue penetration depth and was capable of killing cancer cells inside the tumor.\(^{54}\)

Beyond cancer therapy, LDH nanoparticles have also been applied to many other applications.\(^{55}\) For example, to overcome the limitation of the short half-life of the anticoagulant drug low molecular weight heparin (LMWH), LMWH-LDH nanoparticles were fabricated via a co-precipitation method (adding drug simultaneously during the LDH nanoparticle preparation process, instead of preparing LDH nanoparticles first followed by drug loading via ion-exchange).\(^{55}\) The \textit{in vitro} release test indicated a biphasic and sustainable release profile of LMWH-LDH nanoparticles in pH 7.4 PBS solution at 37 °C. The liberation of the anticoagulant drug was 20% in the first 12 hours, followed by a further 20% release in the following 108 hours. The same group also found that LMWH-LDH nanoparticles had the potential to treat restenosis of an artery that occurs after surgically removing atherosclerotic lesions and restoring blood flow.\(^{56}\) Restenosis is the recurrence of blood vessel narrowing. LMWH-LDH nanoparticles were tested on rat vascular smooth muscle cells (SMC): LMWH-LDH nanoparticles showed a higher ability to inhibit the migration and proliferation of SMC, with over 60% reduction of SMC compared with the control group (only growth medium). The enhanced efficacy of LMWH-LDH nanoparticles was explained as

| Table 1 | IC\(_{50}\) values of cisplatin, DSCP, LDH–Pt(IV), and LDH in different cell lines\(^{19}\) (reproduced from The Royal Society of Chemistry with permission) |
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| Cell type | Cell line | Cisplatin \([\mu M]\) | DSCP \([\mu M]\) | LDH–Pt(IV) \([\mu M]\) | LDH \([\text{mg L}^{-1}]\) |
| Cancer | A549 | 5.0 ± 0.4 | 9.8 ± 0.2 | 0.84 ± 0.28 | >150 |
| Cancer | A2780 | 0.45 ± 0.1 | 1.8 ± 0.2 | 0.34 ± 0.17 | >150 |
| Cancer | NCI-H1299 | 3.3 ± 0.4 | 4.2 ± 0.7 | 0.71 ± 0.07 | N.D. |
| Cancer | MCF-7 | 17 ± 1 | >25 | 1.9 ± 0.6 | N.D. |
| Cancer | HCT116 | 3.7 ± 0.5 | 12 ± 1 | 0.49 ± 0.03 | N.D. |
| Normal | NIH3T3 | 5.1 ± 0.8 | 30 ± 2 | >50 | >150 |
| Normal | HS27 | 7.6 ± 0.7 | 35 ± 2 | >50 | N.D. |
| Normal | BEAS-2B | 2.7 ± 0.2 | 4.6 ± 0.9 | 23 ± 1 | 45 |

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due to the higher cellular uptake through the endocytic pathway, quick escape from late endosomes/lysosomes, and effectively inhibited mitogen-activated protein kinase (MAPK) signal transduction over a prolonged period of time.\(^5^7\) (Fig. 3). LMWH delivery by LDH vectors was further improved by using antibody-targeted LDH nanoparticles for atherosclerosis.\(^5^8\) In the system, an antibody (H93.7C.1D2/48; 1D2) was covalently conjugated to LMWH to form the 1D2–LMWH complex. Subsequently, this 1D2–LMWH complex was loaded into LDH nanoparticles via electrostatic interaction. The results showed that this nanodrug delivery system could be effectively transported to the arterial injury site with the aid of a targeting antibody, consequently achieving the sustained release of LMWH and resulting in minimized luminal loss and reduced thrombotic occlusion in a rat model of arterial injury.

Additionally, LDH nanoparticles were employed as an adjuvant to promote Th1 or Th2 dominant immune responses for vaccination purpose.\(^5^9\) LDH nanoparticles were conjugated with a toll-like receptor ligand CpG, leading to significantly enhanced antibody response and a switch from Th2 towards Th1 response. On Day 21, LDH delivery induced significantly higher IgG2a antibody response than a standard commercial adjuvant Alum. Moreover, the animal test showed that immunization of mice with CpG-OVA-conjugated LDH nanoparticles before challenging with OVA-expressing B16/F10 tumor cells retarded tumor growth and prolonged the survival of tumor-bearing mice.\(^6^0\) Furthermore, LDH nanoparticles loaded with the antigen Intimin \(\beta\) showed significantly enhanced uptake by antigen-presenting cells, promoting immune responses against infections by diarrheagenic \(E. coli\).\(^6^1\) LDH nanoparticles with Intimin \(\beta\) induced strong antibody- and cell-mediated immune responses, which maintained for at least four months in the mouse model with no changes in the histopathology of the animal organs.

Very recently, LDH nanoparticles were used for \(in vivo\) anti-fatigue by incorporating folic acid.\(^6^1\) LDH-folic acid constructs were fabricated via a co-precipitation method. It was found that the constructs were able to prolong the forced swimming time of the tested mice by 32% and 51% in comparison with the folic acid group and the control, with negligible cytotoxicity to normal cells. The forced swimming test was used to evaluate the mice’s physical endurance capacity: reduced susceptibility to fatigue was correlated with longer swimming times.\(^5^6\) Interestingly, it was also found that LDH-folic acid had an obvious effect on decreasing blood urea nitrogen and blood lactic acid, and increasing muscle and hepatic glycogen levels. These results indicated that LDH-folic acid effectively protected the body from fatigue. Wang et al. reported dexamethasone sodium phosphate (Dexa)-LDH nanoparticles for asthma treatment.\(^6^2\) In the allergic asthma rat model, this nanostructure effectively reduced inflammatory cells (macrophages, eosinophils and neutrophils) in the bronchoalveolar lavage (BAL) fluid, inhibited the increase of T-helper-2-type cytokines, and suppressed airway hyperresponsiveness.

For biomedical applications, it is essential for materials to have minimal toxicity. To know this, the bio-safety of LDH nanoparticles was investigated \(in vivo\).\(^6^3\) The study demonstrated that, when LDH nanoparticles were intravenously injected to Sprague Dawley rats at a dose of below 200 mg kg\(^{-1}\), no obvious systemic side-effect could be observed in clinical chemistry and histopathology. This study confirmed the excellent biocompatibility of LDH nanoparticles and indicated their promising applications in biomedicine. The effectiveness and biocompatibility of LDH nanostructures can be controlled through various designs. When tested in mammalian cell lines,\(^5^9\) it was found LDH nanostructures were rapidly uptaken by cells, and the shape had an influence on their
interactions between LDH nanoparticles and drug molecules. The LDH–drug system exhibited fast release of drug due to loose sustained drug delivery; in comparison, the phosphate incorporated allowed interaction with the loaded drug and consequently led to exchangeable anions: nitrate incorporated LDH nanoparticles RH could be tuned from fast to sustained release by altering the profiles was a safer nanovector for cancer therapy.

For rod-like LDH nanoparticles, there is an extra step cytosis to enter cells, and endosomal/lysosomal escape to the cytoplasm. For rod-like LDH nanoparticles, there is an extra step of nuclear translocation\(^{64}\) (Fig. 4). Recently, Senapati et al. loaded a hydrophobic anticancer drug, raloxifene hydrochloride (RH), into a series of Mg–Al LDH nanoparticles with various charge density anions through the ion exchange technique for controlled drug delivery.\(^{65}\) Through this, the release profiles of RH could be tuned from fast to sustained release by altering the exchangeable anions: nitrate incorporated LDH nanoparticles allowed interaction with the loaded drug and consequently led to sustained drug delivery; in comparison, the phosphate incorporated LDH–drug system exhibited fast release of drug due to loose interactions between LDH nanoparticles and drug molecules. The in vivo study suggested that the LDH–drug system with slow release profiles was a safer nanovector for cancer therapy.

### 2.2 Gene delivery

Gene therapy has attracted scientists and clinicians for treating diseases using therapeutic nucleic acids, such as DNA, small interfering RNA (siRNA) and microRNA (miRNA).\(^{66–73}\) For example, siRNA showed great potential for cellular therapy of neuro-degenerative disorders,\(^{74–76}\) cancers\(^{77–79}\) or infectious diseases.\(^{80,81}\) However, the success of gene therapy was hindered by limitations such as ease of degradation, low efficiency of delivery, and high toxicity in systemic transport. To solve these problems, LDH nanoparticles have been used for gene delivery since 2000s. This material could protect the gene from degradation and also enhance intracellular delivery efficacy.\(^{82}\) Li et al. discovered that 20 nm LDH nanoparticles could effectively enter both the cytoplasm and cellular nucleus, while large (180 nm) ones only located in the cytoplasm.\(^{83}\) Therefore, 20 nm LDH nanoparticles were used to deliver the pEGFP-N1 DNA plasmid to transfect NSC 34 cells with minimal cell death. Although LDH nanoparticles could be used for gene delivery, the transfection efficiency was found to be generally low because of the large size of DNA.\(^{84}\) For this reason, LDH nanoparticles would be more suitable for siRNA delivery.\(^{85,86}\) In one study, LDH-siRNA displayed greatly enhanced intracellular delivery of siRNA to mammalian cells\(^{85}\) and cortical neurons.\(^{87}\) The intracellular uptake of LDH-siRNA was demonstrated to be through clathrin-mediated endocytosis. As shown in Fig. 5a, in the absence of chlorpromazine (a clathrin-mediated endocytosis inhibitor), strong fluorescence signals (siRNA duplex was covalently coupled to 6FAM fluorophore at the 5’ end) were observed in cells at 40 min after incubation (Fig. 5a–i). In great contrast, in the presence of chlorpromazine, significantly diminished fluorescence signals were found within the neuronal cytoplasm. These observations indicated that clathrin-mediated endocytosis played an important role in LDH internalization.\(^{86}\)

LDH nanoparticles also show promise for topical application of RNA interference for sustained protection against plant viruses.\(^{87}\) Pathogen-specific double-stranded RNA (dsRNA) can protect plants from viral infection, but its instability limits the application. To address this problem, dsRNA was loaded on LDH nanoparticles. The results demonstrated that the stability of dsRNA could be improved and the dsRNA was able to be slowly released even 30 days after spraying on plant leaves. Very attractively, a single spray of dsRNA loaded on LDH nanoparticles on plant leaves enabled virus protection for at least 20 days (Fig. 5b and c).

#### 2.3 Co-delivery of drugs/genes and bioimaging

Simple delivery of one kind of drug is sometimes not able to satisfy the requirement of an advanced drug delivery system. Taking cancer therapy as an example, multiple drugs are often used to have different targets such as targeting both nucleus and cytoplasm. For this, LDH nanoparticles have been developed to simultaneously transport two types of cancer treatment agents to improve cancer therapy and overcome drug resistance.\(^{91}\) In one example, LDH nanoparticles were used as a vector to deliver the anticancer drug 5-Fluourouracil (5-Fu) and Allstars Cell Death siRNA (CD-siRNA) for potent cancer treatment.\(^{94}\) The experiment outcomes proved...
that the combined chemotherapy and gene therapy significantly enhanced the cytotoxicity of 5-Fu in three cancer cell lines (MCF-7, U2OS and HCT-116). In addition, LDH nanoparticles were reported to simultaneously load a Pt(IV) prodrug and a p53 activator.95 It was discovered that the \textit{in vitro} anticancer activity of cisplatin in p53-wild type cells was dramatically enhanced. The introduction of active p53 protein promoted apoptotic pathways initiated by platinum drugs, thereby substantially increasing the efficacy of the cisplatin prodrug (Fig. 6). Wang \textit{et al.} co-loaded Pt(IV) prodrugs and the Chlorin e6 (Ce6) photosensitizer into LDH nanoparticles to overcome the drug resistance of cisplatin.96 Ce6 photosensitizer and Pt(IV) prodrugs loaded LDH nanoparticles exhibited a synergistic effect in killing cancer cells, and particularly, were highly active towards cisplatin-resistant cells, showing up to 190-fold increased efficiency in comparison with cisplatin alone. It is well known that the failure of cancer chemotherapy could be attributed to the escape of cancer cells from the immune system. In order to tackle this problem, Wang \textit{et al.} fabricated a cisplatin-loaded immunochemotherapeutic LDH nanohybrid that bears an immune checkpoint inhibitor for enhanced cancer therapy97 (Fig. 7). In the study, LDH nanoparticles were co-loaded with the first line chemotherapy drug cisplatin and the immune checkpoint inhibitor indoleamine-2,3-dioxygenase (IDO) inhibitor. The \textit{in vitro} cervical cancer cell study revealed improved cellular accumulation, increased binding of Pt to DNA and higher apoptosis. The IDO inhibitor could inhibit indoleamine-2,3-dioxygenase and activate T cells, thus ultimately boosting the anti-tumor performance of the nanohybrid.97

Most recently, LDH has been fabricated as a multifunctional drug carrier for theranostic applications.98–101 For instance, Li \textit{et al.} reported pH-ultrasensitive Mn(II)-containing LDH nanoparticles with superb longitudinal relaxivity (9.48 mM$^{-1}$ s$^{-1}$ at pH 5.0 and 6.82 mm$^{-1}$ s$^{-1}$ at pH 7.0 vs. 1.16 mm$^{-1}$ s$^{-1}$ at pH 7.4) for magnetic resonance imaging (MRI).102 A two-step method was utilized to synthesize Mn-LDH: Mg$_3$Al-LDH nanoparticles were first obtained through a co-precipitation method followed by isomorphic substitution of partial number of Mg$^{2+}$ with Mn$^{2+}$ ions (Fig. 8). The \textit{in vivo} study revealed that Mn-LDH nanoparticles exhibited clear MRI for tumor tissues in mice for up to 2 days post intravenous injection, and also these nanoparticles were very promising for cancer diagnosis and treatment. Furthermore, Peng \textit{et al.} prepared Gd$^{3+}$-doped monolayered LDH nanosheets with controlled composition and morphology,100
and loaded the nanosheets with DOX (chemotherapy drug) and indocyanine green (photodynamic and photothermal agent) with minimal invasion. This example well demonstrates the powerful function for drug delivery and integrated treatment and diagnosis.

3. Surface modified LDH for biomedical applications

Although LDH nanoparticles are widely used as a vector for enhanced drug and gene delivery, the vast majority of studies have been based on as-prepared pristine (non-surface modified) LDH structures. However, these pristine nanoparticles often suffer from problems of instability in \textit{in vivo} applications due to the lack of surface stabilization. Moreover, besides increasing the stability of LDH nanoparticles, surface modification plays an extremely important role in using nano-materials for biomedical applications.\textsuperscript{103–107} Firstly, surface modification allows convenient attachment of targeting ligands to nanostructures for targeted delivery. Secondly, surface modification renders nanomaterials flexibility in drug loading and versatile functionalities. Thirdly, surface modification is very important for enhancing nanomaterials’ cellular uptake, stability, and biocompatibility.

For this purpose, surface modified and exfoliated layered gadolinium hydroxide nanoparticles were developed for use as multimodal contrast agents for MRI and fluorescence imaging.\textsuperscript{108} In this material, LDH nanoparticles contain MR-active Gd\textsuperscript{3+} ions, which makes the material have potential for MRI. Then LDH nanoparticles were exfoliated by incorporation of long chain oleate ions, as shown in Fig. 9a. The separate individual layers were coated with phospholipids with poly(ethylene glycol) tail groups and fluorescent molecules to achieve multimodal imaging (MRI + fluorescence imaging) application \textit{in vivo} (Fig. 9a). In another work, LDH nanoparticles were coated with liposome, enabling excellent water dispersion and sustained release characteristic, as shown in Fig. 9b.\textsuperscript{109} Recently, the bovine serum albumin (BSA) pre-coating strategy was introduced to increase LDH nanoparticles’ stability in buffered solution. This coating strategy could substantially prevent aggregation of LDH nanoparticles, and increase their cellular uptake in Chinese hamster ovary cells.\textsuperscript{110} Shi \textit{et al.} fabricated bivalent cation \textsuperscript{64}Cu\textsuperscript{2+} and trivalent cation \textsuperscript{44}Sc\textsuperscript{3+} labelled LDH nanoparticles-BSA nanoparticles with excellent labelling efficiency and stability for \textit{in vivo} positron emission tomography (PET).\textsuperscript{111} In this work, PET imaging showed that prominent tumor uptake was achieved in 4T1 breast cancer with \textsuperscript{64}Cu-LDH nanoparticles-BSA via passive targeting alone (7.7 \pm 0.1\% ID g\textsuperscript{-1} at 16 h post-injection; \(n = 3\)). Zuo \textit{et al.} modified LDH nanoparticles with brain tumor targeting ligands angiopep-2 and rabies virus glycoprotein.\textsuperscript{112} The ligands were first covalently linked with BSA through the heterobifunctional cross-linker sulfo-succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate, and then coated onto the surface of LDH nanoparticles through electrostatic interaction, followed by cross-linking with the cross-linker glutaraldehyde to immobilize these BSAs on the LDH nanoparticles’ surface. The ligand conjugated LDH nanoparticles showed dramatically enhanced cellular uptake by
brain tumor cells, and increased the delivery efficacy of 5-FU for effective inhibition of neural U87 and N2a cells.

In addition to the above surface coating method which is mostly via electrostatic attraction, another approach is to modify LDH nanoparticles through covalent bonding by making use of the –OH functional groups on the surface. Park et al. introduced (3-aminopropyl)triethoxysilane (APTES) to tailor LDH nanoparticles through condensation between the hydroxyl groups of LDH and the ethoxy groups of APTES molecules to form a covalent bond. With this method, amine groups were grafted on the surface and interlayer spacing of LDH nanoparticles, making it convenient to attach other guest molecules. For example, the cancer cell specific ligand folic acid and fluorescein-5-isothiocyanate (FITC) were successfully conjugated onto the surface.

Fig. 8 Schematic illustration of (a) the synthetic procedure and (b) structure related multifunctional properties of Mn-LDH nanoparticles. (Reproduced from Wiley-VCH Verlag GmbH & Co. KGaA with permission.)

Fig. 9 The surface modification approach for the fabrication of (a) exfoliated layered gadolinium hydroxide (reproduced from Wiley-VCH Verlag GmbH & Co. KGaA with permission) and (b) liposome coated LDH (reproduced from Elsevier with permission).
of amine-modified LDH nanoparticles through connection with amine functional groups.\textsuperscript{114} Folic acid conjugated LDH nanoparticles showed greatly enhanced selectivity to folate receptor over-expressing cells (KB cells).\textsuperscript{114} Wei et al. incorporated a near-infrared (NIR) dye into amine-modified LDH nanoparticles by electrostatic attraction, and further coated them with chitosan using glutaraldehyde as a cross-linking agent. These chitosan coated LDH nanoparticles had minimal cytotoxicity.\textsuperscript{115} The subsequent in vivo application demonstrated the potential application of chitosan coated LDH nanoparticles for imaging.\textsuperscript{115} Covalent conjugation of Cy5.5 molecules and Polyethylene glycol (PEG)-5000 to amine modified LDH nanoparticles was also reported, and LDH-PEG-5000 showed an enhanced blood circulation time without high fluorescence intensity in the major organs.\textsuperscript{116} Yan et al. developed an approach to exfoliate LDH nanoparticles to thin individual layers so that it is possible to modify both sides of hydroxide nanosheets which are much thinner than conventional LDH nanoparticles. These exfoliated LDH thin layers could be linked with APTES-folic acid and these nanostructures were able to self-assemble into nanoclusters.\textsuperscript{117,118} Through this approach, folic acid modified LDH nanoparticles were prepared for targeting cancer cells with folate receptor overexpression. Folic acid modified LDH nanoparticles possessed a high drug loading capacity of 27 wt% and dramatically improved the therapeutic efficacy of MTX.\textsuperscript{117} A similar approach was also used to modify LDH nanoparticles with fluorescein molecules.\textsuperscript{118} Attractively, this method produced LDH-fluorescein nanoparticles with a very high quantum yield (QY) of 55.1%. This is significantly better than those prepared through the previously reported anion exchange (3.0%) and coprecipitation (12.4%) approaches. Moreover, this type of LDH-fluorescein nanoparticles showed strong fluorescence even in a solid state, while conventional dye molecules completely lost fluorescence in a dry form due to concentration quenching. These nanostructures were also capable of self-assembling into a transparent, free-standing and fluorescent film.\textsuperscript{118} Recently, Park et al. reported biodegradable LDH nanoparticles for active tumor targeting in siRNA transportation. In this work, folic acid conjugated LDH nanoparticles showed 3.0-fold higher suppression of tumor volume than the non-modified LDH system\textsuperscript{119} (Fig. 10). Consistent with the improved siRNA delivery, a low level of Survivin protein and proliferating cell nuclear antigen (PCNA) in tumors was detected in the LDH-folic acid treated group. The results revealed that folic acid modified LDH nanoparticles loaded with siRNA efficiently suppressed Survivin expression at both post-transcriptional and translational levels and induced necrosis in cancer cells.

Recently, it was reported that photosensitizer Ce6 could be covalently conjugated to PEG modified LDH nanoflakes.\textsuperscript{120} In this study, Ce6 molecules were firmly anchored to the stabilized LDH nanoflakes and showed very slow release profiles of Ce6, avoiding premature release of drug molecules in blood and ensuring their efficient delivery to tumors. It was found that LDH nanoparticles–Ce6 significantly inhibited tumor growth as indicated by the size reduction to 13.4%, 14.3% and 26.9% of the tumor volume in the PBS, LDH–Ce6 Mix, and free Ce6 treated groups, respectively. In addition, LDH could be exfoliated to ultrathin nanosheets with a size of 50 nm and a thickness of about 1.4–4 nm.\textsuperscript{121} The fabricated nanosheets were tested for delivery of a negatively charged anticancer drug, MTX, in a mouse model, which demonstrated dramatically improved therapeutic efficacy, indicated by the effective inhibition of tumor growth with minimal side-effects. In comparison, if using the conventional non-surface modified LDH nanoparticles for delivery of MTX, the tumor inhibition efficacy is similar to that of free MTX molecules. Also importantly, with covalent conjugation, LDH nanostructures can not only load negatively charged species like conventional LDH nanoparticles, but also neutral molecules such as PEG and positively charged rhodamine B dyes.

4. LDH nanocomposites for biomedical applications

With the rapid development of LDH nanostructures, extensive research has also been extended to incorporating them with a spectrum of other materials to further broaden their applications. LDH based nanocomposites possess not only advantages and merits of LDH but also those of the combined materials. Therefore, the composites have the potential to satisfy advanced requirements of many practical utilizations.\textsuperscript{122} Next, the biomedical and healthcare applications of LDH nanocomposites will be summarized.

4.1 Nanocomposites with LDH as the core

Silica coating is one of the most commonly used methods to coat nanoparticles because of its ease of further functionalization, optical transparency, good hydrophilicity, excellent stability and high biocompatibility.\textsuperscript{123–128} Therefore, LDH nanoparticles were also decorated with a silica shell, using tetraethyl orthosilicate Si(OCH₂)₄ (TEOS) as a silica source.\textsuperscript{122} Initially, a surfactant template method was reported for an efficient synthesis of an LDH@SiO₂ nanocomposite (Fig. 11a). In the method, CTAB was used as a surfactant-template to fabricate the LDH@SiO₂ nanocomposite. The obtained nanostructures had uniform and accessible mesopores (~2.2 nm), high surface area (~430 m² g⁻¹), and large pore volume (~0.22 cm³ g⁻¹). This nanocomposite was tested for a slow release of ibuprofen.\textsuperscript{129} Then surfactants FC4 and F127 were also introduced to synthesize the LDH@SiO₂ nanocomposite for sustained and pH sensitive release\textsuperscript{130} (Fig. 11b). Li et al. used well-dispersed amine-functionalized SiO₂ nanodots to coat LDH by electrostatic interaction followed by condensation of APTES\textsuperscript{11} (Fig. 11c). These LDH-SiO₂ dot nanostructures were well dispersible in cell culture medium and PBS buffer, and could be used to effectively deliver siRNA into U2OS cells.\textsuperscript{11} Besides silica coating, a Gd-doped LDH@Au nanocomposite was fabricated for drug delivery and diagnosis.\textsuperscript{9} This nanocomposite system displayed high DOX loading capacity, and enabled a pH sensitive release profile of DOX. The Gd-doped LDH@Au nanocomposite was able to efficiently deliver DOX into cells, release DOX in the cytoplasm, and ultimately cause cancer cell death.\textsuperscript{9} Meanwhile, with Gd and Au doping, this nanocomposite could be employed for in vitro Computed Tomography (CT) and T1-weighted MR imaging, with
a better performance than commercial MRI and CT contrast reagents and negligible cytotoxicity. A luminescent Eu-doped LDH nanocomposite was also synthesized with magnetic Fe₃O₄ nanoparticle coating. This LDH could load 31% (w/w) ibuprofen and exhibited sustained release.

4.2 Nanocomposites with LDH as the shell

A LDH based nanocomposite structure was designed using magnetic nanoparticles and LDH as the core and shell, respectively. This structure was fabricated with a simple one-step co-precipitation method. Very attractively, it was explored that a greatly decreased drug release rate of diclofenac and ibuprofen was achieved when the composite structure was placed under a magnetic field. This magnetic field controlled “on–off” phenomenon is due to the instant aggregation of the magnetic nanocomposite induced by an external magnetic field. This structure was then continued to be developed for cancer therapy. In one representative study, the anticancer agent doxifluridine was loaded to monodispersed strongly magnetic Fe₃O₄@LDH nanostructures. This Fe₃O₄@LDH nanocomposite had a well-defined core–shell structure, strong magnetization and magnetic guided controlled release properties. For drug delivery, carboxyl group modified DOX was loaded to the LDH shell via anion-exchange (Fig. 12). This DOX-COOH loaded nanocomposite exhibited high cytotoxicity and powerful inhibition towards cancer cells (HeLa cells), but had low toxicity to normal cells (HEK 293T Cells). The mechanism may be explained as follows: the acidic cytoplasm of HeLa cells stimulated rapid degradation of the LDH shell and converted DOX-COOH to positively charged DOX, thereby rapidly escaping from the positively...
charged intercalation of the LDH shell by strong repulsive interaction, but the relatively neutral cytoplasm of HEK 293T cells caused a smaller amount of DOX to be released.\textsuperscript{135} In addition to anticancer therapy, this type of magnetic nanoparticles@LDH nanocomposite was also used for separation of proteins.\textsuperscript{136} Nanostructures with a SiO\textsubscript{2}-coated Fe\textsubscript{3}O\textsubscript{4} magnetite core and a LDH shell were synthesized by an \textit{in situ} growth method (Fig. 13a). The as-fabricated nanocomposite was 600 nm in diameter, and had a large surface area (83 m\textsuperscript{2} g\textsuperscript{-1}) and uniform mesochannels (4.3 nm) (Fig. 13b and c). The nanocomposite effectively and selectively separated histidine-tagged green fluorescent protein with a binding capacity of 239 m\textsuperscript{g} mg\textsuperscript{-1}, and remained stable in several separation cycles\textsuperscript{136} (Fig. 13d).

SiO\textsubscript{2} nanostructures were also encapsulated with a LDH shell. A 210 nm SiO\textsubscript{2}@LDH core–shell nanocomposite was designed to enhance the immune adjuvant activity.\textsuperscript{137} The zeta potential of this core–shell structure was 0.521 mV, making it easier for DNA binding compared with naked SiO\textsubscript{2} nanostructures which were negatively charged. The larger surface area of this nanocomposite can load a relatively large amount of DNA vaccine. Moreover, the SiO\textsubscript{2}@LDH nanocomposite protected DNA from degradation, increased transfection efficiency, and efficiently promoted macrophage activation, compared with SiO\textsubscript{2} and LDH.

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\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig11.png}
\caption{Schematic illustration of the preparation of LDH core and silica shell nanocomposites\textsuperscript{11,129,130} (reproduced from The Royal Society of Chemistry and Springer with permission).}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig12.png}
\caption{(a) The plausible DOX release mechanism of DOX@MSPs/Ni-LDH-folate in the cytoplasm of tumor cells (acidic environment). (b) The different fluorescence behaviors of HeLa and HEK 293T cells incubated with nano-drugs. (c) The mechanism of pumping positively charged DOX out of the LDH sheets\textsuperscript{135} (reproduced from Elsevier with permission).}
\end{figure}

\begin{figure}[h]
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\includegraphics[width=\textwidth]{fig13.png}
\caption{(a) Schematic illustration of the fabrication of the Fe\textsubscript{3}O\textsubscript{4}@SiO\textsubscript{2}@LDH nanocomposite; (b and c) TEM (b) and SEM (c) images of the Fe\textsubscript{3}O\textsubscript{4}@SiO\textsubscript{2}@NiAl-LDH nanocomposite; (d) photographs of separation of His-tagged GFP by Fe\textsubscript{3}O\textsubscript{4}@SiO\textsubscript{2}@NiAl-LDH microspheres after different times\textsuperscript{136} (reproduced from American Chemical Society with permission).}
\end{figure}
alone. The in vivo immunization study showed that this kind of DNA vaccine loaded SiO2@LDH nanocomposite induced higher serum antibody response and also promoted T-cell proliferation and skewed T helper cells toward Th1 polarization. Later on, a similar structure of SiO2@LDH nanocomposite was used to deliver Newcastle disease virus DNA vaccine. Additionally, the SiO2@LDH nanocomposite was designed for controlled release. Mesoporous silica was used as a core material to load guest molecules of \( \text{Ru(bpy)}_3\text{Cl}_2 \) followed by encapsulation of LDH nanosheets via electrostatic adsorption (Fig. 14). This nanocomposite was used for pH-responsive controlled release by dissolution of the LDH coating in an acidic environment to trigger the release of the drug from mesoporous silica nanoparticles: almost no \( \text{Ru(bpy)}_3\text{Cl}_2 \) release was found from the SiO2@LDH nanocomposite in the physiological environment (pH = 7.4), while slow and fast release took place at pH = 4.0/5.0 and pH = 3.0, respectively (Fig. 14a). Moreover, a multifunctional Y2O3:Er3+,Yb3+@SiO2@LDH nanocomposite system was fabricated for simultaneous tumor optical imaging and therapy. LDH nanosheets were deposited on the surface of monodispersed SiO2 coated Y2O3:Er3+,Yb3+ nanoparticles by precipitation followed by hydrothermal treatment. In this system, Y2O3:Er3+,Yb3+ nanoparticles worked as a near infrared fluorescent nanophosphor which exhibited strong red upconversion fluorescence under 980 nm laser irradiation. The positively charged surface of LDH was utilized as an anticancer drug nanovehicle for efficient intracellular delivery.

Au nanoparticles have also been employed as a core material, wrapped with LDH. In this work, MTX was first attached to citrate-stabilized Au nanoparticles; then, a SiO2 layer was decorated on the surface of the Au nanostructure using tetraethyl orthosilicate (TEOS) as a silica source; later, NaOH was added to the SiO2 shell; and finally, Mg2+ and Al3+ were added to the solution to obtain a LDH shell. By controlling the pH of SiO2 etching, a core/shell structure (pH 11) or a york/shell structure (pH 10.5) was able to be controllably synthesized. MTX loaded to this nanostructure exhibited better anticancer efficiency, compared with free MTX and LDH-MTX.

4.3 LDH thin film coating

LDH based film coating has been widely used for antimicrobial, sensing, electrochemical and optical applications. In a typical work, substrates including metals, ceramics, and glass were coated with oriented LDH films with strong adhesion. Subsequently, silver ions were in situ reduced to metallic silver nanoparticles and evenly distributed on the LDH surface (Fig. 15a and b). The Ag-LDH coating showed excellent and durable antimicrobial activities against both Gram-negative (E. coli and P. aeruginosa) and Gram-positive (B. subtilis and S. aureus) bacteria (Fig. 15c–g). Ciprofloxacin loaded LDH was also coated on a middle ear prostheses implant to combat...
infection with *P. aeruginosa*. The film displayed excellent activity against infection immediately after implantation, also with persisting attenuated activity when re-infection was triggered at 1 week after surgery. Similar techniques were as well applied to decorate porous titanium implants with antibiotics for prolonged antibiotic release, improved cell adhesion and good *in vitro* and *in vivo* antibacterial effects. It was found that middle ear prostheses coated with LDH showed little signs of degradation after being implanted into the middle ear of rabbits for 10 days, indicating that the LDH has excellent biocompatibility and stability in the middle ear. Moreover, Peng et al. reported Mg-Al LDH and Mg(OH)\(_2\) co-coated Mg-Nd-Zn-Zr alloy to improve its corrosion resistance and biocompatibility. The LDH coated alloy also showed greatly enhanced cell adhesion, migration and proliferation and a decreased hemolysis ratio to a level suitable for clinical application. The *in vivo* study demonstrated that the Mg(OH)\(_2\) and Mg-Al LDH coating exhibited better histocompatibility than pure Mg(OH)\(_2\) coatings or untreated magnesium alloy.

### 4.4 Other types of LDH based composites

As a ceramic material, LDH has superior mechanical, optical and thermal properties which pure polymers normally lack. Taking advantage of these merits, LDH nanoparticles can be regarded as a filler to incorporate into the polymer matrix to form nanocomposites and thus improve the desirable property of a pure polymer matrix. In one application, LDH nanoparticles were added to carboxymethylcellulose (CMC) to fabricate mechanically strengthened dissolvable polymer microneedles for improved transdermal delivery to human skin. The addition of LDH nanoparticles dramatically improved the mechanical property of weak dissolving polymers: the elastic modulus of CMC was measured to be 0.993 ± 0.065 and 2.878 ± 0.123 GPa before and after the addition of 5 wt% LDH to CMC (Fig. 16a). With the improved mechanical strength, LDH-CMC microneedles could penetrate pig and human skin much more uniformly, also with a substantially greater penetration depth than CMC microneedles (Fig. 16b and c). The advantage of this mechanically strengthened LDH-CMC nanocomposite is that it still dissolves very rapidly within the skin (Fig. 16d and e), while previously reported mechanically strengthened microneedles suffer from slower dissolution rates, which can affect their clinical use as dissolvable microneedles.

In addition, Mg-Al LDH nanoparticles were added to chitosan to fabricate a porous scaffold and loaded with Pifithrin-\(\alpha\) (PFT\(\alpha\)) to enhance stem cell osteogenic differentiation and bone regeneration. PFT\(\alpha\) molecules exhibit a potent ability to induce osteogenesis of hBMSCs via the GSK3/\(\beta\)-catenin pathway. Here the LDH-CS-PFT\(\alpha\) scaffold was demonstrated to significantly enhance the osteogenic differentiation of hBMSCs. When used in an *in vivo* test, the scaffold greatly increased the repair and regeneration of bone tissue in cranial defect model rats in comparison with that in the control rats at 12 weeks post-implantation. Thus, the development of a LDH-chitosan based scaffold provided a promising strategy for tissue engineering applications. Recently, a bone remodeling drug, risedronate (RS), was loaded into LDH nanoparticles, followed by the formation of a sheet with poly(lactic-co-glycolic acid) on a clinically approved bioabsorbable bone plate (Fig. 17). This design, the presence of metals in LDH enabled *in vivo* X-ray images for up to 4 weeks after implantation. In the meantime, the sustained release of drug greatly improved bone regeneration.

### 5. Discussion

#### 5.1 Advantages of LDH nanostructures

LDH nanostructures have been extensively studied for about 20 years with constantly increasing interest as indicated in the introduction. For biomedical applications, LDH nanostructures offer many advantages. First, these nanostructures possess pH sensitivity. LDH nanostructures are made of hydrous oxides, making them sensitive to acidic environments with very good biodegradability. This enables LDH nanostructures to be ideal for cancer therapy as tumor tissues and cancer cells possess lower pH. Using pH as a stimuli has constantly been a popular strategy for controlled drug delivery and release. Biodegradability is also very important for body clearance of the material and consequently for enhancing biocompatibility and safety profile. Second, the metal component of LDH nanostructures could be easily tuned for different applications. The consideration of biocompatibility, metal ions like Zn\(^{2+}\), Mg\(^{2+}\) and Al\(^{3+}\) are the typical candidates for divalent and trivalent metal cations, respectively. Other types of metal ions like Mn\(^{2+}\) can also...
be incorporated for MRI observation. Compared with other materials such as semiconductor quantum dots for fluorescence imaging, MRI is much more clinically relevant. Third, LDH nanostructures are competent for cell membrane penetration due to their positive charge, thereby greatly improving intracellular delivery efficacy. Biological barriers are the major obstacles of effective delivery of drugs, genes and biomolecules into the cell cytoplasm. Fourth, the drug loading process can be very straightforward in the case of negatively charged species and the drug loading capacity is relatively high. Fifth, the fabrication is cheap and the size tunability can vary with a wide range from 20 nm to 10 μm, suitable for different biological and clinical applications. This size tunability is far greater than for most other types of nanomaterials.第六, our research showed that ultra-thin LDH nanosheets tend to preferably accumulate in the kidney instead of liver like most other nanomaterials. This may be good for subsequent clearance and more suitable for clinical applications. Seventh, LDH has a great flexibility to form numerous types of composites for various utilizations. Furthermore, LDH is a type of ceramic nanoparticles with very high mechanical strength and therefore can be utilized as a filler to modify the mechanical strength of a polymer matrix for specific applications and also as a thin film coating on various kinds of implantation devices.

5.2 Challenges of LDH nanostructures for biomedical applications

Although LDH nanostructures possess superior advantages that seem very promising for future applications, several challenges are still remaining. For example, because of the positive charge of LDH nanostructures, they may interact with negatively charged biomolecules in the biological environment and lead to loss of surface charge and aggregation. This challenge becomes crucial when LDH nanostructures are administrated via the blood system. Therefore, improvement of the stability of LDH nanostructures is a major challenge. Second, compared with other nanomaterials, there is less flexibility in functionalizing the surface of LDH nanostructures. For conventional LDH nanoparticles, due to the bulky thickness, the surface modification of the 2 outermost LDH layers is not enough for high stability in the biological environment. Therefore, it is important to develop new generations of ultra-thin LDH nanosheets with flexibility of surface modification for practical applications. Third, in comparison with other inorganic materials such as silica and gold nanoparticles, the controllability of the fabrication of LDH nanostructures and the reproducibility need to be substantially improved. To tackle this issue, continuous synthesis has been employed instead of the traditional flow chemistry approaches that allow reducing the residence time and maintaining almost constant supersaturation to produce LDH in large amounts.

6. Conclusions and future outlook

This review summarizes the development of LDH nanomaterials including pristine LDH nanostructures, surface modified LDH nanostructures, and LDH based nanocomposites for biomedical applications. LDH shows good biocompatibility, pH sensitivity, biodegradability, high intracellular delivery efficacy, and great flexibility in size tunability and nanocomposite formation. Initially, pristine LDH nanostructures were used for drug and gene delivery and photodynamic therapy. Although effective, it is desirable to surface modify these LDH nanostructures for improved properties due to the importance of the surface of nanomaterials. Surface modification not only makes LDH nanostructures more stable in the biological environment. Therefore, it is important to develop new generations of ultra-thin LDH nanosheets with flexibility of surface modification for practical applications. Third, in comparison with other inorganic materials such as silica and gold nanoparticles, the controllability of the fabrication of LDH nanostructures and the reproducibility need to be substantially improved. To tackle this issue, continuous synthesis has been employed instead of the traditional flow chemistry approaches that allow reducing the residence time and maintaining almost constant supersaturation to produce LDH in large amounts.
need to be established to ensure improved properties and minimize the influence of drug loading conditions such as temperature, time and pH on loaded drugs and genes. Thirdly, it is required to fabricate highly stable LDH nanomaterials in biological solution so that the drug delivery system can efficiently transport drugs and genes to target sites with minimal pre-mature drug and gene release. The high stability will prevent aggregation of nanostructures which can result in low therapeutic efficacy and possible precipitation in blood circulation. In this regard, it is desirable to develop ultra-thin LDH nanosheets. Fourthly, it is needed to thoroughly understand the in vivo biodegradability, biodistribution, and clearance of LDH nanomaterials. Fifthly, the flexibility of formation of LDH nanocomposites is an important advantage of LDH. This area of research should be significantly expanded, particularly by combining with materials which can circumvent the limitation of LDH nanostructures or enhance properties and performance. Moreover, there are many other types of nanostructures for biomedical applications such as bioceramic nanoparticles. However, there is a lack of studies that compare different types of nanomaterials with the same standard protocols for direct comparison so it is difficult to identify the best candidate for practical applications.

Conflicts of interest

There are no conflicts.

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