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Fabrication of Ultrathin Zn(OH)₂ Nanosheets as Drug Carriers

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Abstract

Ultrathin two-dimensional (2D) porous Zn(OH)₂ nanosheets (PNs) have been fabricated by using one-dimensional Cu nanowires as backbones. PNs have thicknesses of about 3.8 nm and pore sizes of 4–10 nm. To form “smart” porous nanosheets, DNA aptamers were covalently conjugated on the surface of PNs. These ultrathin nanosheets show good biocompatibility, efficient cellular uptake, and promising pH-stimulated drug release.

Graphical abstract

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Electronic Supplementary Material: Supplementary material (please give brief details, e.g., further details of characterization of Cu NWs, EDX, Mapping, BET, FTIR, Confocal fluorescence images, Photographs of Dox adsorption on SPNs, and Zeta potential) is available in the online version of this article at http://dx.doi.org/10.1007/s12274-***-****-*.

Keywords

Aptamer; Porous $\text{Zn}(\text{OH})_2$ nanosheets; Cancer cell; Cell-targeted delivery

1 Introduction

Since graphene first became readily available, various two-dimensional (2D) graphene-like nanosheets have been developed, including metal chalcogenides (e.g., MoS_2), metal oxides (e.g., MnO_2), and carbon nitrides (C_3N_4), with lateral dimension up to micrometers.¹ Based on the high percentage of surface atoms and active sites on the exposed surfaces, they have shown great potential in drug delivery,² catalysis,³ biomolecular detection,⁴ energy storage,⁵ water treatment,⁶ and cancer cell imaging.⁷ Approaches to fabricate ultrathin 2D nanosheets (thickness of < 5 nm) include electro-deposition, thermal evaporation, chemical exfoliation, delamination of layered precursor, and ligand-assisted oriented attachment of nanocrystal building blocks,⁸ during which high temperature, complicated procedures, and toxic organic surfactants are almost unavoidable. Developing simple procedures to obtain ultrathin 2D nanosheets in an environmentally benign system, e.g., in water or ethanol, could further enhance appeal for their application, especially in the biorelated areas.

$\text{Zn}(\text{OH})_2$ is appealing in biomedical applications, such as surgical dressings and separation of proteins, based on its nonhazardous nature and stability.⁹ Although $\text{Zn}(\text{OH})_2$ can be readily precipitated by tuning the pH, a special treatment, such as hydrothermal synthesis, thermal evaporation or dissolution-recrystallization,⁹ is always required to achieve the 2D structure. Facile synthesis of ultrathin $\text{Zn}(\text{OH})_2$ nanosheets at room temperature has never been reported. Therefore, we present, for the first time, the fabrication of 2D ultrathin porous $\text{Zn}(\text{OH})_2$ nanosheets (PNs) by a facile strategy (Scheme 1). The synthetic procedure is rapid, green and energy-efficient. More significantly, upon further conjugation with sgc8 aptamer, these PNs show excellent biocompatibility and selective binding ability to target cancer cells, thus providing a platform for pH-responsive delivery of anticancer drugs.

2 Experimental

2.1 Chemicals

The following chemicals were obtained as indicated: Sodium hydroxide (Fisher Scientific (99.9%), Copper (II) nitrate trihydrate ($\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$, 99.5%, Merck), ethylenediamine (EDA, 99.9%, Sigma-Aldrich), anhydrous hydrazine (98%, Sigma-Aldrich), sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$, 99%, Sigma-Aldrich), zinc chloride (ZnCl_2 , 99.9%, Sigma-Aldrich), sodium borohydride (NaBH_4 , 98%, Sigma-Aldrich), dimethylformamide (DMF, 99.9%, Sigma-Aldrich), polyvinylpyrrolidone (PVP, MW = 1,300,000, Sigma-Aldrich), polyvinylpyrrolidone (PVP, MW = 24000, 99.9%, Sigma-Aldrich), (3-aminopropyl) triethoxysilane (APTES, 99.9%, Sigma-Aldrich), methanol (absolute for analysis, 99%, Sigma-Aldrich), dimethyl sulfoxide (DMSO, anhydrous, 99.9%, Sigma-Aldrich), sulfosuccinimidyl-4-(N-maleimido methyl) cyclohexane-1-carboxylate (Sulfo-SMCC, 90%, Thermo Scientific Pierce), doxorubicin (Dox, 98%, Sigma-Aldrich), acetone and ethanol (absolute for analysis, ACS, 99.9%, Merck). All materials were used without further purification.

2.2 Samples Preparation

Synthesis of copper nanowires—The synthesis of copper nanowires follows the approach developed by Cai and coworkers,¹⁰ which involves reducing $\text{Cu}(\text{NO}_3)_2$ with hydrazine in an aqueous solution of NaOH and some ethylenediamine (EDA). In a typical experiment, NaOH (36 g), DI-water (70 mL), EDA (563 μL), and hydrazine (263 μL , 34 wt %) were added to a 120 mL flat bottom flask one by one. After 10~15 min stirring, 4 mL aqueous solution of 0.1 M $\text{Cu}(\text{NO}_3)_2$ was added to the solution drop-by-drop under vigorous stirring. Then, the solution was transferred to a 100 mL Teflon-lined stainless steel autoclave. After that, 5mL aqueous solution of PVP (molecular weight 1,300,000) (2g/L) was gently added to the top of the solution. The autoclave was finally sealed and placed in an electric oven at 80 °C for 3 hours. The autoclave was then cooled to room temperature. The Cu nanowires floated on top of the reaction solution, and were then washed with DI-water and ethanol containing hydrazine (0.01 wt %) before drying in a 50 °C vacuum oven overnight.

Synthesis of ultrathin porous $\text{Zn}(\text{OH})_2$ nanosheets

In a typical experiment, Cu nanowires (6.35 mg), DI-water (6 mL), and ethanol (6 mL) were added sequentially to a 50 mL flat bottom flask. After ultrasonication for 15 min, 300 mg PVP (molecular weight 24000) was added to the solution. The solution was vigorously stirred for 15 min, after which 0.6 mL of ZnCl_2 (8 mg) aqueous solution was added. The mixture was stirred for an additional 10 min. Then, 4 mL of 1M $\text{Na}_2\text{S}_2\text{O}_3$ aqueous solution was added dropwise (drop/2s) to the above mixture. Finally, the mixture was stirred for 10 min. The red mixture gradually changed to transparent gray, and the products were collected by centrifugation and washed with acetone and methanol three times.

DNA Synthesis—All DNA sequences (see Table S1) were synthesized on an ABI 3400 DNA/RNA synthesizer (Applied Biosystems, Foster City, CA, USA) by the standard phosphoramidite method. Fluorescein (FITC) (or TAMRA) was coupled on the 5' - end of

primers and templates (see Table S1 for sequences), if applicable. DNA sequences were deprotected according to the manufacturer's guidelines. The synthesized DNA oligos were further purified using reversed-phase high-pressure liquid chromatography (HPLC) (ProStar, Varian, Walnut Creek, CA, USA) with a C18 column and acetonitrile-TEAA solvent. Finally, the concentrations of these DNA oligomers were determined by measuring their absorbances at 260 nm with a UV-1800 spectrophotometer (Shimadzu, Japan).

Preparation of smart porous Zn(OH)₂ nanosheets (SPNs, conjugation of sgc8 aptamers to nanosheets)—In order to obtain SPNs, the porous Zn(OH)₂ nanosheets (PNs) were first functionalized with an amino-group using (3-aminopropyl) triethoxysilane (APTES). In a typical procedure, 6 mg PNs was dispersed in 15 mL anhydrous toluene by ultrasonication for 3 hours. Then, 15 μ L 97% APTES was added to the toluene mixture and stirred for 24 hours. The final product was washed several times using ethanol and centrifugation. An aliquot of these particles (amino-functionalized PNs, APTES-PNs) was dried in an oven at 40 °C for characterization.

Second, APTES-PNs were dispersed in 1 mL Dulbecco's PBS. One mg Sulfo-SMCC (Sulfo succinimidyl-4-N-maleimidomethyl cyclohexane-1-carboxylate) and 50 μ L DMSO (dimethyl sulfoxide) were added to the mixture, which was shaken for 1 hour. Then, the mixture was collected by centrifugation and washed with Dulbecco's PBS solution three times. The product was redispersed in 500 μ L Dulbecco's PBS solution, 100 μ L sgc8 aptamer (160 μ M) was added, and the solution was shaken for another 24 hours.

Cell culture—Cell lines CCRF-CEM (ATCC® CRM-CCL-119™, Human T-cell ALL) and Ramos (ATCC® CRL-1596™, Human B-cell Burkitt's lymphoma) were obtained from the American Type Culture Collection (Manassas, VA). Cells were cultured in RPMI 1640 medium (Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS) (heat inactivated, GIBCO) and 100 IU/mL penicillin-streptomycin (Cellgro) at 37 °C in a humid atmosphere with 5% CO₂. Cell density was determined using a hemocytometer prior to each experiment.

Specific recognition ability of sgc8 aptamer and SPNs to target cancer cells—The binding abilities of sgc8 aptamers or sgc8 aptamer-incorporated SPNs were determined using flow cytometry on a FACScan cytometer (BD Immunocytometry Systems). A green laser at 488 nm with different excitation voltages (650, 700, and 750 V) was used as the excitation source. 400 nM of sgc8 aptamers and sgc8 aptamer-incorporated SPNs were incubated with cells (3×10^5) in binding buffer [200 μ L; 4.5 g/L glucose, 5 mM MgCl₂, 0.1 mg/mL yeast tRNA (Sigma-Aldrich) and 1 mg/mL BSA (Fisher Scientific, Pittsburgh, PA) in Dulbecco's PBS] on ice for 30 min in the dark. Cells were washed twice with 1 mL washing buffer (4.5 g/L glucose and 5 mM MgCl₂ in Dulbecco's PBS). Cells were suspended in washing buffer (200 μ L) prior to flow cytometric analysis. Data were analyzed with FlowJo software (Tree Star, Inc., Ashland, OR).

2.3 Internalization Study

To investigate the internalization of SPNs (TAMRA-labeled sgc8 aptamer) into different cell lines, samples containing CEM or Ramos cells with a concentration of 10^6 cells/mL were incubated with the desired concentrations of SPNs at 37 °C in a volume of 200 μ L binding buffer for 4 hours with 5% CO₂ atmosphere. The cells were then centrifuged, washed three times with 200 μ L of washing buffer, resuspended in 200 μ L of binding buffer, and subjected to confocal fluorescence microscopy analysis using an Olympus FV500-IX81 confocal microscope (Olympus, Center Valley, PA, USA) having a 60 $\times$ oil-dispersion objective.

2.4 Drug loading into SPNs

Doxorubicin (Dox) (200 μ M, 1.5mL) was incubated with 1mg SPNs dispersed in 1 mL Dulbecco's PBS (Sigma Aldrich, St. Louis, MO) at room temperature for 24 hours, followed by centrifugation at 10000 rpm for 15 min. Free Dox in the supernatant was isolated and quantified by measuring the absorption of Dox at 480 nm on a Cary Bio-100 UV/Vis spectrometer (Varian). The precipitate (SPNs-Dox complexes) was then dispersed in Dulbecco's PBS (600 μ L).

2.5 Dox release kinetics study

The Dox release kinetics study was carried out using dialysis membrane tubing (MWCO = 3500). The released Dox could cross the dialysis membrane, but not the SPNs. In order to study the release kinetics of Dox from Dox-SPNs, 3 mg of Dox-loaded SPNs (Dox-SPNs) dispersed in 0.5 mL of PBS buffer (pH 7.4) in dialysis membrane tubing were floated in 20 mL of PBS buffer (pH 7.4) at 30 °C. After 72 hours, 200 μ L aliquots were removed for absorbance measurement at predetermined time intervals. Each 200 μ L aliquot was replaced with 200 μ L of fresh PBS buffer to maintain the total volume. The amount of Dox released was determined by measuring the absorption of Dox at 480 nm on a Cary Bio-100 UV/Vis spectrometer at selected time intervals. In order to study the release kinetics of Dox from Dox-SPNs in PBS buffers with different pH values (pH 5, pH 6), a similar experimental setup with the same temperature and release times was used.

2.6 Cytotoxicity assay

The cytotoxicity of SPNs, Dox-SPNs, or free Dox to CEM and Ramos cells was evaluated using the **Cell Titer 96®** Proliferation Assay (Promega, Madison, WI, USA). A sample of 1×10^5 cells in 50 μ L of fresh cell culture medium was seeded into each test well on a 96-well plate. Then SPNs only, Dox-SPNs, or free Dox (0~1.25 μ M) in 50 μ L of fresh cell culture medium was added to the respective test well. The resultant cell mixture was incubated at 37 °C in a 5% CO₂ atmosphere for 4 hours. Then, 75 μ L of cell culture medium was removed from the test well after centrifugation, and another 75 μ L of fresh cell culture medium was added. The 96-well plate was then returned to the incubator for another 48 hours. Finally, 20 μ L of Cell Titer reagent was added to each test well, and the 96-well plate was subjected to absorption measurement at 490 nm using a Versa Max tunable microplate reader (Molecular Devices, Inc., Sunnyvale, CA).

2.7 Characterization

Morphology of the samples was characterized with a transmission electron microscope (TEM) system (JEOL Model JEM-2010F) operating at 200 kV. The crystal phase of samples was investigated using a Bruker D8 Advance diffractometer X-ray diffraction (XRD) at the 2θ range of 10° to 80° with Cu K α radiation. FTIR measurements were conducted on a Perkin Elmer Instruments Spectrum GX FTIR spectrometer at room temperature from 400 to 4000 cm^{-1} . A total of 32 scans were recorded at a resolution of 2 cm^{-1} for averaging each spectrum. Nitrogen adsorption/desorption (Quantachrome Instruments, Autosorb AS-6B) was used to investigate the specific surface area. UV-Vis measurements were performed with a Cary Bio-100 UV/Vis spectrometer (Varian) for DNA and Dox quantification. The specific surface area was calculated by the Brunauer-Emmett-Teller (BET) method using nitrogen adsorption/desorption (Quantachrome Instruments, Autosorb AS-6B), and atomic force microscopy (AFM) (Digital Instruments) was used to determine the thickness of the PNs. Zeta potential analysis was performed with a Malvern Zetasizer Nano ZS (Model No. ZEN 3600, Malvern Instruments Ltd., Worcestershire UK) at 25 $^\circ\text{C}$.

3 Results and Discussion

As revealed by transmission electron microscopy (TEM), the as-prepared products were well-dispersed nanosheets (Fig. 1a). High-magnification TEM (Fig. 1b) images show the highly porous structure of the nanosheets, with pore sizes ranging from 4 to 10 nm. The crystal phase of the product was determined to be hexagonal $\text{Zn}(\text{OH})_2$ (JCPDS card no. 48-1066) from the corresponding powder X-ray diffraction (XRD) pattern shown in Fig. 1d. The observed lattice fringe of 0.247 nm in the high-resolution TEM (HRTEM) image (Fig. 1c) corresponds to the d spacing of the (113) lattice planes in $\text{Zn}(\text{OH})_2$. The ring-type selected-area electron diffraction (SAED) pattern (inset in Fig. 1c) indicates the polycrystalline nature of these PNs. The thickness of one typical nanosheet was measured to be 3.8 nm by atomic force microscopy (AFM) (Fig. 1e). To determine the specific surface area and pore size distribution, full nitrogen sorption isotherms of these PNs were measured. According to the Brunauer-Emmett-Teller (BET) model in Fig. S2, the pore sizes display a relatively wide distribution centered at 2.9 nm. The specific surface area of the PNs was measured to be 127 m^2g^{-1} .

To investigate the growth process of PNs, the intermediate products were collected at different reaction times and systematically investigated (Fig. 2). Uniform Cu nanowires with an average diameter of around 100 nm (Fig. 2a) were used as backbones. Thirty seconds after the addition of ZnCl_2 and $\text{Na}_2\text{S}_2\text{O}_3$ solution, the surfaces of the nanowires became rough, and many tiny “buds” emerged (Fig. 2b). One minute later, the buds grew up from the nanowire surfaces to form worm-like nanostructures, which were then transformed into ultrathin nanosheets within 2~3 minutes (Fig. 2c), generating a heterostructure by the grafting of nanosheets onto the surface of the nanowires (Fig. 2d). Prolonging the reaction time to 6 minutes led to gradual decay of the inner solid parts of the nanowires, resulting in a nanosheet/nanotube heterostructure (Fig. 2e). When the nanotube backbones were fully consumed, which took less than 10 minutes, large-scale and uniform nanosheets were then well-dispersed in the solution (Fig. 2f).

Based on the above observations, a model was proposed to illustrate the formation of PNs, as shown in Scheme 1. The following summarizes the growth mechanism of the PNs. (1) Before the reaction, PVP was mixed with Cu nanowires, and the adsorbed PVP molecules later acted as a passivating layer to control the dissolution rate of Cu nanowires.¹¹ (2) Upon addition of ZnCl₂ solution, several coordination reactions occurred. First, the hydrolysis product of ZnCl₂ ($\text{ZnCl}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}[\text{Zn}(\text{OH})\text{Cl}_2]$) slowly reacted with Cu and oxygen to form a soluble Cu[Zn(OH)Cl₂] complex ($4\text{Cu} + \text{O}_2 + 4\text{H}[\text{Zn}(\text{OH})\text{Cl}_2] \rightleftharpoons 4\text{Cu}[\text{Zn}(\text{OH})\text{Cl}_2] + 2\text{H}_2\text{O}$, $2\text{Cu}^+ + \frac{1}{2}\text{O}_2 + \text{H}_2\text{O} \rightarrow 2\text{Cu}^{2+} + 2\text{OH}^-$) (see *standard electrode potentials*, ESI) by strong interaction between Cu⁺ and [Zn(OH)Cl₂]⁻.^{12,13} This process, in combination with Cu oxidation ($\text{Cu} \rightarrow \text{Cu}^+ \rightarrow \text{Cu}^{2+}$), led to partial dissolution of the Cu nanowire surfaces.¹² (3) Then, Na₂S₂O₃ reacted with Cu⁺ and H₂O: $\text{Cu}^+ + x\text{S}_2\text{O}_3^{2-} \rightleftharpoons [\text{Cu}(\text{S}_2\text{O}_3^{2-})_x]^{1-2x}$,¹⁴ $\text{S}_2\text{O}_3^{2-} + \text{H}_2\text{O} \rightleftharpoons \text{HS}_2\text{O}_3^- + \text{OH}^-$. (4) The OH⁻ released from reaction (3) then facilitated the precipitation of Zn(OH)₂ ($[\text{Zn}(\text{OH})\text{Cl}_2]^- + \text{OH}^- \rightarrow \text{Zn}(\text{OH})_2 + 2\text{Cl}^-$).¹⁵ In particular, at the reacting interface of Cu nanowires, where the local concentration of OH⁻ is the highest, rapid precipitation of Zn(OH)₂ formed tiny “buds” on the nanowire surfaces (Fig. 2b–c). These Zn(OH)₂ “buds” gradually grew with the continuous supply of OH⁻ generated in reaction (3).¹⁶ In addition, driven by the differences in the concentration gradients in solution, Cu²⁺, Zn²⁺ and S₂O₃²⁻ could diffuse freely through the interface. As a result, the inner parts of the Cu nanowires gradually decayed as the reaction proceeded, leading to the formation of a tube-like nanostructure, as shown in Fig. 2d–e.¹⁷ (5) Finally, given sufficient time, most Cu nanowires were “consumed” by the continuous oxidation reaction, and with their collapse, ultrathin nanosheets were formed and dispersed in the solution. TEM element mapping and Energy dispersive X-ray spectroscopy (EDX) results also confirm that the as-prepared product is pure Zn(OH)₂ (Fig. 3 and Fig. S3). Although the lateral size of nanosheets could grow to a few hundred nanometers, it is interesting to note that the thickness of the final nanosheets was still in the narrow range of 3.5~4 nm (Fig. 1e). This could be attributed to the passivation effect of PVP, which could effectively hinder the growth of Zn(OH)₂ crystals in the perpendicular direction.^{11,18}

To construct smart porous nanosheets (SPNs) with specific cell-targeting capability, fluorescein (FITC)-or TAMRA-labeled sgc8 aptamer with an extra sulfhydryl group at the 3'-terminus (Table S1), which specifically binds to cell membrane (protein tyrosine kinase 7 (PTK7)) with high affinity, was chosen as a model in this study.¹⁹ The as-prepared PNs are hydrophilic because of the many hydroxyl (-OH) groups attached on the surface (Fig. S4a). Therefore, in order to improve the conjugation efficiency of the sgc8 aptamer, APTES was covalently bonded on the surface of PNs (size: 10 nm ~ 150 nm, Fig. S5) to produce APTES-PNs (Fig. S6). Moreover, the APTES equipped PNs with an active amino group (Fig. S4b), which could react with Sulfo-SMCC, a sulfhydryl- and amine-reactive crosslinker, to generate a maleimide-activated surface on the APTES-PNs. Finally, FITC-or TAMRA-labeled sgc8 aptamer with a sulfhydryl group was anchored onto the surface of the maleimide-activated APTES-PNs to obtain SPNs (Fig. S6–S7).^{20,21} These SPNs contained three components: porous nanosheets as a carrier, APTES and Sulfo-SMCC as linker, and an aptamer as the targeting moiety.

Compared with Ramos cells, CEM cells show high expression of PTK7, which can specifically interact with sgc8 aptamers with high affinity on the cell membrane.¹⁹ Therefore, Ramos cells were used as negative control in the following experiments. The selective binding affinity of SPNs was verified by flow cytometry. Fig. 4a shows a very strong signal shift for CEM cells targeted with SPNs, while no obvious signal shift was observed for Ramos cells (Fig. 4b and Fig. S8). Given their two-dimensional nature,²² we assumed that the large contact area and multivalent effect of the PNs would enhance binding ability, consequently endowing SPNs with a high potential for application in targeted drug delivery.

Having confirmed the targeting ability of SPNs as smart nanocarriers, we next investigated whether SPNs could be internalized into target cancer cells. Here, TAMRA was used as the fluorophore to label sgc8 because FITC is pH-sensitive, and its fluorescence could therefore be dramatically reduced in the acidic environment inside living cells.¹⁹ After incubating CEM cells with TAMRA-labeled SPNs at 37 °C for 4 hours, a strong red fluorescence was observed by confocal fluorescence microscopy (Fig. 4c), but no distinct red fluorescence was observed for Ramos cells (Fig. 4d). Fig. 4c shows red fluorescence inside CEM cells in overlapped images, indicating that the SPNs could enter the cells by receptor-mediated endocytosis (Fig. 5a).²³ As shown above, the properties displayed by the SPNs, i.e., selective targeting and internalization, make them a potential platform for targeted drug delivery.

Dox (doxorubicin) is one of the most utilized chemotherapeutic drugs for a wide spectrum of cancers.²⁴ It was therefore used here to investigate the drug delivery and release kinetics of SPNs. To start, Dox was noncovalently loaded onto SPNs by a simple mixing process (Fig. S9). Unbound Dox was later removed by centrifugation. Here, the low negative zeta potential (-55.6 mV, Fig. S10) and the unique 2D porous structures with large specific surface area for electrostatic interaction between Dox molecules and nanosheets contribute to the high loading efficiency of the Dox molecules. A kinetic study was then performed to determine the drug release efficiency of Dox-SPNs. The results, as shown in Fig. 5b, indicate the rapid release of Dox under lower pH conditions (pH5), with the percentage of released Dox reaching about 79% after incubation for 72 hours. However, slower release was observed at higher pH values. Only 55% and 18% of Dox was released within the same time at pH6 and pH7, respectively. By the acid sensitivity of SPN surface, a low pH environment can enlarge pore size,¹⁹ which, in turn, will accelerate the release of Dox from Dox-loaded SPNs. These results indicate that Dox-SPNs are highly efficient in releasing Dox in the acidic conditions of most cancer cells, especially those found in lysosomes (pH≈5).²⁵ **In addition, confocal microscopy examined the distribution of Dox delivered via SPNs in CEM cells** (Fig. S11), and results verified the Dox distributes a most of the cell range. MTS assay results showed that 96% of CEM and Ramos cells remained alive in the presence of SPNs, indicating the excellent biocompatibility of these SPNs (Fig. S12).

We further evaluated the selective cytotoxicity of Dox-SPNs against CEM and Ramos cells in culture medium. Free Dox and Dox-SPNs exhibited dose-dependent cytotoxicity to CEM, as well as Ramos, cells (Fig. 5c–d). Significant cytotoxicity of Dox delivered by the Dox-SPNs occurred in target CEM cells, but much less was observed in nontarget Ramos cells, in contrast to nonselective cytotoxicity of free Dox in both target and nontarget cells. Fig. 5c

shows a dramatic decrease of cell viability for CEM cells by Dox-SPNs, with one-fold lower IC_{50} of 0.56 μ M compared to free Dox (1.2 μ M). This can be attributed to the maximal internalization of Dox-SPNs and larger amount of Dox released. However, for Ramos cells, relatively weak drug potency of Dox-SPNs was observed (Fig. 5d). This can be attributed to the minimal internalization of Dox-SPNs to Ramos cells and the resulting smaller amount of Dox released from Dox-SPNs to binding buffer (pH 7.4) during the 4-hour cell treatment. By using Dox-SPNs instead of Dox alone, the results from the cytotoxicity assay demonstrate that Dox-SPNs could achieve better selectivity, more killing efficacy, and improved targeting specificity.

4 Conclusions

In summary, we have demonstrated a facile strategy to fabricate cell-targeted ultrathin porous $Zn(OH)_2$ nanosheets at room temperature. We demonstrated the engineering of these nanosheets by incorporation of aptamers, which showed pH-responsive delivery of anticancer drugs. The advantages of these ultrathin 2D nanosheets include good biocompatibility, efficient cellular uptake, and excellent drug release kinetics in acidic conditions, in particular the delivery of chemotherapeutic drugs, such as Dox. In addition, the fabrication strategy demonstrated in this work could provide a paradigm for the synthesis of more functional materials for biomedical applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Characterization of porous Zn(OH)_2 nanosheets: a) low-magnification and b) high-magnification TEM images; c) HRTEM image (the inset is SAED); (d) XRD patterns; (e) AFM image of typical Zn(OH)_2 nanosheets (scale bar: 200 nm).

Figure 2. Samples prepared from the synthesis for TEM at a) 0s; b) 30s; c) 1min; d) 3 min; e) 6 min; f) 10 min.

Figure 3.
TEM elemental mapping and elemental distribution scheme for porous Zn(OH)_2 nanosheets.

Figure 4.

Flow cytometry histograms which compare the binding of DNA library, free sgc8 aptamers and SPNs (400 nM) with (a) CEM cells and (b) Ramos cells. Confocal laser scanning microscopy images of the internalization of (c) CEM cells (target cells) and (d) Ramos cells (control cells) incubated with SPNs (TAMRA-labeled sgc8) at 37 °C for 4 hours.

Figure 5.

(a) Aptamer-based SPNs allow for targeted cancer chemotherapy. As a consequence of surface binding of sgc8 aptamers, Dox-SPNs specifically enter target cancer cells through receptor-mediated endocytosis. (b) Cumulative release of Dox from Dox-SPNs at different pH values. Cytotoxicity assay: viability of CEM cells (c) and Ramos cells (d) treated with free Dox and Dox-SPNs.

Scheme 1.
Schematic illustration of the formation of PNs.