Therapeutic Bioapplications with Layered Double Hydroxides Nanohybrids

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Cancer treatment using layered double hydroxides nanohybrids for bioimaging and therapy has been researched for enhanced clinical methods. In the case of tumor targeting and cancer therapy based on biomedical materials, synergistic combinations of therapy and bioimaging have been applied for the diagnosis and treatment of cancer at once

Keywords: layered double hydroxide ; nanohybrid ; drug delivery system

1. Introduction

In general, LDHs consist of positively charged brucite-like cationic layers with octahedral structure and unit cell of $M(OH)_6$ and negatively charged anionic species inside the interlayer space. Therefore, the chemical formula of LDHs is described as $[M^{2+}_{1-x}\ M^{3+}_{x}(OH)_2]^{x+}\ A^{n-}_{x/n}{\cdot}mH_2O$, where M^{2+} cations (Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, etc.) in the layer are partially substituted by M^{3+} cations (Al^{3+}, Cr^{3+}, Co^{3+}, Ca^{3+}, Gd^{3+}, etc.) [1][2][3][4][5][6]. As a result of the isomorphic substitution, the host layers generate the positive charges so that negatively charged guests, A^{n-} (Cl^-, NO_3^-, CO_3^{2-}, etc.), are intercalated in the interlayer region to neutralize the charges through electrostatic interaction.

The unique physicochemical properties (large anionic exchange capacity, biocompatibility, and controlled release of biomolecules and drugs in a pH-dependent manner, the hybrid systems), which are based on the LDHs, have also been considered promising inorganic materials for various bioapplications with medical functions [7][8][9][10][11][12][13].

2. Therapeutic Bioapplications with LDH Nanohybrids

2.1. Gene Therapy

Gene therapy is one of the most promising cancer treatment methods. It is normally difficult to deliver naked gene molecules into target tumors and cancer cells due to low stability, high toxicity, and poor delivery efficiency. In 1999, soluble inorganic LDH material was demonstrated as a gene reservoir for the first time by Choy et al. [14], and then it was realized as a promising gene delivery carrier by showing therapeutic efficacy in vitro experiments [15]. Such intercalation phenomena of genetic molecules including deoxyribonucleic acid (DNA) into LDH materials were theoretically proved by Thyveetil et al. [16]. Since those research results were published, much attention has been paid to the study of efficient gene delivery nanosystems through the bio-LDH nanohybrids with core–shell structures, the manipulation of particle size and/or the anisotropic properties of LDH nanosheets for enhancing the cellular uptake efficiency and reducing the cytotoxicity of LDH nanohybrids as well [17][18].

Although many attempts have been made to use LDHs for gene reservoir and cancer therapy, the successful demonstration of gene delivery remained a challenge in in vivo experiments. The In vivo target delivery system for small interference ribonucleic acid (siRNA) using LDH conjugated with folic acid (LDHFA), a cancer-overexpressing receptor-targeting ligand was reported for the first time by Park et al. in 2016 [19]. For the proof-of-principle, various weight ratio of Survivin siRNA (siSurvivin) with either LDH or LDHFA was loaded by a self-assembly process and the biological and chemical stabilities of each nanohybrids are confirmed by gel retardation assay. The prepared LDHFA/siSurvivin with a particle size of 100 nm completely suppressed the movement since the positively charged LDHFA are spontaneously assembled with negatively charged siRNA through electrostatic attraction. The reduction in mean sizes and tumor volumes was achieved by up to 66.4% on xenograft mice transfected with KB cells after treatment with the LDHFA/siSurvivin. Such a 3.0-fold higher anti-tumor effect was understood through the tumor-targeting effect by both the clathrin-mediated endocytosis of intrinsic LDH nanoparticles with enhanced permeability and retention (EPR) and folate receptor-mediated endocytosis. With this research, the engineered LDH nanohybrids have fruitfully accelerated the evolution of the next-generation gene delivery system for cancer treatment.

Furthermore, advanced gene delivery with the bioimaging function was proposed through lattice engineering technique and partial replacement of transition metal elements into the LDH layer [20][21]. For example, Manganese-based LDH nanoparticles (Mn-LDH) were reported for gene delivery systems together with bioimaging to obtain not only a therapeutic advantage but also T_1-weighted magnetic resonance imaging (MRI). The successful substitution of divalent Mn element inside the LDH layer was investigated by X-ray photoelectron spectroscopy (XPS) analysis. It was also confirmed that the Mn-LDH is able to produce a brighter MRI contrast agent for cancer imaging with an r_1 value of around 4.47 $mM^{-1}s^{-1}$, which is higher than that of commercial MRI contrast agents on the basis of Gd(III) complexes. Those Mn-LDH materials also showed low cytotoxicity and therapeutic function as well by delivering the cell-death siRNA (CD-siRNA) with Mn-LDH in order to kill the brain cancer cells more efficiently, where the high gene delivery efficacy with a simultaneous cancer diagnosis of LDH are proven for dual biofunctional performance.

2.2. Chemotherapy

Chemotherapy with LDHs is most often developed and utilized to treat cancer using many different anti-cancer drugs including methotrexate (MTX), doxorubicin (DOX), 5-fluorouracil (5-FU), dacarbazine (DAC), etc. [22][23][24][25]. Chemotherapy is a common powerful cancer treatment approach that uses chemicals to inhibit fast-growing cancer cells in the human body.

Since the MgAl-LDH intercalated with MTX drug was applied to chemotherapy in vitro cell-line experiment in 2004 [26], a series of MTX-LDH nanohybrids have been studied on drug contents, controlled release profile, toxicity, and improvement of colloidal stability as delivery systems. Li et al. reported SiO_2 dot-coated LDH for MTX carriers and showed effective inhibition in U2OS cell lines compared with MTX-LDH [27]. Furthermore, 5-FU/LDH nanohybrids were utilized with PEGylated hyaluronic acid for targeted drug delivery [28]. In addition to drug delivery, the attempts to apply chemotherapy with bioimaging were made through doping Gd on DOX/LDH-Au [29], coating MnO_2 on the surface of LDHs [30], using Manganese-iron LDH [31].

For the proof-of-concept of LDH-MTX, the X-ray diffraction pattern is typically investigated at first to confirm a series of (00l) reflective peaks, which indicates accommodation and well-oriented arrangement of MTX drug molecules inside the space of LDH materials. The image of high-resolution transmission electron microscopy of the MTX-LDH nanohybrids showed that the interlayer distance of the MTX-LDH nanohybrids was approximately 21.0 Å. The preparation method of MTX-LDH is well described by co-precipitation and subsequent hydrothermal treatment for chemically, structurally, and morphologically controlled production of drug-LDH nanohybrids.

Very recently, the anti-tumor effect of MTX-LDH was demonstrated in an orthotopic breast cancer model for potential preclinical trials [22]. The tumor growth inhibition was proven by comparing with MTX only, PBS, and LDH to know the efficiency of the therapeutic performance of MTX-LDH. The mean tumor volumes which are treated with the PBS and pristine LDH were 3374.6 mm^3 and 3638.5 mm^3 on day 32, respectively. In the case of MTX-LDH, the mean volume of the tumor was determined to be a numerical value of 627.8 mm^3, whereas the tumor volume treated with MTX only was 2447.6 mm^3. The MTX-LDH nanohybrids also showed an increased survival rate which was associated with tumor growth. The survival rate of the mice injected with MTX-LDH on days 0, 7, 14, 21, and 28 was 100% until day 32. In contrast, the mice treated with PBS, MTX only, and LDH showed survival rates of 16.7%, 33.3%, and 66.6%, respectively. In the case of the TUNEL assay to analyze induced apoptosis in vivo, few positive spots in tumors were detected in tumors treated with PBS or LDH. Quantification of the TUNEL-positive spots in tumors treated with MTX-LDH was 49.6%, whereas tumors treated with PBS, LDH, and MTX showed 0.8%, 0.6%, and 14.0% TUNEL-positive spots, respectively.

Due to the limited application for in vivo imaging of the MTX-LDH, certain contrasting agents can be accommodated to trace drug delivery carriers instead of fluorescent dyes. As an example study, single-photon emission computed tomography (SPECT) and positron emission tomography (PET) are applied to LDH nanohybrid for radioisotope (RI)-based tumor imaging. Among RI, Co-57 was utilized for contrasting agents with LDH (Co-57/LDH) [32].

The X-ray diffraction (XRD) demonstrated that the MTX was intercalated into LDH layers. Furthermore, the information that Co^{2+} moiety could increase the (006) peak intensity compared to MTX was demonstrated from the XRD patterns of Co@MTX-LDH. It was clearly confirmed that the particle size and shape of Co@MTX-LDH were around 150 nm with hexagonal plate-like morphology. X-ray absorption spectroscopy (XAS) was studied to confirm the partial substitution of Co^{2+} into the LDH layer of the MTX-LDH nanohybrid. At the Co K-edge, the X-ray absorption near-edge structure (XANES) spectra exhibited that the Co^{2+}@MTX-MgAlLDH is similar to the $Co^{2+}Al^{3+}$-LDH rather than Co^{2+}-MTX complex in accordance with edge position, white-line intensity, and overall spectral shape. The location incorporated Co^{2+} element into the hydroxide layer of MTX-LDH was observed by Fourier transform extended X-ray absorption fine structure (FT-

EXAFS) spectra as well. The overall spectral shapes of Co@MTX-LDH were also similar to those of CoAl-LDH; however, they were not comparable to that of the Co^{2+}-MTX complex in view of the local environment around the Co element.

The time-dependent in vitro cellular uptake of Co-57/LDH was researched in mouse colon carcinoma cell line (CT-57) and human hepatocellular carcinoma cell line (HepG2) by using a γ-counter to measure the radioactivity. The anticancer activity of the Co@MTX-LDH was further suggested indirectly by treating CT-26 cells with MTX-LDH, which strongly proved the substantial apoptosis and cancer-cell suppression after 48 h of incubation. The bioimaging function of Co@MTX-LDH was clearly evaluated in vivo SPECT/CT images on CT-26 xenografted mouse model, where the SPECT signal after 3 h became stronger than 1 h signal in tumor tissue and then disappeared after 6 h injection by metabolism. As Oh et al. found, the radioisotope-labeled drug-LDH nanohybrids are able to be potential candidates for dual-function biomedical nanomaterials with a high cancer-cell suppression effect and cancer imaging.

Other anti-cancer drugs have been applied for chemotherapy as well. The DOX is generally used as a DNA-targeting anti-cancer agent with a positive charge, while MTX and 5-FU are anionic anti-cancer drugs. Such DOX have limits to clinical use since the DOX can cause damage to the heart. To reduce cell toxicity, the DOX drug was intercalated into MgAl-LDH via a base-catalyzed co-precipitation reaction [25]. Typical plate morphology of LDH was observed in both TEM images of MgAl-LDH and DOX@MgAl-LDH. Additionally, the test of pH-responsive DOX drug release from DOX@MgAl-LDH was performed in PBS buffer with different acidic conditions until 12 h at 37 °C. After 12 h, the DOX release from the LDH matrix was around 7% at pH 7.4, 46% at pH 6.5 and 80% at pH 4.5, respectively. The DOX@MgAl-LDH had an impact on more effective cell viability than free DOX in both human hepatocarcinoma HepG2 and murine hepatocarcinoma H22 cell lines.

The in vivo anti-cancer activity of prepared DOX@MgAl-LDH was successfully further evaluated in H22 tumor-bearing mice at a DOX dose of 4 mg kg^{-1} once every other day for 5 times. The DOX@MgAl-LDH demonstrated a significant reduction in tumor growth compared to free DOX. Such inhibition rate of tumor growth of DOX@MgAl-LDH was 64%, whereas free DOX was 36%. Moreover, in vivo biodistribution was investigated to know the biological activity and behavior of LDH nanohybrid. The fluorescence intensity of Cy5-conjugated MgAl-LDH showed much stronger than that of free Cy5 in tumors over time. The DOX-loaded LDHs have been considered promising efficient chemotherapeutic drug delivery in tumor treatment.

A variety of LDH hybrid systems has still been developed in chemotherapy techniques; however, those LDH nanohybrids must be more studied to prove the safety and efficacy in orthotopic tumor xenografts with metastasis of various tumor types.

2.3. Immunotherapy

Cancer immunotherapy differs from other common cancer therapy strategies in its stimulation of the immune system. Immunotherapy targets usually antigens and antigen-presenting cells (APCs) to activate immunogenicity. Therefore, a selection of proper therapeutic agents must be a significant process for immunotherapy to target immune cells such as dendritic cells (DCs), B cells, T cells, macrophages, and natural killer (NK) cells [33][34][35][36].

Li et al. synthesized DNA/LDH nanohybrids to activate DCs [37]. They used MgAl-LDH with a ratio of 1 to 1 for the efficient loading of DNA. Their pcDNA3-ovalbumin (OVA)/LDH with CpG nanohybrids showed more effective immunotherapy results than that of CpG-free nanohybrids, more effective inhibition of B16-OVA melanoma tumor growth and higher survival rate of tumor-bearing mice than treated CpG free nanohybrids.

The immune systems of the human body make it difficult to efficiently deliver immunotherapeutic agents to immune cells. EL4 transfection cells (EG7-OVA) were chosen as the tumor model. LDH-based adjuvant-antigen nanohybrid with CpG and OVA (CO-LDH) was prepared by co-precipitation and surface-adsorption. The Mg_2Al-Cl-LDH displayed approximately 106 nm diameter and hexagonal-shaped morphology with sizes ranging from 40 to 200 nm. A bicinchoninic acid assay was performed to determine the OVA protein adsorption capacity of LDH nanoparticles.

2.4. Combination Therapy

Although each therapy mentioned above has a significant effect on cancer treatment, there have been many efforts to combine more than two cancer therapies for highly efficient clinical treatment.

A representative example of combination therapy is a co-delivery of chemotherapeutic drugs and genes such as 5-FU with Allstars Cell Death siRNA (CD-siRNA) using LDHs [38]. The 5-FU/LDH was synthesized by co-precipitation and ion

exchange, the CD-siRNA-5-FU/LDH nanocomplexes were then prepared via the electrostatic interaction between negatively charged siRNAs and positively charged 5-FU/LDH. The XRD patterns of pristine LDH demonstrated (00l) reflections, corresponding to (003) and (006) that revealed the formation of crystalline layered structures. In the case of 5-FU/LDH, (00l) reflection was a weaker and broader peak than pristine LDH because 5-FU was intercalated into the layer of pristine LDH. The shape of 5-FU/LDH nanohybrids displayed regular hexagonal morphology with sizes ranging from 50 to 150 nm. From the agarose gel retardation assay, the ability of binding siRNA for 5-FU/LDH was investigated, where 5-FU/LDH was adsorbed effectively with dsDNA when the mass ratio of 5-FU/LDH to dsDNA over 10:1.

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