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Aptamer- CaCO_3 nanostructures: A facile, pH-responsive, specific platform for targeted anticancer theranostics

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Abstract

Application of cancer theranostics depends on the development of multifunctional nanostructure platforms for accurate cell targeting and controlled drug release, imaging and therapy. In this work, a comprehensive, easily fabricated anticancer theranostic platform with high drug-loading capacity, termed aptamer-functionalized calcium carbonate (CaCO_3) nanostructure (apt-CCN), is reported. Flow cytometry and confocal fluorescence microscopy studies demonstrated that apt-CCNs can specifically bind to target cancer cells, but not to control cells, and that they possess highly efficient internalization to target cancer cells. This smart nanostructure selectively reaches the lysosomes through receptor-mediated endocytosis and is responsive to the relatively low lysosome pH (4.5–5.5), facilitating the release of doxorubicin. The apt-CCN platform offers targeted and efficient drug transport, as well as target-specific delivery of imaging agents for cancer diagnosis and therapy.

Keywords

aptamer; anticancer drugs; CaCO_3 nanostructure; targeted delivery; theranostics

Introduction

Targeted cancer therapy remains a major challenge because most anticancer drugs are unable to differentiate between diseased and healthy cells, leading to undesired side effects, low maximal tolerated dose, and reduced therapeutic efficacy.^{1–4} These challenges can be addressed through multifunctional nanostructure platforms with targeted and efficient drug transport, as well as target-specific delivery of imaging agents for cancer diagnosis and therapy.^{5–10}

Tumors are characterized by poor lymphatic drainage and abnormal vessel development. These factors promote the retention of macromolecules via the enhanced permeability and

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retention (EPR) effect, which allows drug-carrying nanostructures to specifically accumulate at the tumor site by the mechanism of passive targeting.⁶ On the other hand, according to Torchilin, active targeting “is based on the attachment of specific ligands to the surface of pharmaceutical carriers to recognize and bind pathological cells.”¹¹ Approaches involving aptamer molecules, which are integrated with the drug-loaded nanostructure, take advantage of overexpressed antigens or cancer-specific protein receptors on the target cells for specific ligand binding.

Among all targeting ligands, including folic acids, peptides, antibodies and aptamers, the latter are of particular interest and have been studied for active targeted transport of conventional chemotherapeutic drugs.^{12–15} Generated by a process called SELEX (Systematic Evolution of Ligands by Exponential Enrichment), aptamers are single-stranded oligonucleotides with unique intramolecular conformations, allowing target-specific recognition of cancer cells with equilibrium dissociation constants in the nanomolar-to-picomolar range.^{16,17} Aptamers offer many advantages over antibodies in chemical stability, structural flexibility, facile storage, and lack of immunogenicity in therapeutic applications.^{11–17} As a consequence of multivalent binding, aptamer-conjugated nanostructures have higher binding affinities to targeted cells than those of free aptamers (up to 170,000-fold).^{11–18} Furthermore, aptamers on the surface of nanostructures can be conjugated with luminescent groups for target cell imaging. More importantly, cell-surface aptamer binding may result in receptor-mediated internalization and highly differential uptake efficiency of the nanostructures in target cells instead of nontarget cells.^{11–13} Moreover, nanostructures with larger surface-to-volume ratios may have a higher capacity for drug loading than large particles, thereby permitting more drug molecules to be delivered into cancer cells.^{5,6}

Apart from effective drug transport, therapeutic efficacy depends on controlled release of drugs from a given theranostic platform, which is the governing principle here. To facilitate drug release inside cancer cells, smart nanostructures responsive to external stimuli, such as heat, light, or acidic conditions, have been developed.^{12,13,19,20} Examples include gold nanorods with heat-triggered release,²⁰ porous hollow magnetite nanoparticles (PHMNPs) with pH-triggered release,¹¹ and polymer nanogels with redox-triggered release.¹⁹ However, these strategies have intrinsic limitations that may compromise their application in cancer therapy, including: (i) complicated design, leading to laborious and costly preparation of nanomaterials as drug carriers; (ii) laborious and inefficient linkage between nanostructures and aptamers; and (iii) poor biodegradability, causing chronic accumulation of nanomaterials *in vivo*.

Calcium carbonate, a common inorganic material in nature, has shown potential as a smart carrier for anticancer drug delivery because of its excellent biocompatibility, biodegradability and pH-sensitive properties.^{21–23} For example, hierarchical hollow CaCO₃ particles with diameters of about 500 nm were used as an anticancer drug carrier for doxorubicin (DOX) and achieved localized drug release by their pH-sensitive structure.²⁴ However, in the absence of any targeting ligands, these particles were unable to differentiate between diseased and healthy cells. Lipid-CaCO₃ nanoparticles were reported to mediate the specific delivery of peptide EEEEpYFELV (EV) and then induce severe apoptotic effects in

lung cancer cells *in vitro*.²⁵ However, a time-consuming and labor-intensive multistep organic synthesis was required to introduce targeting ligands onto the surface of lipid-CaCO₃ nanoparticles. In addition, both types of functional CaCO₃ particles carried positive charges, leading to nonspecific electrostatic interactions with negatively charged cell membranes.

In the present study, we designed and engineered a smart aptamer-CaCO₃ nanostructure (apt-CCN) able to overcome these limitations. The apt-CCN has three major components: negatively charged porous CCN as an anticancer drug carrier, cross-linked avidin membrane as an active and universal linker between CCN and aptamer, and aptamer as the targeting moiety. The apt-CCNs have high negative surface charge density with a porous nanostructure that allows high loading capacity of therapeutics and bioimaging agents. The apt-CCNs exhibit low cytotoxicity and can specifically recognize target cancer cells. In addition, multiple aptamers on the surface of CCNs produce a multivalent effect, leading to enhanced specific binding and efficient internalization of CCNs by target cancer cells through receptor-mediated endocytosis. Importantly, the acidic environment of the lysosomes facilitates the release of DOX from the pH-sensitive CCNs. These features, which can be accomplished through a simple, universal preparation, all make the apt-CCN platform a promising tool for potential applications in cancer cell theranostics.

Results and Discussion

Strategy of apt-CCN for cancer cell targeting, imaging and therapy

As a proof-of-concept, negatively charged porous CCNs were prepared through a facile water-in-oil emulsion method^{25,30} (Figure 1). Briefly, a well-dispersed water-in-oil emulsion containing a low concentration of calcium chloride (30 mM) was used as the source of calcium. A high concentration of sodium carbonate (2.92 M) was then added as a precipitant to create porous CCNs with negative charges by adsorbed CO₃²⁻. CCN particles have smaller size and larger specific surface area, when compared to the reported calcium carbonate particles produced by a starch template method.²⁴ These properties allow high-capacity loading of therapeutics and imaging agents.

The loading of positively charged DOX and avidin on negatively charged CCNs was easily accomplished through electrostatic interaction. An avidin membrane was formed on the surface of CCNs by a cross-linking reaction using glutaraldehyde. Biotin-labeled aptamers, which were prepared through solid phase synthesis on an automatic DNA synthesizer, could also be linked to CCNs by avidin-biotin interaction. Specifically, aptamers were anchored to the CCNs by the high binding affinity ($K_d \approx 10^{-15}$ M) between biotin and avidin.²⁶ To preclude any interference with the specificity and affinity of aptamer binding, as well as internalization of CCNs into target cancer cells, a polythymine (T₁₀) spacer was inserted between the biotin and the aptamer sequences. The interchangeability of aptamer, combined with facile modification with various imaging agents, enables this platform to perform a wide range of applications in cancer theranostics.

Sgc8 aptamer can specifically bind to protein tyrosine kinase 7 (PTK 7), which is overexpressed on target CEM (human T-cell acute lymphocytic leukemia) cell membranes,

but not nontarget Ramos cells, with a nanomolar (nM) dissociation constant.¹⁶ Sgc8 aptamer can also be internalized into target cells through receptor-mediated endocytosis.¹⁶ The addition of sgc8 aptamers on the surface of CCNs provides for more specific nanoparticle-cancer cell surface interactions and enhanced cellular uptake compared to CCNs alone or CCNs modified with a peptide ligand. Since CCNs are stable at physiological pH, but soluble in an acidic environment, the low lysosomal pH condition will decompose the nanostructure, thereby accelerating the release of DOX from CCNs to kill target cancer cells (Figure 1).

Characterization and high drug-loading capacity of apt-CCNs

TEM images indicated that CCN building units of about 10 nm started to form and self-assembled into spherically shaped, porous nanoparticles (Figure 2B) with an average diameter of 288 ± 80 nm (Figure 2A), which is smaller than that of previously reported calcium carbonate microparticles (ca. 500 nm) formed by the starch template method.²⁴ In addition, there was no observable structural difference between apt-DOX-CCNs and CCNs. XRD spectra showed characteristics of crystalline vaterite (Figure S1). As quantified by measuring the fluorescence intensity of DOX at 591 nm, the drug payload was 44.5 ± 0.06 μ mol per one gram of CCN, which is better or comparable to reported results.^{24,25} This finding can be explained by the smaller size and higher specific surface area of CCNs for high-capacity loading. It is worth noting that the small size of CCNs should also facilitate internalization into target cancer cells in contrast to larger particles formed by the starch template method. In addition, although CCNs exhibit a negative zeta potential of -26.3 mV, the greatly enhanced zeta potential of DOX-CCNs clearly indicated that the negatively charged drug-loaded CCNs would have a strong electrostatic interaction with positively charged molecules like DOX and avidin (Figure 2C). Based on absorbance data, the avidin and aptamer payloads were 553 ± 16 nmol and 620 ± 39 nmol per one gram of CCN, respectively. The negatively charged apt-DOX-CCNs should also reduce the self-self interaction commonly found in nanostructures, as well as nonspecific interactions with the local environment, either of which can clog the bloodstream.

Selective recognition capability of apt-CCNs

To determine the capability of apt-CCNs to specifically recognize their cognate target cancer cells, the binding of apt-CCNs toward target and control cancer cells was studied using flow cytometry. Sgc8 aptamer, which specifically binds to the cell membrane receptor PTK 7¹⁶, was used as the targeting aptamer. CEM cells, which have a high expression of PTK 7, were chosen as the target cancer cells, whereas Ramos cells, with no PTK 7 expression on the membrane surface, were used as control cancer cells. In addition, a control DNA (ctl) was also used to evaluate the specificity of apt-CCNs. The ctl-DNA is another aptamer for targeting Ramos cells that does not bind to the CEM cells, but can render the particle surface properties more similar to the (Sgc 8) apt-CCNs. The aptamer and ctl DNA were labeled with fluorescein isothiocyanate (FITC) fluorophore. As shown in Figure 3, a strong green fluorescence was observed for CEM cells, but not for Ramos cells, demonstrating that apt-CCNs exhibited specific binding capability toward target cells. This is in agreement with the visualized fluorescence images (data not shown). Moreover, compared with aptamer alone, the fluorescence signal intensity of CEM cells treated with

apt-CCNs was greatly amplified as a result of the presence of multiple building blocks on each CCN. In contrast, ctl-CCNs did not cause any shift for CEM cells. Thus, the selective recognition capability of apt-CCNs to target CEM cells was demonstrated.

Specific and efficient internalization

Next, using confocal microscopy, the ability of apt-CCNs to selectively internalize into the target cancer cells, but not nontarget Ramos cells, was investigated. Here, 3'-TAMRA was used as the fluorophore because FITC is pH-sensitive, and its fluorescence can be dramatically reduced in an acidic environment such as that of lysosomes inside living cells. Aptamer sgc8 has previously been reported to specifically enter target cells through receptor-mediated endocytosis⁷, with the lysosome as its final destination inside living cells. Therefore, a colocalization study was carried out to determine if the apt-CCNs could also enter the lysosomes. After incubating CEM cells with apt-CCNs at 37°C for 2 h, a strong red fluorescence was observed by confocal fluorescence microscopy, but no distinct red fluorescence was seen for Ramos cells (Figure S2), thus demonstrating that apt-CCNs could specifically internalize into the target CEM cells. In addition, we also compared the internalization amount of DOX between free DOX and apt-DOX-CCNs into target CEM cells. It was observed that the fluorescence signal of apt-DOX-CCNs was much higher than that of free DOX. While incubating the cells with apt-CCNs, the lysosomes of CEM cells were labeled with a green dye known as LysoSensor™. Confocal fluorescence microscopy images show green fluorescence from the lysosensor in the FITC channel, while they show red fluorescence from either aptamer or the apt-CCN in the TAMRA channel (Figure 4). In addition, the images overlapped to produce a yellow fluorescence inside the CEM cells, indicating that both apt-CCNs and the aptamer could enter the cells and reside in their lysosomes. It was observed that apt-CCN's signal is higher than aptamer's. Based on their higher signal, apt-CCNs showed enhanced internalization efficiency over that of aptamer alone, most likely the result of multiple CCN building blocks for aptamer incorporation, leading, in turn, to multivalent binding. The selective internalization and the low pH inside lysosomes, coupled with the instability of apt-CCN structure in an acidic environment, should facilitate efficient release of the targeted anticancer drug.

In vitro selective cytotoxicity of apt-DOX-CCNs

To test the feasibility of apt-DOX-CCNs for anticancer therapy, we monitored their release kinetics in PBS buffer at different pH values (pH = 4.5, 5.5, 7.5), using fluorescence spectroscopy to measure DOX fluorescence. DOX release was shown to be pH-dependent, being significantly lower under physiological conditions (pH 7.5) than under acidic conditions (pH 4.5, 5.5) (Figure S3). The half-life, or $t_{1/2}$ (i.e., the time needed for the release of 50% of the maximal loading), was approximately 70 h, which is much longer than that of previous reports,¹¹ indicating that DOX loaded inside apt-CCNs is relatively stable under physiological conditions. At lower pH values, however, the $t_{1/2}$ was shorter (10 h at pH 4.5 and 30 h at pH 5.5), corresponding to approximately 75% release at pH 4.5 within the first 48 h. We attribute the observed pH-dependent release behavior to the low pH environments, which can break down CCN to accelerate the rate of DOX release from apt-DOX-CCNs.

Having demonstrated slow drug release under physiological conditions, but rapid release in acidic environments, such as that found in the lysosome,^{28,29} the selective cytotoxicity to target CEM cells was next evaluated. In this assay, both target CEM cells and nontarget Ramos cells were treated with free DOX and apt-DOX-CCNs, respectively. DOX is the most utilized anticancer drug against a range of neoplasms, including acute lymphoblastic and myeloblastic leukemias, as well as malignant lymphomas. It was confirmed that free DOX showed dose-dependent cytotoxicity in both CEM cells and Ramos cells (Figure 5). However, DOX-loaded apt-CCNs induced an efficient and dose-dependent cytotoxicity in targeted CEM cells, but not in nontarget Ramos cells (Figure 5). The cytotoxicity of DOX-loaded apt-CCNs was evaluated to be ~13.1-fold over that of free DOX at the same concentration of CEM cells (0.19 μ M) (Figure 6). In contrast, no obvious decrease of cell viability was observed when those cells were incubated with previously reported calcium carbonate microparticles, instead of free DOX, even at a concentration higher than 0.19 μ M.²⁴

The enhanced killing efficacy of apt-DOX-CCN can be attributed to efficient drug delivery and high internalization of apt-CCNs to target CEM cells. On the other hand, free DOX could not sufficiently permeate the cancer cell membrane during the 2 h treatment, and without aptamer-guided targeting, DOX-loaded ctrl-CCNs showed only negligible cytotoxicity with about 90% cell viability (Figure 6). It should be noted that apt-CCNs produced little effect on both cell lines, indicating the excellent biocompatibility of CCNs. Based on these results, it can be concluded that apt-DOX-CCN is a potentially promising anticancer theranostic platform, possessing such properties as excellent biocompatibility and biodegradability, enhanced internalization, bioimaging capability, and controlled drug release for highly selective cell killing ability.

Conclusions

In summary, the apt-CCN platform presents some remarkable features not previously reported for calcium carbonate nanoparticles, including: (1) high payload of toxic anticancer drug accommodated by the porosity of the CCN structure; (2) rapid release of anticancer drug under the acidic conditions of the lysosome, owing to the pH-sensitive property of CCN structure; (3) real-time signaling of CCN behavior at target cells *via* bioimaging agents coupled to apt-CCNs; (4) specific targeting, as demonstrated by selective cancer cell recognition, selective anticancer drug delivery, and selective *in vitro* cytotoxicity; and (5) decreased nonspecific killing of control cancer cells. While the use of CCNs takes advantage of passive targeting from the EPR effect, the additional use of target-specific aptamers drives active targeting for a significantly improved therapeutic efficacy over that of free anticancer drugs. Finally, the preparation method of apt-CCN is simple and universal, allowing for the anchoring of various therapeutic agents and aptamers in or on these nanostructures, and, hence, providing potential for wide application in cancer theranostics.

Experimental section

Preparation of apt-CCN

To form the CCNs, cyclohexane (7.5 mL), Triton X-100 (1.77 mL) and 1-hexanol (1.6 mL) were mixed thoroughly, followed by addition of 400 μ L of 30 mM calcium chloride solution to form a well-dispersed water-in-oil emulsion. Finally, the solution of sodium carbonate (40 μ L, 2.92 M) was added, and the mixture was kept under moderate stirring overnight before the CCNs were collected by centrifugation.

DOX in DMSO (7 μ L, 11.3 mM) was mixed with CCN (1 mg) dissolved in 400 μ L of water and then kept overnight until DOX-loaded CCNs were collected by centrifugation after washing three times with deionized water. Then, avidin solution (50 μ L, 5 mg/mL) was mixed with 1 mg of CCNs with or without DOX and incubated for 4 h, followed by centrifuging and rinsing three times to obtain avidin-loaded CCNs. Next, 50 wt% glutaraldehyde (10 μ L) was mixed with avidin-loaded CCNs (1 mg) in PBS buffer (pH = 7.5) for 2 h to prepare a cross-linked avidin membrane on the CCNs' surface. Finally, biotin-modified aptamer (7 μ L, 650 μ M) was reacted with 1 mg CCN for 4 h to prepare aptamer-modified and DOX-loaded CCNs.

Transmission electron microscopy (TEM) images were measured using a Hitachi H-7000 transmission electron microscope with a working voltage of 100 kV. Zeta potential (Zetaplus, Zeta Potential Analyser, Brookhaven Instruments Co.) was used to characterize the surface charge of the CCNs. X-ray diffraction patterns were recorded on a DX-2000 X-ray diffraction (XRD) instrument (Dandong, China).

Aptamer synthesis

The sgc8 aptamer and control DNA with biotin group or desired dye modifications were synthesized on an ABI 3400 DNA/RNA synthesizer (Applied Biosystems, Foster City, CA, USA). The aptamer with FITC labeling was used primarily for flow cytometry, while the aptamer with TAMRA labeling was used for confocal fluorescence microscopy. The aptamer without dye labeling was used for the cytotoxicity assay. Detailed sequence information is provided in the Supporting Information (Table S1). The aptamers with and without FITC labeling were deprotected in 3 mL of AMA solution (ammonium hydroxide: 40% aqueous methylamine = 1:1) at 65°C for 25 min. The TAMRA-labeled aptamers were deprotected in 3 mL of TAMRA deprotection solution (methanol:tert-butylamine:water = 1:1:2) at 65°C for 4 h. All deprotected sequences were precipitated by adding 250 μ L of 3 M NaCl and 6 mL of cold ethanol. Then the precipitated aptamers were collected by centrifugation and dissolved in 400 μ L of triethylammonium acetate (TEAA) for further purification by reversed-phase high-pressure liquid chromatography (ProStar, Varian, Walnut Creek, CA, USA) using a C18 column and acetonitrile-TEAA solvent. Finally, these aptamers were quantified by measuring their absorbance at 260 nm.

DOX release from apt-DOX-CCNs

DOX payload determination and release kinetics studies were carried out using dialysis membrane tubing (MWCO = 3500). The released DOX could cross the dialysis membrane,

but not the CCNs. In order to study the release kinetics of DOX from CCNs, 1 mg of DOX-loaded CCNs dispersed in 0.2 mL of PBS buffer in dialysis membrane tubing was equilibrated with 3 mL of PBS buffer with different pH values (pH = 4.5, 5.5, 7.5) at room temperature. Then, 100 μ L aliquots were removed for fluorescence measurements at predetermined time intervals. Each 100 μ L aliquot was returned to maintain the total volume and total DOX amount. The maximal loading of DOX was determined after dissolving the DOX-CCNs in HCl solution (pH = 1) by measuring the emission intensity at 591 nm.

Confocal Fluorescence Microscopy Imaging

All cellular fluorescence images were collected on a FV500-IX81 confocal microscope (Olympus America Inc., Melville, NY) with a 40 $\times$ oil immersion objective (NA = 1.40, Olympus, Melville, NY). A 488 nm argon laser was the excitation source for DOX or FITC dye, and a 543 nm argon laser was used for excitation of TAMRA dye.

Binding test

To demonstrate the specific targeting of aptamer and CCN toward different cell lines, fluorescence measurements were obtained on a FACScan cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA). Samples containing CEM or Ramos cells with a concentration of 1×10^6 cells/mL were incubated with the desired concentrations of aptamer, apt-CCN, or ctl-CCN on ice in a 200 μ L volume of binding buffer for 30 min. The cells were centrifuged, washed three times with 200 μ L of washing buffer, redispersed in 200 μ L of binding buffer, and subjected to flow cytometry analysis by counting 20000 events.

Internalization and colocalization study

To investigate the internalization of apt-CCNs into different cell lines, samples containing CEM or Ramos cells with a concentration of 1×10^6 cells/mL were incubated with the desired concentrations of aptamer or apt-CCNs at 37°C in a volume of 200 μ L binding buffer for 2 h in a 5% CO₂ atmosphere. The cells were then centrifuged, washed three times with 200 μ L washing buffer, resuspended in 200 μ L of binding buffer, and examined by confocal fluorescence microscopy. For the colocalization study, 10 μ M lysosensor was added for specific staining of the lysosomes of cancer cells during the last 0.5 h of a 2 h incubation.

Cytotoxicity assay

The cytotoxicities of apt-CCNs, apt-DOX-CCNs, ctl-DOX-CCNs and DOX only to CEM and Ramos cells were evaluated using the CellTiter 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 DOX only, apt-CCNs, apt-DOX-CCNs (0–2.5 μ M), or ctl-DOX-CCNs (0–2.5 μ M) in 50 μ L of fresh cell culture medium were added to the test wells. The resultant cell mixture was incubated at 37°C in a 5% CO₂ atmosphere for 2 h. Then, 75 μ L of cell culture medium was removed from the test wells 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 h. Finally, 20 μ L of CellTiter reagent was

added to each test well, and the 96-well plate was subjected to absorption measurement at 490 nm, using a VersaMax tunable microplate reader (Molecular Devices, Inc., Sunnyvale, CA).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Schematic illustration of apt-CCNs for targeted delivery of anticancer drug in theranostic applications. Construction of apt-CCN with four steps: fabrication of negatively charged porous CCN nanostructures; loading of DOX inside CCN by electrostatic interaction; loading and crosslinking of avidin on the surface of CCN; modification with aptamer on the cross-linked avidin membrane of the CCN. The apt-DOX-CCNs specifically enter target cancer cells through receptor-mediated endocytosis, followed by release of drugs inside the acidic lysosome by the pH-sensitive structure of CCNs, inducing cytotoxicity to the target cells. Target recognition was reported *via* the fluorescence of drugs loaded inside the apt-CCN platform.

Figure 2. Characterization of CCNs. TEM image (A, B) of CCNs. (C) Zeta potential of CCNs, DOX-CCNs and apt-DOX-CCNs.

Figure 3.

Mean fluorescence calculated from flow cytometry histograms to monitor the binding of apt-CCN with CEM cells (target cells) and Ramos cells (nontarget cells). Fluorescence of CEM cells treated with apt-CCNs was significantly stronger than that of aptamer alone, whereas no observable fluorescence was noted for Ramos cells.

Figure 4.

Colocalization study of (A) apt-CCNs and (B) aptamer with lysosensor in CEM cells (target cells). A strong TAMRA fluorescence signal was obtained from apt-CCNs over that of aptamer alone, in agreement with the flow cytometry results. Apt-CCNs show an improved internalization into target CEM cells.

Figure 5. Cytotoxicity assay of CEM cells (A, target cells) and Ramos cells (B, control cells) treated with apt-DOX-CCNs and free DOX. DOX concentration ranges from 0 to 0.6 μM .

Figure 6.

Cytotoxicity compared among CEM cells (target cells) treated with apt-DOX-CCNs, ctl-DOX-CCNs, apt-CCNs and DOX alone. All DOX concentrations were 0.19 μ M. All apt-CCN concentrations were 6.0 ng/mL.