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Self-assembled Hybrid Nanoparticles for Targeted Co-delivery of Two Drugs into Cancer Cells

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Abstract

A therapeutic aptamer-lipid-poly(lactide-co-glycolic acid) hybrid nanoparticle-based drug delivery system was prepared and characterized. The hybrid can co-deliver two different drugs with distinct solubility characteristics and different anticancer mechanisms to target cancer cells with high specificity and efficiency.

Nanobiotechnology, in particular methods based on nanoparticles, has made a significant contribution to the improvement of drug delivery in cancer therapy. Several types of nanoparticle-based drug delivery systems have been developed for this purpose.^{1–3} Among these, drug delivery systems based on poly(lactide-co-glycolic acid) nanoparticles (PLGA NPs) play an important role in cancer therapy. PLGA is a biocompatible and biodegradable polymer, which has been approved by the Food and Drug Administration (FDA), with an established clinical safety record.⁴ Based on their hydrophobic nature, PLGA nanoparticle-based drug delivery systems have been mainly used to carry hydrophobic drugs.^{5–7}

A new type delivery system with efficient hydrophobic drug loading capacity combined with good stability was recently introduced with the fabrication of PLGA-lecithin-polyethylene glycol (PEG) core-shell nanoparticles.^{8–14} PEG-passivated PLGA NPs are especially desirable because their extended systemic circulation time allows preferential

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accumulation at the tumor site.^{15–17} Furthermore, the surface of PLGA NPs can be modified with various molecular recognition moieties, including folates, peptides, antibodies and aptamers, for specific targeting to reduce side effects.^{11, 18–25} Among these ligands, aptamers generated from cell SELEX²⁶ exhibit both high specificity and high binding affinity. Furthermore, aptamers can be easily synthesized and chemically modified for molecular conjugation. This study demonstrates that the aptamer modified PLGA-lecithin-PEG nanoparticles can be prepared via one-step self-assembly and then used for targeted co-delivery of two types of drugs.

Because of their excellent antitumor efficiency against various solid tumors, doxorubicin hydrochloride (DOX) and paclitaxel (PTX) are commonly used as chemotherapeutic drugs.^{27, 28} Some clinical studies have shown that the incorporation of both DOX and PTX increases antitumor efficiency compared to the individual drugs^{28, 29} and these two drugs with different release rates still show a synergistic effect.²⁴ However, these drugs have distinct solubility characteristics and different anticancer mechanisms. DOX is a hydrophilic drug which binds to DNA and induces a series of biochemical events leading to apoptosis.³⁰ In contrast, PTX is a highly hydrophobic drug, which inhibits microtubule disassembly and promotes the formation of unusually stable microtubules, thereby causing cell apoptosis.^{31, 32} Thus, designing a simple co-delivery system is the key point for successful combinational therapy. However, by their differences in solubility, it is not easy to realize targeted co-delivery of DOX and PTX with one carrier.²⁷

To address these issues, we synthesized aptamer-coated PLGA hybrid nanoparticles with core-shell lipid-polymeric structures via simple nanoprecipitation and self-assembly (Scheme 1). Aptamer sgc8, which can bind human protein tyrosine kinase 7 (PTK7) overexpressed on target CEM (human T-cell acute lymphocytic leukemia) cell membranes, but not nontarget Ramos cells^{26, 33}, was chosen as a model ligand. The sgc8 aptamer was then designed to hybridize with a diacyllipid-modified DNA strand via a tail with repetitive 5'-GCA-3' sequences, where DOX can be intercalated by preferential interaction with double-stranded GC/CG regions.^{34, 35} In our previous study, the lipid-modified DNA was used for rapid and simple modification of hydrophobic particles.³⁶ In this new design, after self-assembly, hydrophobic PLGA with encapsulated hydrophobic PTX constitutes the core structure, while lecithin, DSPE-PEG and lipid-PEG-aptamer loading DOX form the hydrophilic shell. The average NP size measured by dynamic light scattering was 117 ± 5 nm. Transmission electron micrograph (TEM) images obtained with 1 mg/mL nanoparticles stained with uranyl acetate solution showed, as expected, spherically shaped nanoparticles with core-shell structures (Fig. S1).

To ensure that DOX was successfully loaded into the shell part of particles and that PTX was successfully encapsulated inside the core, both fluorescence spectroscopy and reversed-phase HPLC were used to characterize the NPs. FAM fluorescence (labeled in the DNA-formed shell part) showed a significant decrease in intensity after DOX drug loading as a consequence of fluorescence resonance energy transfer (FRET) from FAM to DOX (Fig. S2), indicating that DOX had been successfully loaded into the particle shells. The HPLC results indicate that NPs dissolved in acetonitrile eluted at the same time as pure PTX dissolved in the same solvent (Fig. S3), indicating that PTX had, indeed, been encapsulated

into the NPs. According to the HPLC peak area of pure PTX and the PTX peak area of the NPs, the PTX encapsulation efficiency was calculated to be 35% of the drug input weight, and the weight percentage of paclitaxel in the loaded PLGA was determined to be ~3.5%.

After successfully synthesizing these hybrid particles, we further verified the selective binding of the NPs to target CEM cells through flow cytometric analysis (Fig. 1) and confocal laser scanning microscopy (Fig. 2 a, b). Compared to control Ramos cells, the CEM cells showed a larger fluorescence signal shift and a higher TAMRA fluorescence (Fig. 2 a), indicating that the NPs could selectively interact with target cells. For efficient drug delivery, it is essential that NPs be internalized by diseased cells. Previous work showed that *sgc8* is specifically internalized by target CEM cells via endocytosis³⁷ and aptamer modified NPs are internalized into the target cells through ligand-receptor interaction-induced endocytosis.³⁸ Compared with the results for Ramos cells (37 °C; Fig. 2 d), a higher TAMRA fluorescence signal in the CEM cells showed that TAMRA-labeled NPs were selectively internalized into target CEM cells at physiological temperature (37 °C; Fig. 2 c).

To further visualize cell uptake of the NPs and to see the distribution of both core and shell using fluorescence microscopy, the hydrophobic dye TRITC-DHPE (excitation/emission=555 nm/580 nm) was encapsulated inside the FAM-labeled hybrid NPs. Figure S4 shows that PTK7-targeted TRITC DHPE-encapsulated NPs were selectively taken up by CEM cells, which express the PTK7 protein (Fig. S4 a, c), but not by Ramos cells, which do not express the PTK7 protein (Fig. S4 b, d). As can be seen, the TRITC-DHPE and FAM signals are colocalized, indicating that the NPs were taken up by CEM cells in their entirety. The small TRITC- DHPE and FAM signals from Ramos cells may represent a portion of free TRITC-DHPE and free FAM-labeled lipid-DNA released from the NPs during incubation or, alternatively, may represent nonspecific uptake of particles by the Ramos cells.

Finally, the selective cytotoxicity of anticancer drugs transported by hybrid NPs was tested. The DOX/PTX pair was used to demonstrate the synergistic effect of co-delivery of two chemotherapeutic drugs in the same nanoparticles. Having established that drugs could be selectively transported into target cells by NPs, the resultant cytotoxicity was evaluated by a 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay. In this assay, both target CEM cells and nontarget Ramos cells were treated with NPs with different drug loading (Fig. 3). As shown in Figure 3, co-delivery of DOX and PTX significantly reduced the targeted CEM cell viability, but Ramos cells were not affected. This demonstrated the synergistic cytotoxic efficacy of NPs carrying two drugs into target cells. In contrast, the lack of cytotoxicity of NPs without drug loading in either CEM or Ramos cells indicates the biocompatibility of these transporters under our experimental conditions.

In summary, biocompatible NPs with core-shell structures prepared by self-assembly from PLGA and lipid-modified DNA aptamer were employed as carriers for targeted co-delivery of DOX and PTX in antitumor therapy. The hydrophobic drug PTX can be easily encapsulated into the hydrophobic core through hydrophobic interaction, while hydrophilic

DOX can be loaded by intercalating into double-stranded DNA on the hydrophilic shell. The cellular uptake results show that the NPs can be selectively taken up by target CEM cancer cells. Using this concept, we have successfully co-delivered these two drugs with distinct solubility to target cancer cells. The cytotoxicity results show that this targeted co-delivery system selectively enhances antitumor efficacy. Other NPs of this type can be used for targeted co-delivery of other hydrophobic drugs and other intercalation drugs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Flow cytometry analysis of hybrid nanoparticles targeting cells. a) CEM (positive) cells were treated with 10 μ g nanoparticles (black line) for 30 min at 4 °C, along with the control unstained cells (red line). b) Ramos (negative) cells were used as a control cell line for target specificity.

Fig 2. Confocal laser scanning microscopy images of CEM and Ramos cells treated with 10 μg nanoparticles. a, b) Cells treated with NPs for 30 min at 4°C ; c, d) Cells treated with NPs for 2 h at 37°C. The scale bars correspond to 20 μm .

Fig 3.
Cytotoxicity assay of CEM and Ramos cells treated with nanoparticles with different drug loading.

Scheme 1.

Schematic illustration of self-assembled hybrid nanoparticles for targeted co-delivery of two different drugs into cancer cells. The nanoparticles have a core-shell structure: lecithin, DSPE-PEG and lipid-PEG-aptamer loading DOX form the hydrophilic shell; PLGA encapsulating PTX forms the hydrophobic core.