ATP-Responsive Hybrid DNA/Graphene Nanoassemblies for Anticancer Drug Delivery

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Statement of Purpose: Adenosine-5'-triphosphate (ATP), the primary energy molecule for cell function, is attracting extensive attentions, which can be used as a promising trigger for enhanced release of preloaded drugs form the carriers responding to the intracellular ATP concentration.[1,2] ATP has a very high concentration inside the cells (1-10 mM), which is much greater than that outside the cells (< 5 μM).[3] Such a pronounced gradient is the premise of design of the ATP-triggered intracellular drug delivery systems. However, the existing ATP-responsive anticancer drug delivery methods are often limited by complicated formulation design and relatively low loading capacity of drugs.

Methods: We developed a new ATP-responsive anticancer drug delivery strategy utilizing DNA-graphene crosslinked hybrid nanoaggregates as carriers (**Figure 1**). This nanoaggregate consists of graphene oxide (GO), two single-stranded DNA (ssDNA, denoted as DNA1 and DNA2) and ATP aptamer. The GO nanosheet is applied to carry doxorubicin (DOX), a model small-molecule anticancer drug, with a high loading efficiency *via* supramolecular π-π stacking between GO and DOX. The single-stranded DNA1 and DNA2 together with the ATP aptamer serve as the linkers upon hybridization for controlled assembly of the DNA-GO nanoaggregates (DNA-GA). Both DNA1 and DNA2 are composed of "head" and "tail" sequences, which are a target-specific sequence (the head one) complementary to the target ATP aptamer and a repeated GT sequence (the tail one) to facilitate the binding of DNA1 or DNA2 on the GO nanosheets, respectively. DNA1 and DNA2 are separately added to the DOX-loaded GO (DOX/GO) solution to form the DOX-loaded DNA-GO complex (DOX/DNA-GC) *via* strong interactions including van der Waals forces, π-π stacking and hydrogen bond, respectively. When the ATP aptamer is added in the mixture of DOX/DNA1-GC and DOX/DNA2-GC, the hybridization of the ATP aptamer with both DNA1 and DNA2 results in the assembly of the GO nanosheets to form the layered-structural DOX-loaded DNA-GO nanoaggregates (DOX/DNA-GA). Such aggregates, with an increased average size and a decreased specific surface area toward the surrounding medium, can effectively inhibit DOX release from the GO nanosheets. The ATP aptamer has been widely used for ATP detection based on its specific and stable binding to ATP. In the presence of ATP, the responsive formation of the ATP/ATP aptamer complex causes the dissociation of DOX/DNA-GA into DOX/DNA-GC that has a decreased size and an increased surface area exposing to the medium, which promotes the release of DOX in the environment with a high ATP concentration such as cytosol compared with that in the ATP-deficient extracellular fluid. This supports the development of a novel ATP-responsive platform for targeted on-demand delivery of anticancer drugs inside specific cells.

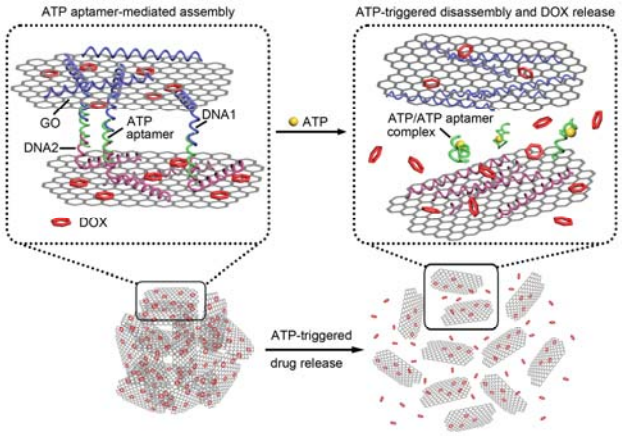

Figure 1. Schematic illustration of ATP-responsive DNA-GA for anticancer drug delivery.

Results: DNA-GC displayed a clear single-layer structure with the thickness of 2 nm, whereas the thickness of DNA-GA was 5-15 nm, with a well-packed layered structure. The DOX-loading capacity was about 10% as a mass ratio of DOX to GO. When DNA-GA was incubated with different concentrations of ATP and the release DOX was notably promoted, especially at high ATP concentrations comparable to the intracellular ATP level. The relative cellular uptake efficiency of DOX/DNA-GA was about 16%. After 2 h of incubation, DOX/DNA-GA was endocytosed by the cancer cells and evenly distributed within the cells. As the incubation time was prolonged to 6 h, DOX was efficiently released from GO, and the released DOX was specifically accumulated into the nuclei for subsequently inducing cytotoxicity.

Conclusions: In summary, we have developed a new ATP-mediated controlled drug release system comprised of a 2D nanomaterial (GO) assembled nanoaggretes crosslinked by ATP-responsive DNA strands. The straightforward formulation design, high loading capacity of drugs and capability of site-specifically promoting drug release render this formulation as a promising strategy for enhanced therapeutic efficacy in cancer treatment.

References:

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