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ATP mediated rolling circle amplification and opening DNA-gate for drug delivery to cell

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ABSTRACT

Here, we have developed a facile fluorometric system for the detection of adenosine triphosphate (ATP) by a rolling circle amplification (RCA) based on proximity ligation mediated amplification, and simultaneously achieved the release of the anticancer drug doxorubicin (DOX) through the mesoporous silicon system. Once the ATP molecule is present, the linker DNA will be released from the graphene oxide (GO) surface and hybridized to the template DNA of the GO surface joining with ligation enzyme. RCA reaction is followed by the addition of the phi29 DNA polymerase. The product of RCA reaction contains a base fragment complementary to the signal DNA, allowing the fluorescent oligonucleotide probe to be released from the GO surface and fluorescence is recovered. The strong fluorescence signal realized the sensitive detection of ATP. Gate DNA were modified to the surface of the mesoporous silica (MSN) by electrostatic attraction to encapsulate DOX. After the above-mentioned RCA process, its result that long DNA chain containing a base fragment complementary to gate DNA, would be hybridized to the gate DNA strand on the surface of MSN, which opened the MSN hole and released the drug DOX into cell for HeLa cell therapy. And the specificity to folate receptor overexpressed on cell surface was satisfactory which would be beneficial for cancer therapy.

1. Introduction

As the most basic energy substance, adenosine triphosphate (ATP) has an important position and influence in cell signaling and many intracellular reactions. Due to the significance of ATP in living systems, deep study of ATP aptamer was made for its configuration, sequence, functions and structure in the past period. ATP was utilized as a trigger for the controlled release of guests based on ATP-ATP aptamer recognition [1,2]. However, many similar studies have been reported that usually only one aptamer bond to one target molecule. The 1:1 binding ratio limits the amplification of signal and thus infects the sensitivity of the assays. Inspired by this, the emergence of rolling circle amplification (RCA) provides an exciting and new possibility to achieve the high sensitivity. RCA has also been shown to be adaptable to a wide variety of analytical schemes and devices [3–5].

Mesoporous silica-based drug delivery system (MSDS) offers a new idea to prolong bioavailability of drugs and improves bioactivity in cancer therapy [6]. To further heighten the therapeutic efficacy and biological specificity, valuable efforts have been bended to discover an active targeting preparing and stimuli-responsive MSDS [7], which particularly were gathered at the tumour site and stimulated to release drugs within tumour cells. These stimuli includes multifarious physiological factors, for example, glutathione [8,9], telomerase [10], pH [11], RNA [12] as well as external signals, such as temperature [13,14], light [15,16].

Inspired by these researches, ATP mediated proximity ligation for RCA amplification was designed for ATP detection in this paper. Graphene oxide (GO) modified folate (FA) was employed as a DNA transporter to target folate receptors (FR)-positive cells. So ATP mediated proximity ligation for RCA amplification was used for ATP detection. Besides, mesoporous silica nanoparticle modified FA (MSN/FA) as a drug cargo carried DOX through FR-positive cell membranes. And RCA product opened the door of MSN/FA to release the DOX for cell drug delivery.

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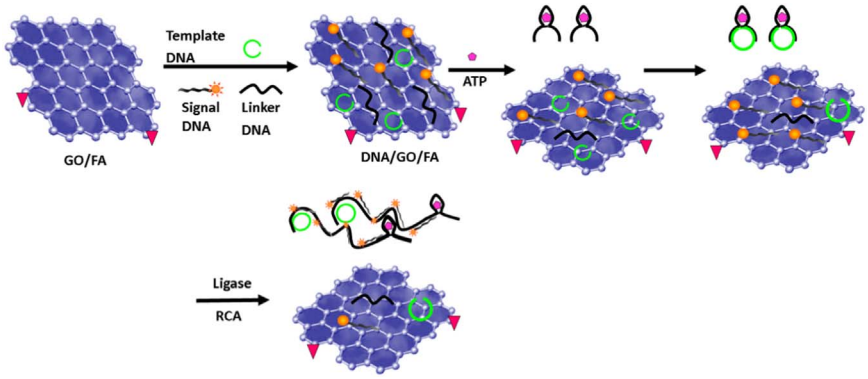


Scheme 1. ATP-controlled proximity ligation for amplification response to ATP.

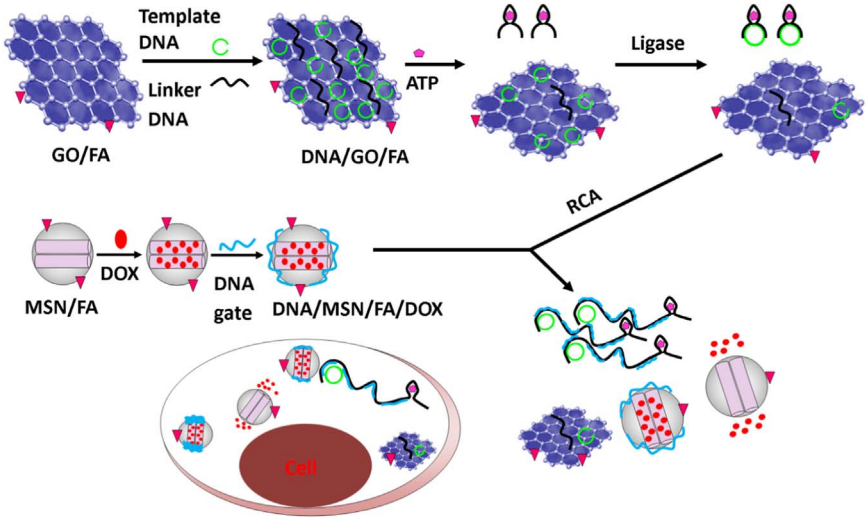
We fabricated an ATP-controlled proximity ligation for amplification to respond ATP and open DNA-gate on mesoporous silica for drug delivery. As an aptamer and a DNA-protective nanomaterial, graphene oxide (GO) has been proven that can vigorously combine with single-stranded DNA [17]. Template DNA, signal DNA and linker DNA were initially attached to the surface of GO (DNA/GO) because of the strong noncovalent combining capacity between nucleobases and GO. Furthermore, GO can quench the fluorescence of Cy3 conjugated with DNA3 (signal DNA) resulting from the outstanding electronic transference of GO. Template DNA contained ATP-binding domain. Upon the specific recognition of ATP, the template DNA formed an allosteric construction and was released from the GO surface. The allosteric template DNA could be circularized upon proximity ligation after hybridizing to linker DNA. RCA was initiated from the 3′-end of the template DNA, and the elongated sequence was hybridized with thousands of signal DNA3. The elongated sequence was frequently thousands of nucleotides in length, leading to a large amplification of the template DNA and thereby the fluorescent signals were generated (Scheme 1). To enhance the ability of specific recognition for cancerous cells with over-expression of folate receptors (FR), GO was modified with FA (GO/FA).

To expand its applications, GO/FA was used to carry DNA into cell. MSN modified with FA (MSN/FA) was blocked by a DNA-gate (DNA4), and DOX was packaged into MSN (DNA/MSN/FA/DOX) (Scheme 2). The RCA resultant was complementary with DNA4. By entrapping DOX in the mesopores of MSN/FA and covalently immobilizing quencher (BHQ) on the inner walls of the mesopores, fluorescent was turn-off. The hybridization of the complementary sequences between RCA resultant and DNA4 led to the detachment of the DNA4 from MSN/FA and the DOX was released to turn-on the fluorescence. In short, when challenging with DOX which as a drug molecules, after the RCA resultant and DNA/MSN/FA/DOX composite were taken in by cells, the DNA4 was detached due to the hybridization, eventually uncapping of pores to release DOX. The fluorescence was recovered. Drug was not only allowed to be contained within the nanovechicle due to this novel approach to drug delivery but leads to controlled release of the therapeutic agent to specifically induce apoptosis of cancer cells.

2. Materials and methods

2.1. Reagents and materials

DNA oligonucleotides (Table S1) used in this work were synthesized and purified by Sangon Biotech (Shanghai, China) Co., Ltd. Adenosine 5′-triphosphate (ATP), disodium salty trihydrate, doxorubicin hydrochloride (DOX), Folic acid (FA) and dNTPs were obtained from Bio Basic Inc.. E.coli DNA Ligase and phi29 DNA polymerase were bought from Takara Bio Inc. Tetraethylorthosilicate (TEOS), n-cetyltrimethylammoniumbromide (CTABr), 3-aminopropyltriethoxysilane (APTES) were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). Black hole quencher carboxylic acid (BHQ) was obtained from Biosearch Technologies, Inc. (USA). 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were purchased from Aladdin. Poly (ethylene glycol) (PEG) was purchased from Pierce. All other reagents were of analytical grade and used without further purification. All aqueous solutions were prepared using ultrapure water.



Scheme 2. ATP-controlled proximity ligation for amplification to open DNA-gate on mesoporous silica for drug delivery.