Colloids and Surfaces B: Biointerfaces 134 (2015) 40–46

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

# Aptamer-functionalized hydrogel as effective anti-cancer drugs delivery agents

Zonghua Wang*, Jianfei Xia, Feng Cai, Feifei Zhang, Min Yang, Sai Bi, Rijun Gui, Yanhui Li, Yanzhi Xia

*Collaborative Innovation Center for Marine Biomass Fiber Materials and Textiles, Laboratory of Fiber Materials and Modern Textile, The Growing Base for State Key Laboratory, College of Chemical Science and Engineering, Shandong Sino-Japanese Center for Collaborative Research of Carbon Nanomaterials, Qingdao University, Ningxia Road 308, Qingdao 266071, Shandong, China*

ARTICLE INFO

*Article history:*
Received 17 February 2015
Received in revised form 2 May 2015
Accepted 12 June 2015
Available online 22 June 2015

*Keywords:*
Hydrogel
Aptamer
Targeted release
Drug carrier

ABSTRACT

An aptamer-functionalized hydrogel has been developed, which can be regulated by the AS1411 aptamer with the sol–gel conversion. Also the hydrogel can be further utilized for the controlled encapsulation and release of the cancer drugs. Specially, the AS1411 initiates the hybridization of acrydite-modified oligonucleotides to form the hydrogels and the presence of the target protein nucleolin leads the gel to dissolve as a result of reducing the cross-linking density by competitive target-aptamer binding. Based on the rheology of hydrogels, it is possible to utilize this material for storing and releasing molecules. In this research, the cancer drug doxorubicin is encapsulated inside the gel during the formation of the hydrogel and then released in the presence of nucleolin. Further experiments are carried out to prove the specific recognition of target matter. In vitro researches confirm that the aptamer-functionalized hydrogels can be used as drug carriers in targeted therapy and other biotechnological applications.



## 1. Introduction

Strategies for target drug delivery systems with polymeric materials have been intensively investigated in recent years for the applications in such areas as biomedicine and tissue engineering [1,2]. Hydrogel is a three-dimensional polymer network, in which the individual hydrophilic polymer chains are connected by the physical or chemical bond. It has been one of the most appealing polymeric materials used for preparing sustained-release systems that have a great impact on pharmaceutical development and regenerative medicine [3,4]. As a drug carrier, hydrogel has very obvious advantages, such as good biocompatibility, no side-effects, good biodegradability performance [5,6] and so on. Also, smart hydrogels, which are susceptible to physicochemical changes in response to stimuli such as pH [7,8], light [9], molecular recognition [10–12], and temperature [13], have attracted significant attention. Although smart hydrogels can undergo conformational or chemical changes based on the changes of the surrounding medium, they still have problems such as low affinity and specificity.

Recently, nucleic acid aptamers have been applied to approach this challenge [14,15]. Aptamers, created by in vitro selection [16,17], are able to fold into three-dimensional structures with specific molecular binding motifs or specific binding protein. They have been used for molecular detection [18], cell detection [19,20] and targeted therapeutic applications [21–23]. It is reported that aptamers have been applied in the preparation of hydrogels for bioanalysis [24] and drug delivery due to the excellent molecular recognition properties. For example, a sustained-release polyacrylamide hydrogel system was achieved by using the anti-PDGF-BB aptamer to retain the protein drugs [25]. To slow the rate of drug release and achieve pulsatile release, the protein drugs were released with the aid of complementary oligonucleotide [26]. And also a photoresponsive DNA-cross-linked hydrogel was developed by grafting azobenzene-incorporated DNA into polymer to form a hydrogel, which encapsulated different loads in the sol state [27]. However, the controlled release of the drugs was achieved with relatively cumbersome method.

In this research, we present a straightforward strategy for target cancer cell recognition and target drug delivery by using an aptamer-functionalized hydrogel system. A 26-nucleotide guanosine-rich (G-rich) DNA sequence (AS1411), known as anti-nucleolin aptamer, is selected as the principal part of the target function. It can bind to the nucleolin receptors, the targets for

* Corresponding author. Tel.: +86 13853219173; fax: +86 53285950873.
*E-mail addresses:* wangzonghua@qdu.edu.cn, 13853219173@126.com (Z. Wang).

http://dx.doi.org/10.1016/j.colsurfb.2015.06.031
0927-7765/

delivery of various anti-cancer ligands and nanoparticles [28], which are normally seen over expressed on the tumor cells. The AS1411 initiates the hybridization of acrydite-modified oligonucleotides to form the hydrogels. Subsequently, the aptamer-cross-linked hydrogel was used as a drug carrier by encapsulating the cancer drug doxorubicin (dox) [29] to test the release properties. The presence of the target protein nucleolin leads the aptamer to melt and bind with nucleolin, thus to the drug release. It is indicated that the as-made aptamer-functionalized hydrogel is a promising biomaterial for drug loading and targeted release.

## 2. Reagents, instruments and methods

### 2.1. Reagents and instruments

DNA and the aptamer AS1411 were purchased from Sangon Biotech. The sequences of S-A, S-B and Linker-Apt were shown in Table 1. Dox was also purchased from Sangon Biotech. The target protein nucleolin was purchased from Abcam. Other reagents were purchased from Aladdin Industrial Corporation. Human plasma was taken from a healthy body and stored at −20 °C. All reagents were of analytical grade.

Transmission Electron Microscope (TEM) observations were performed on a JEM-2100F electron microscope. Ultraviolet visible (UV–vis) absorption and fluorescence spectra were recorded at room temperature on a Hitachi 3100 spectrophotometer and an F-96 spectrophotometer, respectively. Fluorescence spectra were obtained on a Model FluoroMax-4 spectrofluorometer (Horiba, Japan).

### 2.2. Hydrogel formation

1 mM solutions of acrydite-modified S-A and S-B were prepared separately in centrifuge tubes containing 4% acrylamide in 10 mM Tris–HCl buffer (pH 7.4, 200 mM NaCl). After vacuum desiccation for 10 min to remove air at 37 °C, 1.4% of freshly prepared initiator consisting of 0.5 mL $H_2O$, 0.05 g ammonium persulfate (APS) and catalyst consisting of 0.5 mL $H_2O$ and 25 μL *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) was added to this stock solution. The polymerization reaction also took place in the vacuum desiccator again for 15 min at 37 °C to form polymer strand A (PS-A) and polymer strand B (PS-B). After that, PS-A, PS-B and AS1411 were mixed in stoichiometric ratio (1:1:1), immediately forming the hydrogel.

### 2.3. Characterization of aptamer-functionalized hydrogel

For visualizing the gelling formation and transition, gold nanoparticles (AuNPs) were employed as indicating agents. Model drug AuNPs, modified with bovine serum albumin (BSA) to avoid aggregation due to high salt concentration, were added into the sol system and mixed well with PS-A and PS-B before adding AS1411. After introduction of aptamers, homogenous red hydrogel formed with AuNPs trapped inside and distributed well.

**Table 1**
Sequences of oligonucleotides.

| Name | Sequence |
|---|---|
| S-A | 5′-Acrydite-AAA ACA CCA CCG AGA T-3′ |
| S-B | 5′-Acrydite-AAA ATC ACA GAT GAG T-3′ |
| Linker-Apt | 5′-ACT CAT CTG TGA ATC TC***G GTG GTG GTG GTT GTG GTG GTG GTG G***-3′ |

AS1411 aptamer with underline.

### 2.4. The measured method and loading efficiency of dox

Initially, a standard absorbance curve was established using the UV–vis spectrophotometer from a series of standard dox solution with different concentrations at the wavelength of 480 nm. The exact amounts of drug within the hydrogel were determined with the aid of the standard absorbance curve. The relationship between concentration ($w$) and absorbance (Abs) could be described by the following equation:

$$w = 52.63 \times \mathrm{Abs} - 0.03 \quad (1)$$

correlation coefficient $R = 0.9927$.

The loading efficiency of dox was calculated in the following equation:

$$\Phi = \frac{(M_{0\mathrm{dox}} - M_{\mathrm{dox}})}{M_{\mathrm{carrier}}} \quad (2)$$

where $\Phi$ is the loading efficiency, $M_{0\mathrm{dox}}$ is the initial amount of dox, $M_{\mathrm{dox}}$ is the amount of dox in the upper layer, and $M_{\mathrm{dox}}$ could be obtained with certain volume multiplied by concentrations of dox and $M_{\mathrm{carrier}}$ is the total amount of drug carrier.

### 2.5. Loading dox on hydrogel

Before adding AS1411, 100 μM dox was added to the mixed solution with different pH to encapsulate drug molecules inside the hydrogels. The uncaptured molecules were removed by several centrifugation/washing steps. Also the drug loading capacity of the hydrogel at different temperature was investigated.

### 2.6. In vitro drug release response

The drug release of the synthesized hydrogel was studied at different pH 6.3 (the pH on the surface of cancer cells) and 7.4 (the physiological pH) at the physiological temperature of 37 °C. The dox loaded hydrogel solution was put into a centrifuge tube and then the configured targeted receptor protein solution was added. The absorbance and the fluorescence intensity of dox in the supernatant were determined in order to monitor the release kinetics of the dox. All the drug release experiments were repeated at least three times.

Also, the release of drug triggered by target nucleolin in the serum was investigated. Aliquots of 2.0 mL of Tris–HCl buffer (pH of 6.3) were added to 1.0 mL of human plasma. After vortexing for 3 min, the mixture was centrifuged at 10,000 rpm for 10 min, the supernatant was collected and used as the sample solution. Then the dox loaded hydrogel was put into the solution and also the configured targeted receptor protein solution was added. The absorbance of the dox in the supernatant was determined in order to monitor the release kinetics of the dox in biological media.

## 3. Results and discussion

### 3.1. The synthesis process and working principle of the aptamer-functionalized hydrogel

The synthesis process and the working principle of aptamer-functionalized hydrogel are schematically shown in Fig. 1. As shown in Fig. 1A, at first, two pieces of DNA, acrydite-modified oligonucleotides A (S-A) and acrydite-modified oligonucleotides B (S-B), are copolymerized with acrylamide to form linear DNA–polyacrylamide conjugates, PS-A and PS-B, respectively. When mixed in equal amounts, the polymers grafted with S-A and S-B are in transparent liquid form. The addition of AS1411