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Biodegradable graphene oxide and polyaptamer DNA hybrid hydrogels for implantable drug delivery

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ABSTRACT

Here, we report an injectable and biodegradable hydrogel based on drug-specific DNA polyaptamer networks using graphene oxide nanosheets as a physical crosslinker. Polyaptamer DNA and graphene oxide (PA-GO) hybrid hydrogels were constructed by single-step rolling-circle amplification (RCA) of a DNA template in the presence of GO nanosheets. The DNA template for RCA was designed to contain a kanamycin (Kan)-aptamer sequence for specific and efficient drug loading and a GO-binding 12-mer oligo A sequence. PA-GO hybrid hydrogels exhibited a bird's nest-like surface morphology, a swelling ratio of 657% at 2 h, and viscoelasticity suitable for injection and retention. PA-GO hydrogel was degraded by deoxyribonuclease I. PA-GO hybrid hydrogels specifically bound Kan, exhibiting a drug loading efficiency of 58.0% for Kan compared with 1.5% for gentamicin. Kan-loaded PA-GO (Kan/PA-GO) hybrid hydrogels exerted antibacterial activity against gram-negative (*Escherichia coli*) and gram-positive (*Staphylococcus aureus*) bacteria. In mice, subcutaneously injected, fluorescent Kan-loaded PA-GO hybrid hydrogel was retained at the injection site and degraded with time. Our findings suggest the potential of PA-GO hybrid hydrogels constructed by single-step RCA for biomedical applications.

1. Introduction

DNA has recently emerged as a new material for biomedical applications. One of biomedical applications using DNA is three-dimensional hydrogel. DNA hydrogels are natural, biocompatible and biodegradable [1]. DNA hydrogels have been studied as drug-delivery [2] and gene-delivery [3] vehicles, and as biosensors [4,5]. Although DNA hydrogels have attracted interest for possible biological and medical applications, the development of DNA-based hydrogels with designable properties is still in its early stages.

DNA hydrogels have been prepared by hybridization of nucleic acid units with chemical or DNA cross-linkers. Ethylene glycol diglycidyl ether [6,7] and azobenzene [8] have been used as DNA hydrogel chemical cross-linkers. DNA molecules with sticky ends also serve as cross-linkers to form three-dimensional DNA hydrogels [9,10].

Until now, little attention has been paid to the functionality and drug specificity of DNA molecules used for DNA hydrogel matrices. Recently, CpG sequence-containing DNA hydrogels were studied for their ability to confer immunomodulation [2]. A previous study reported DNA hydrogels designed to deliver doxorubicin [11]. In the study, drug loading onto DNA hydrogels was produced by non-specific electrostatic interactions between the positively charged drug molecule and negatively charged DNA, which may limit the application of hydrogels to positively charged drugs.

In this study, we propose an injectable DNA and graphene oxide (GO) hybrid hydrogel using single-stranded DNA (ssDNA) and polyaptamer sequences as a drug-specific hydrogel matrix and GO nanosheets as a physical cross-linker. Drug-specificity was provided to DNA hydrogel matrices by introducing a drug-specific polyaptamer sequence between GO-binding ssDNA. Drug-specific polyaptamer and GO-binding ssDNA sequences were obtained by rolling-circle amplification (RCA), and then cross-linked in the presence of GO.

We designed RCA template to contain 12-mer oligo A, for generation of 12-mer oligo thymine (oligo T) after amplification. GO has been reported to have greater binding affinity toward ssDNA of certain sequences than double-stranded DNA (dsDNA) [12,13].

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Moreover, the interaction of GO with ssDNA can vary depending on the nucleotide of the sequences. Adenine and thymine were found to have comparable graphene binding energies [14]. However, poly-A and poly-T were reported to differ in their three dimensional interaction with graphene. On graphene surfaces, poly-thymine formed a network, whereas poly-adenine formed spherical particles [15]. Therefore, we exploited oligo T sequences of RCA products to facilitate networking interaction with GO to form hydrogels. Here, we report the construction, rheology, drug specificity, and in vivo retention of polyaptamer DNA and graphene oxide hybrid (PA-GO) hydrogels using an antibiotic kanamycin (Kan) as a model drug.

2. Experimental section

2.1. Synthesis of GO nanosheets

GO nanosheets were prepared from graphite powder according to a modification of Hummer's method, as described previously [16]. In brief, graphite powder (0.5 g; Sigma–Aldrich, St. Louis, MO, USA) was added to cold H_2SO_4 (23 mL) and stirred on ice, after which $KMnO_4$ (3 g) and $NaNO_3$ (0.5 g) were added slowly. Following continuous stirring for 1 h at 35 °C, 46 mL of triple-distilled water (TDW) was added and the solution was incubated for 1 h at 90 °C. The reaction was stopped by adding 140 mL TDW and 10 mL 30% H_2O_2. The product was thoroughly washed and purified by repeated centrifugation of the resulting mixture. The purified product was suspended in TDW and sonicated for 2 h to exfoliate the agglomerated GO layers into single GO nanosheets. Unexfoliated GO was removed by centrifugation at 1600 × g for 10 min, and the supernatant containing GO nanosheets was collected.

2.2. Preparation of circular ssDNA template for RCA

Circular DNA templates for RCA were prepared using a 5′-phosphorylated linear ssDNA template. The phosphorylated linear ssDNA template was composed of an RCA primer-complimentary sequence for circle formation, a GO binding 12-mer oligo A sequence, and a Kan-binding aptamer to provide drug specificity. The sequence of the primer was 5′-GTA CGT GCT AGC CGA TAT GCC T-3′, and the sequence of the polyaptamer template was 5′-phosphate-ATC GGC TAG CAC GTA CAG AAC TAA AAA AAA AAA AGT CGG CTT AGC CTC AAC CCC CAA GGC AAA AAG GCA T-3′, where the underlined 21-mer is an aptamer sequence for interacting with Kan [17]. In some experiments, a control RCA template in which the Kan-specific aptamer sequence was replaced with a scrambled random sequence with the same number of bases (5′-phosphate-ATC GGC TAG CAC GTA CAG AAC TAA AAA AAA AAA AGT GCG CTC GGT CAT AGT ACG GAA GGC AAA AAG GCA T-3′). In experiments designed to test the role of 12-mer oligo A, a polyaptamer template lacking the 12-mer oligo A (5′-phosphate-ATC GGC TAG CAC GTA CAG AAC TGT CGG CTT AGC CTC AAC CCC CAA GGC AAA AAG GCA T-3′) was used. The linear ssDNA template and circular-form–inducing RCA primers were from Bioneer Co. (Daejeon, Republic of Korea). For RCA, 5′-phosphorylated linear ssDNA template (0.5 µM) was mixed with 0.5 µM of its corresponding primer in 1 mL of hybridization buffer (10 mM Tris–HCl, 1 mM EDTA, 100 mM NaCl, pH 8.0). The solution was thoroughly vortexed, heated for 5 min at 95 °C, and then allowed to cool slowly to 30 °C over a 3-h period. The primer-hybridized circular template (50 nM) was reacted with 25 µl (equivalent to 125 Weiss units) of T4 DNA ligase (Thermo Fisher Scientific Inc., Waltham, MA, USA) in 1 mL of T4 DNA ligase reaction buffer for 12 h at 4 °C. The solution was then incubated for 10 min at 70 °C to inactivate the ligase and stored at −20 °C until use.

2.3. RCA-mediated synthesis of PA-GO hybrid hydrogels

Kan-specific polyaptamer-based hydrogels with GO as a cross-linker were synthesized by amplifying circular RCA templates in the presence of GO nanosheets. Circular ssDNA template (10 nM) containing a 21-bp Kan-specific aptamer sequence or scrambled DNA sequence was treated with a 10-mM dNTP mix (Applied Biosystems, Seoul, Republic of Korea) and ϕ29 DNA polymerase (volume equivalent to 1% of the total reaction volume) in 1.2 mL of ϕ29 DNA polymerase reaction buffer (Thermo Fisher Scientific Inc.). The mixture was vortexed, and the RCA reaction was allowed to proceed at 30 °C for 0.5 h. GO (200 µg) was then added and, after vortexing the mixture for 30 s, the RCA reaction was continued for 12 h. The resulting PA-GO hybrid hydrogels or SC-GO hydrogels were kept at room temperature until use. In some experiments, various amounts of GO (100 µg, 200 µg, or 400 µg) were added to RCA reaction solutions. The RCA reaction was then continued for 12 h and photographs were taken to test the formation of the PA-GO hydrogels.

2.4. Scanning electron microscopy

The morphology of PA-GO hybrid hydrogels was observed by scanning electron microscopy (SEM). PA-GO hydrogels were spray-coated with Au/Pd. The coated samples were then placed onto a 12-mm diameter copper grid (FESEM Finder grid; Ted Pella Inc., Redding, CA, USA), and SEM images were obtained using a Field-Emission SEM/FIB (focused ion beam) system (Carl Zeiss Inc., Thornwood, NY, US).

2.5. Characterization of GO nanosheets

The morphologies of GO nanosheets were examined by transmission electron microscopy (TEM, JEM1010, Jeol Ltd, Tokyo, Japan). The GO nanosheets for TEM imaging were prepared by drying a droplet of the GO suspension onto a carbon grid (Agar Scientific Ltd., Stanstead, United Kingdom). The lateral sizes of GO nanosheets were measured using an ELSZ-1000 instrument (Photal, Osaka, Japan). The synthesized GO nanosheets (3 mg) were dispersed in 1 mL of triple distilled water and their lateral sizes were assessed using He–Ne laser (10 mW) light scattering.

2.6. Measurement of PA-GO hybrid hydrogel swelling ratios

The swelling ratio of hydrogels was calculated from their water uptake capacity. PA-GO hybrid hydrogel was dehydrated by freeze-drying (FreeZone 2.5; Labconco, MO, USA). The dehydrated hydrogel was then immersed in 2 mL of TDW and incubated at 37 °C. At predetermined time points, the hydrogel was removed, blotted with tissue paper, and weighed. The swelling ratio of the hydrogel was calculated from the following equation: Swelling ratio (%) = $[(W_s - W_i)/W_i] \times 100$, where W_i represents the weight of the dehydrated hydrogel and W_s the weight of the rehydrated hydrogel.

2.7. Rheology and X-ray diffraction pattern measurements

A rotational rheometer (DHR-1; TA Instruments Ltd., DE, USA) was used to measure rheological properties of the PA-GO hybrid hydrogel. To assess the rheological recovery of injectable PA-GO hydrogel, the rheological properties of PA-GO hydrogel were measured before and after extrusion through a 1-mL insulin syringe (BD Ultra-fine II insulin syringes; Becton Dickinson and Company, Franklin Lakes, NJ, USA). The hydrogels were placed on