Magnetic Graphitic Nanocapsules for Programmed DNA Fishing and Detection

Zhi-Ling Song, Xu-Hua Zhao, Wei-Na Liu, Ding Ding, Xia Bian, Hao Liang, Xiao-Bing Zhang, Zhuo Chen,* and Weihong Tan*

**Graphene** nanomaterials are typically used in biosensing applications, and they have been demonstrated as good fluorescence quenchers. While many conventional amplification platforms are available, developing new nanomaterials and establishing simple, enzyme-free and low-cost strategies for high sensitivity biosensing is still challenging. Therefore, in this work, a core–shell magnetic graphitic nanocapsule (MGN) material is synthesized and its capabilities for the detection of biomolecules are investigated. MGN combines the unique properties of graphene and magnetic particles into one simple and sensitive biosensing platform, which quenches around 98% of the dye fluorescence within minutes. Based on a programmed multipurpose DNA capturing and releasing strategy, the MGN sensing platform demonstrates an outstanding capacity to fish, enrich, and detect DNA. Target DNA molecules as low as 50 pM could be detected, which is 3-fold lower than the limit of detection commonly achieved by carbon nanotube and graphene-based fluorescent biosensors. Moreover, the MGN platform exhibits good sensing specificity against DNA mismatch tests. Overall, therefore, these magnetic graphitic nanocapsules demonstrate a promising tool for molecular disease diagnosis and biomedicine. This simple fishing and enrichment strategy may also be extended to other biological and environmental applications and systems.

1. Introduction

Graphene is a 2D nanomaterial composed of a honeycomb-like single layer of carbon atoms. It has distinct electrical,[1] and spectroscopic properties,[2] rich chemical and robust mechanical properties,[3] and it has emerged as one of the most extensively studied nanomaterials.[4] Numerous applications have been developed based on graphene, such as optical modulators,[5] integrated circuits,[6] transparent conducting electrodes,[7] ultracapacitors,[8] detection and diagnostic biodevices,[9] drug delivery,[10] and DNA sequencing.[11] Recently, researchers have utilized graphene as a nanoquencher and nanoscaffold for biomolecular sensing, and it has consistently exhibited considerable potential.[12] Graphene nanomaterials have been demonstrated as good fluorescence quenchers.[9,12] Fluorescence dye-labeled single-stranded DNA or aptamer molecules can noncovalently bind to graphene surfaces and can be used as a platform for the detection of different analytes. Various sensing strategies and systems have been explored, and they have all demonstrated good selectivity and sensitivity.[9,12] Nonetheless, the application of a graphene sensing platform in complicated matrices remains problematic. Thus, investigators are seeking ways to further improve selectivity and sensitivity, reduce detection time and lower the cost of new graphene-based biosensors. DNA enzymes, rolling circle and polymerase chain reaction (PCR) amplification strategies have thus far been developed, and they have improved the limit of detection (LOD) of DNA biosensors.[13–15] However, they are all relatively expensive enzyme-based sensing
platforms, and the enzyme is easily deactivated in complicated systems. Although rolling circle and PCR amplification can rapidly synthesize multiple copies of DNA or RNA molecules, DNA replication involves an elaborate process and requires specific instrumentation. Therefore, developing new nanomaterials and establishing simple, enzyme-free and low-cost strategies for high-sensitivity biosensing remain challenging.

Magnetic materials, especially magnetic nanoparticles, are of particular significance, and a wide variety of applications have been envisaged for them. Magnetic nanoparticles have been used in the separation and detection of DNA, cells and other biological entities therapeutic drug, gene and radionuclide delivery contrast agents for magnetic resonance molecular imaging and as heat mediators for the catabolism of tumors via hyperthermia applications. Combining the unique properties of magnetic nanoparticles and graphene will offer some attractive possibilities in biomedicine. Different magnetic nanoparticle carbon coating strategies have been developed, such as sonochemical procedures, pyrolysis of iron stearate, and vapour deposition approaches, and they have all demonstrated great potentials.

Herein, we developed a magnetic graphitic nanocapsule nanomaterial for nucleic acid detection. Combining the unique properties of graphene and magnetic nanoparticles, a core–shell magnetic graphitic nanocapsule material was first synthesized. Then, by means of π-stacking interactions between the nucleotide bases and the magnetic graphitic nanocapsule material was formed. DLS measurement further confirmed the average size of the MGN, and ζ-Potential measurements indicated the negative charge of the MGN which originated from the carboxyl groups present on the MGN surface (Figure S1 and S2 in the Supporting Information). The graphite shell was further proved through Raman spectroscopy (Figure 1B), showing a graphitic carbon (G) peak at ∼1590 cm⁻¹ and a disordered (D) peak at ∼1300 cm⁻¹. The high Raman D peak reflects the high strain of the graphite shells caused by the small size of the MGN. Figure 1C demonstrated the enrichment of MGN under an external magnetic field. The acid-treated MGN was suspended in water as a stable black solution (Figure 1C, left), which could remain stable for weeks. After magnetic manipulation, the MGNs were enriched around the sidewall of the vial (Figure 1C, right). The strong magnetic force overcame the gravitational force and attracted the MGN around the magnet, aligned according to the magnetic field gradient. This effectively demonstrated the detection and enrichment capabilities of MGN.

2. Results and Discussion

2.1. Characterization of Magnetic Graphitic Nanocapsules

Magnetic graphitic nanocapsules prepared after mixed-acid polishing display high water-solubility by the massive suspended hydroxyl and carboxyl groups present at the MGN surface. Transmission electron microscopy (TEM) was utilized to characterize MGN morphology and construction. As shown in Figure 1A, the average size of the MGN is around 29 nm, and its structure consisted of a metal nanocrystal core with several graphite shells. DLS measurement further confirmed the average size of the MGN, and ζ-Potential measurements indicated the negative charge of the MGN which originated from the carboxyl groups present on the MGN surface (Figure S1 and S2 in the Supporting Information). The graphite shell was further proved through Raman spectroscopy (Figure 1B), showing a graphitic carbon (G) peak at ~1590 cm⁻¹ and a disordered (D) peak at ~1300 cm⁻¹. The high Raman D peak reflects the high strain of the graphite shells caused by the small size of the MGN. Figure 1C demonstrated the enrichment of MGN under an external magnetic field. The acid-treated MGN was suspended in water as a stable black solution (Figure 1C, left), which could remain stable for weeks. After magnetic manipulation, the MGNs were enriched around the sidewall of the vial (Figure 1C, right). The strong magnetic force overcame the gravitational force and attracted the MGN around the magnet, aligned according to the magnetic field gradient. This effectively demonstrated the detection and enrichment capabilities of MGN.
on the MGN surfaces through π-stacking interactions between the nucleotide bases and the MGN outer graphitic shells. The fluorescence of FAM is quenched by the graphitic shell through fluorescent resonance energy transfer (FRET).[9,12] DNA1 was designed to have a dual function. One part of the DNA1 molecule is able to specifically recognize the target DNA (T1) molecules. The other part is used as an anchor to keep the whole molecule (with T1 molecules bound) on the MGN surface during the T1 fishing process. After the T1 molecules were fished by the DNA1-MGN complexes, a magnet was utilized to enrich and concentrate them, as illustrated in Scheme 1A. With respect to applications in clinical medicine, this strategy also permits the fishing of target molecules from a complicated sample solution like serum and urine, even at a very low concentration. After the fishing and enriching process, we next used a releasing DNA (P1) for programmed detection of the T1 DNA molecules. P1 molecule was designed to bind to the tail of the DNA1. When T1 bound to DNA1 and concentrated, the P1 was added to hybridize with the FAM-DNA1/T1 complex to form a complete duplex helix. The helix has weaker interaction with the MGN which results in creating distance between the FAM molecules and MGN and, thus, recovering fluorescence. Both T1 and P1 DNA must be present to activate the release of FAM-DNA1 from the MGN surface and recover fluorescence (Scheme 1B).

2.3. Fluorescence Quenching Efficiency of the Magnetic Graphitic Nanocapsules

Different tests were conducted to verify the quenching efficiency of the MGN nanomaterials. Increase in MGN concentration correlates with increased quenching of FAM-DNA1 fluorescence intensity (Figure 2A), until MGN concentration approached 0.09 mg/mL, when the fluorescence intensity of 50 nM FAM-DNA1 decreased sharply. As shown in Figure 2B, more than 98% of the fluorescence can be quenched when...

Figure 2. Quenching efficiency of the magnetic graphitic nanocapsule materials. (A) Fluorescence spectra of 50 nM FAM-DNA1 mixed with various concentrations of MGN in 20 mM Tris-HCl buffer (pH 7.0) for 30 min. (B) Quenching efficiency vs. concentration of MGN. (C) Fluorescence quenching of FAM-DNA1 (50 nM) in Tris-HCl buffer by MGN as a function of time. (D) Fluorescence restoration of FAM-DNA1/MGN complexes with (red triangles) and without (dark dots) T1 present in Tris-HCl buffer as a function of time. T1 enrichment factor is 5. FAM-DNA1 concentration: 50 nM. Initial T1 concentration: 10 nm. P1 concentration: 100 nM. Excitation wavelength: 494 nm, and emission wavelength: 518 nm.
Figure 3. MGN for fishing and enrichment of DNA molecules. (A) Fluorescence-emission spectra of DNA1 (final concentration of 50 nM) under different conditions: a) MGN+DNA1, without enrichment; b) MGN+DNA1+T1 (10 nM), without enrichment; c) MGN+DNA1, with 5 times enrichment; d) MGN+DNA1+T1 (10 nM), with 5 times enrichment. (B) F/F0 (relative fluorescence intensity, where F0 and F are the fluorescence intensity without and with the presence of 10 nM T1, respectively) with different enrichment factors.

the concentration of MGN is higher than 0.09 mg/mL. FAM-DNA1 interacts with MGN through π-stacking interactions between the nucleotide bases and the MGN outer shells to guarantee the close proximity of FAM with MGN. The MGN collectively quenched FAM fluorescence through energy-transfer or electron-transfer processes. A typical MGN possesses an absorption spectrum that overlaps the photoluminescence spectra of FAM, allowing resonant energy transfer or electron-transfer processes. A typical MGN possesses an absorption spectrum that overlaps the photoluminescence spectra of FAM, allowing resonant energy transfer (RET) to occur. MGN, which contains highly delocalized π electrons on the outer graphitic shell, acts as the acceptor in the energy-transfer process, and photoexcited fluorophores act as the donors to transfer energy to the ground-state MGN acceptor.\[12\]

We further investigated the quenching efficiency of the MGN as a function of time. Figure 2C shows the changes of fluorescence intensity of 50 nM FAM-DNA1 relative to time after addition of 0.09 mg/mL MGN. Within 2 min, the fluorescence intensity dropped to around 80%. After 15 min, more than 98% fluorescence had been quenched by MGN, and no significant fluorescence recovery was observed, indicating good quenching efficiency. Figure 2D shows the fluorescence recovery curve with and without 10 nM target DNA T1. Without target, most of the fluorescence was still quenched, and only a slight increase of intensity occurred as a result of the background from the addition of PI releasing DNAs. However, with the addition of T1 DNA, an increase in fluorescence recovery was observed. After 1.5 h of incubation, the recovered fluorescence intensity reached a plateau, and no obvious further increases were observed, indicating equilibrium of the hybridization between the FAM-DNA1 and T1, PI molecules. The hybridized DNA formed a duplex helix which had weaker interaction with the MGN. This resulted in separating the FAM molecules from the MGN, and a strong fluorescence was recovered.

2.4. MGN for Fishing and Enrichment of DNA Molecules

We next investigated the concentration capacity of MGN nanomaterials. Figure 3 shows the detection of 10 nM T1 DNA molecules through the programmed MGN strategy. Different enrichment factors were investigated. As shown in Figure 3A, curves a and b were the fluorescence intensity with and without 10 nM T1 present, respectively, when no magnet was utilized. On the other hand, curves c and d were the fluorescence change after 5 times enrichment and fishing with the magnet. The intensity of curve d significantly increased when compared to that of curve b. Interestingly, the fluorescence intensities of curve c were slightly lower than those of curve a. This decrease in background intensity after magnetic fishing and enrichment contributed to the improvement in sensing sensitivity. The free FAM-DNA1 molecules in the sample solution were excluded during the enrichment step. By reducing the background signal, such exclusion of free FAM-DNA1 molecules was speculated to be the mechanism underlying the lower fluorescence intensity. Systematic investigation of enrichment times and DNA detection was conducted, and the findings are reported in Figure 3B. F/F0 was the relative fluorescence where F0 and F correspond to the fluorescence intensity without and with target T1 molecules, respectively. Without enrichment (1 time) and only magnetic fishing, the relative fluorescence F/F0 was similar to that without enrichment. Fishing and enriching of T1 molecules by 2 times and 5 times resulted in significantly increasing the F/F0 value. More than 3 times relative fluorescence improved after 5 times enrichment. This MGN sensing strategy was simple, and no enzyme was needed. This also indicated the potential of using the programmed MGN for sensitive detection of molecules from a complicated sample solution. Higher relative fluorescence improvement could certainly be achieved through optimization of the magnetic enriching operation process, and this will be the direction of our future studies.

2.5. DNA Detection with Programmed MGN Platform

Nucleic acid detection was utilized as the model system to demonstrate the sensing capabilities of the MGN nanomaterials. The effect of the concentration ratio of PI over DNA1
on the sensing response was investigated. When the concentration of the releasing DNA P1 was 2 times higher than that of the capturing DNA1, the programmed MGN sensing platform demonstrated its highest sensitivity (Figure 3S in Supporting Information). The programmed MGN DNA fishing and detection assay is sensitive and specific.

Figure 4A shows the fluorescence emission spectra of the FAM-DNA1/MGN complex upon adding target T1 DNA molecules at different concentrations and releasing with P1 molecules. A dramatic increase in FAM fluorescence with the increasing concentration of T1 molecules was observed. All the tests were done under an enrichment of 5 times target molecules. The plot of fluorescence intensity with T1 concentration from 0 to 50 nM is shown in Figure 4B. The linear range is from 50–1000 pM (Figure 4B inset). The linear equation is \( y = 1.11 + 0.0018x \), where \( y \) is the relative fluorescence \( F/F_0 \), and \( x \) is the concentration of T1 (regression coefficient \( R = 0.994 \)). The detection limit was 50 pM, which is 3-fold lower than LODs commonly reported for CNT- and graphene-based fluorescent biosensors. \([9,12,25]\) Several possible reasons may explain this excellent performance. First, MGN is a superquencher and possesses long-term nanoscale RET properties between MGN and FAM molecules. The high fluorescence quenching efficiency leads to low background and results in high sensitivity. Second, through our programmed MGN fishing and enriching strategies, we reduced the background and increased the positive signals, which further contributed to the high sensitivity of the MGN sensing assay. Third, the folding of DNA will be more favorable on the MGN surface, as opposed to a 1D nanotube surface. Therefore, the DNA on the MGN surface is more sensitive to the target molecules vis-à-vis fluorescence responses.

### 2.6. Specificity of MGN DNA Sensor

The programmed MGN sensing assay is also specific. To evaluate this property, we challenged the system with nucleotide polymorphisms, which represent promising disease markers because they are stably inherited sequence variations in the human genome. Both single-base and double-base mismatches have been investigated. In a typical experiment, the FAM-DNA1/MGN complex was incubated with 10 nM target T1 DNA and single-base mismatch M1, M2 or double-base mismatch M3 DNA molecules, respectively, then released with P1 DNA molecules. The mismatched base of M1 and M2 were G and A, respectively. As shown in Figure 5, remarkably higher fluorescence was observed with the complementary target T1 than with the single- or double-mismatch nucleotides. These results clearly demonstrate the high specificity of our programmed MGN sensing assay. Double-base mismatches indicated better differentiation than the single-base mismatches. For maximum efficiency and selectivity, more effort should be directed toward optimizing hybridization time, designing a hairpin capturing DNA structure, or further amplifying the sensing with other strategies.

### 3. Conclusion

In summary, we demonstrated the utilization of magnetic graphitic nanocapsule nanomaterials for programmed DNA
fishing and detection. The MGN combined the unique properties of graphene and magnetic particles. Synthesized MGN can be enriched by an external magnet and aligned according to the magnetic field gradient. The MGN exhibited good quenching efficiency and could quench around 98% of the fluorescence of the noncovalently bound FAM-DNA1 within minutes through energy-transfer or electron-transfer processes. After the designed FAM-DNA1 functionalization, target DNA could be released and enriched from the original sample solution with the MGN platform. Different enriching factors were investigated, and all showed high potential for DNA fishing and detection. It is only in the presence of releasing DNA P1 that the sensing platform operates, with FAM-DNA1, T1 and P1 hybridizing to form a duplex helix. The helix has weaker interaction with the MGN which results in separating the FAM molecules from the MGN and, thus, recovering fluorescence. The programmed MGN DNA fishing and detection assay was demonstrated to be sensitive and specific. With this simple, nonenzymatic MGN sensing strategy, target DNA molecules down to 50 pM can be detected, which is 3-fold lower than the limits of detection reported for common CNT and graphene-based fluorescent biosensors. Moreover, DNA mismatch tests were investigated, and the MGN platform exhibited good sensing specificity for both single and double mismatches. This simple fishing and enrichment strategy may also be extended to other complicated clinical and environmental applications and systems. The reliable, highly sensitive, and selective MGN platform was demonstrated to be a promising tool for molecular disease diagnosis and biomedicine.

4. Experimental Section

Materials and Chemicals: All DNA sequences were synthesized by Shanghai Biotech (China). Sequences of oligonucleotide probes used in this work are as follows: Capturing DNA1: 5′-FAM-TTT GTC CAG GGT CTC TAT ATT GTG CAA GG-3′; releasing DNA P1: 5′-CTT TGC ACA ATA-3′; single-base mismatch DNA M1: 5′-TAG AGA CCC TGG ACA AA-3′; single-base mismatch DNA M2: 5′-TAG AGA CCC TGG TCA AA-3′; double-base mismatch DNA M3: 5′-TAG ACA CCC TGG CCA AA-3′. Tris-HCl buffer solutions (20 mM Tris, 100 mM NaCl, and 2 mM MgCl2, pH 7.4) were used as working solutions. Magnet was purchased from Changsha Chemical Reagents Company (Changsha, China). All other chemicals of analytical reagent grade were obtained from Changsha Chemical Reagents Company (Changsha, China) and used as received without further purification. Doubly distilled water (resistance >18 MΩ cm−1) was used throughout all experiments.

Preparation of Magnetic Graphitic Nanocapsules: Magnetic graphitic nanocapsule was produced in a chemical vapor deposition (CVD) system. First, fumed silica (1.00 g, Aladdin) was impregnated with Co(NO3)2·6H2O (0.210 g) in methanol and sonicated for 1 h. Then the methanol was removed, the mixture was dried at 80 °C, and the powder was ground. Typically, 0.50 g of the powder was used for methane CVD in a tube furnace. The sample grew with a methane flow of 200 cm3 min−1 for 6 min. After growth, the sample was etched with 10% HF in H2O (80%) and ethanol (10%) to dissolve the silica. The graphite-coated magnetic nanoparticle solid product was then washed thoroughly and collected through centrifugation. Before DNA detection, a solution of sulfuric and nitric acid was utilized to polish the as-grown MGN and solubilize it in water.

DNA Fishing and Sensing: DNA1 was prepared as 10 nM in 20 mM Tris-HCl buffer (pH 7.4, containing 100 mM NaCl and 2 mM MgCl2) and mixed with 0.09 mg/mL MGN for 30 min prior to the addition of target T1. Then T1 was added to the prepared DNA1-MGN solution. The final T1 concentration in detection solution ranged from 50 pM to 50 nM. After allowing hybridization for about 15 min at 37 °C, the mixture was exposed to external magnetization for 2 h. The fished and enriched T1 molecules on the MGN were collected. Then a solution of 100 nM P1 was added to the collected MGN and incubated for 1.5 h at room temperature to complete the hybridization. Finally, the fluorescence of the mixture was detected with a fluorescence analyzer.

Characterization: We characterized the MGN by transmission electron microscopy (TEM, JEOL 3010, operated at 120 to 200 kV) and Raman spectroscopy (Horiba Jobin Yvon LabRAM-010 Raman microscope with 632 nm He-Ne laser excitation). All fluorescence spectra were collected with a Hitachi F-7000 spectrophotometer equipped with a xenon lamp excitation source. Dynamic light scattering (DLS) was measured on the Malvern Zetasizer Nano ZS90 (Malvern Instruments, Ltd., Worcestershire, UK).

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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