Fast DNA Extraction with Polyacrylamide Microspheres for Polymerase Chain Reaction Detection

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ABSTRACT: The fast and cost-effective DNA extraction is critical for all DNA-based detections. Here, we fabricated a new kind of polyacrylamide microsphere (PAMMP) in various sizes with two methods, spot polymerization (large size but low yield) and modified inverse microemulsion polymerization (small size but high yield). The fabricated PAMMPs have strong autofluorescence (fPAMMPs), including both visible fluorescence (VF) and near-infrared fluorescence (NIRF), which can remain very stable in various stringent conditions including strong acid and alkali and high temperature. The fabricated fPAMMPs were also highly positively charged, which could be used to effectively capture various biomolecules such as IRDye 800-labeled streptavidin and DNA. We thus developed a new method for rapid extraction (3−5 min) of DNA from various samples including bacteria, mammalian cells, plant and animal solid tissues, and human blood plasma using fPAMMPs. Moreover, the DNA captured on fPAMMPs (fPAMMP@DNA) could be effectively detected by both normal and quantitative PCR amplifications. Finally, we showed that NaBH₄ treatment removed autofluorescence in fPAMMPs (PAMMPs), which could also be applied to DNA extraction and PCR detection. In conclusion, we here fabricated new kinds of fPAMMPs and PAMMPs, developed a new rapid DNA extraction method, and demonstrated their useful applications in PCR detection.

INTRODUCTION

As a powerful tool for DNA detection, polymerase chain reaction (PCR) amplification is widely used in basic biological research, medical diagnostics, forensic science, agricultural science, and other fields. In the application of these fields, nucleic acid extraction is inevitable in PCR detection. However, extracting DNA from a sample is a complicated and speed-limited task that requires specially trained technicians to perform, involving many processing steps. In addition, some very specialized materials (such as filtering tubes or magnetic beads) and reagents (such as component-complicated solutions of lysis, binding, washing, and elution) are needed. For clinical technicians who need to test many samples every day, DNA purification is a tedious job. Therefore, a method that can extract DNA more simply and quickly is still demanded.

Recent publications report rapid nucleic acid extraction methods based on different types of solid substrates including paper, alumina membrane, silica, and cellulose. These methods simplify the nucleic acid extraction process by directly amplifying from a solid matrix and do not require a separate nucleic acid elution step. Although a solid matrix, although a solid matrix.

Received: March 17, 2020
Accepted: May 25, 2020
Published: June 4, 2020

Finally, we showed that NaBH₄ treatment removed autofluorescence in fPAMMPs (PAMMPs), which could also be applied to DNA extraction and PCR detection. In conclusion, we here fabricated new kinds of fPAMMPs and PAMMPs, developed a new rapid DNA extraction method, and demonstrated their useful applications in PCR detection.
synthesized, polyacrylamide/sodium alginate composite microspheres with a double-network structure were prepared, and their absorption capacity for dye was tested. The polyacrylamide/chitosan composite microspheres have been used for controlled delivery of anti-inflammatory drug. Functionally modified polyacrylamide-graft-carrageenan pH-sensitive composite microspheres have been realized to achieve colon targeted drug delivery.

The surface properties of polyacrylamide nanoparticles (PAMNPs) can be modified through introducing functional reactive groups by adding different monomers during the preparation process such as introducing the carboxyl groups by adding acrylic acid monomer and introducing the amino groups by adding APMA. We have previously synthesized a kind of PAMNP with autofluorescence of both visible fluorescence (VF) and near-infrared fluorescence (NIRF). This kind of autofluorescence is generated by glutaraldehyde (GTA) cross-linking, which produces two different double bonds, C=NO and C≡C. In the synthesis of PAMNPs, we also added ε-poly-L-lysine (ε-PL) for further increasing amino groups in GTA cross-linking, further enhancing the autofluorescence of PAMNPs. In addition, due to the use of APMA and ε-PL, PAMNPs had positive charges. Our study also demonstrated that PAMNPs had high biocompatibility in vitro and in vivo.

In this study, based on the previous study, we synthesized positively charged PAMMPs with autofluorescence (PAMMPs) via spot polymerization. The different-size PAMMPs were synthesized by changing the spotting volume (Figure 1). These PAMMPs have excellent VF and NIRF. However, this kind of synthesis has a low yield. To enhance the yield, we then tried to synthesize PAMMPs via modified inverse microemulsion polymerization. The synthesized PAMMPs also have excellent VF of green, red, and blue (Figure 2A-a). Spectrofluorimetry analysis revealed that PAMMPs have two excitation/emission peaks, 450 nm/509 nm and 459 nm/671 nm, respectively (Figure 2A-b). Additionally, PAMMPs showed NIRF at both emission wavelengths of 720 and 820 nm; however, the NIRF at an emission wavelength of 720 nm was much stronger than that at 820 nm (Figure 2A-d). The size analysis revealed that the synthesized PAMMPs had a size between 10 and 60 μm (Figure 2B-a), indicating a good monodispersity of PAMMPs.

We next checked the stability of these physical features of PAMMPs. We checked the stability of PAMMPs’ fluorescence by irradiating PAMMPs with excitation light under a fluorescence microscope for different times (Figure S1). The irradiated PAMMPs were immediately imaged with a fluorescence microscope, and the mean fluorescence intensity was analyzed with Image-Pro. The results showed that the VF of PAMMPs can endure the photobleaching for several minutes (Figure 2B-b), indicating the stability of PAMMPs’ fluorescence. More importantly, PAMMPs could retain their morphology and autofluorescence (both VF and NIRF) after a

**RESULTS AND DISCUSSION**

**Synthesis and Characterization of PAMMPs.** We first synthesized PAMMPs via spot polymerization. The different-size PAMMPs were synthesized by changing the spotting volume (Figure 1). These PAMMPs have excellent VF and NIRF. However, this kind of synthesis has a low yield. To enhance the yield, we then tried to synthesize PAMMPs via modified inverse microemulsion polymerization. The synthesized PAMMPs also have excellent VF of green, red, and blue (Figure 2A-a). Spectrofluorimetry analysis revealed that PAMMPs have two excitation/emission peaks, 450 nm/509 nm and 459 nm/671 nm, respectively (Figure 2A-b). Additionally, PAMMPs showed NIRF at both emission wavelengths of 720 and 820 nm; however, the NIRF at an emission wavelength of 720 nm was much stronger than that at 820 nm (Figure 2A-d). The size analysis revealed that the synthesized PAMMPs had a size between 10 and 60 μm (Figure 2B-a), indicating a good monodispersity of PAMMPs.

In this study, based on the previous study, we synthesized positively charged PAMMPs with autofluorescence (PAMMPs) via spot polymerization and inverse microemulsion polymerization. The prepared PAMMPs have high stability under various harsh conditions such as acid, alkali, and high temperature. Utilizing these excellent features of the synthesized PAMMPs, we developed a simple and fast DNA extraction method based on PAMMPs. This method can be used to extract DNA from various samples such as microorganisms, animals, humans, and plants. The entire extraction process can be finished in 30 s and requires no complicated instruments. Importantly, the DNA-absorbed PAMMPs (PAMMP@DNA) can be directly detected by both normal and quantitative PCR. This PAMMP-based DNA extraction method and the subsequent PCR detection of PAMMP@DNA provide a promising method for rapid DNA detection technology.

**Figure 1.** Preparation of PAMMPs by microspotting. (A) Visible fluorescence (VF) image of PAMMPs. Scale bars are 200 μm. (B) Diameter analysis of PAMMPs. (C) NIRF images of PAMMPs. The PAMMPs with different diameters were synthesized via altering the spotting volume.
long-time (as much as 60 min) treatment of strong acid (0.2 M HCl), alkali (0.2 M NaOH), or high temperature (95 °C) for (Figure S2), indicating that fPAMMPs can tolerate harsh physical environments.

Due to the utility of amino-rich chemicals, APMA and ε-PL, fPAMMPs have strong positive charges as we previously characterized PAMNPs. We thus speculated that two properties, the porous structure and positive charge, make fPAMMPs an ideal vector of different biomolecules or small-molecule drugs. To investigate this potential, we performed a post-loading of IRDye 800CW streptavidin (LI-COR Bioscience). As a result, this biomolecule was successfully loaded on fPAMMPs (Figure 2B-c). In addition, this loading was very stable even after keeping the loaded fPAMMPs for 60 days (Figure S3).

**Ability of fPAMMPs to Capture DNA.** Inspired by the post-loading of IRDye 800CW streptavidin, we speculated that DNA that has high negative charges should be much easier to be loaded or captured on fPAMMPs. To verify this speculation, we tried to load the genomic DNA (gDNA) of SiHa cells on fPAMMPs by mixing the gDNA with fPAMMPs in a tube in which the loading was promoted by just inverting the tube several times. The fPAMMPs were then washed to remove the excess gDNA and detected by gel electrophoresis. As a result, the gDNA was rapidly captured on fPAMMP in a few seconds (Figure 3A-a). Importantly, this kind of DNA loading on fPAMMPs was very stable because DNA captured on fPAMMPs did not disassociate from fPAMMPs after a long-time electrophoresis. Due to the large size of fPAMMPs, fPAMMPs cannot enter the gel. Therefore, the DNA-bound fPAMMPs (fPAMMP@DNA) still remained in loading wells after electrophoresis. To further explore the strength of DNA–fPAMMP interaction, we incubated fPAMMP@DNA in boiling water and washed fPAMMP@DNA with the elution

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**Figure 2.** Preparation of fPAMMPs by the modified inverse microemulsion polymerization. (A) Analysis of fPAMMPs’ fluorescence. (a) Microscopy and VF images of fPAMMPs; scale bars are 200 μm. (b) Red and (c) green VF excitation and emission curves of fPAMMPs. (d) NIRF images at 720 and 820 nm emission wavelengths. (B) Analysis of fPAMMPs’ size. (a) Number of fPAMMPs and size distribution of fPAMMPs. (b) VF stability of fPAMMPs. The fPAMMPs were irradiated for different times with excitation light under a VF microscope and then photographed (Figure S1). Mean optical density was analyzed to represent the VF stability. (c) NIRF image of fPAMMPs and fPAMMP@IRDye 800CW streptavidin. (1) IRDye 800CW streptavidin, (2−7) the first to sixth washing solutions, (8) fPAMMPs, and (9) fPAMMP@IRDye 800CW streptavidin.
buffer from Axygen DNA purification kit. The results demonstrated that DNA still remained on fPAMMPs (Figure S4).

**DNA Extraction and Direct PCR Amplification with fPAMMPs.** Because fPAMMPs can effectively capture DNA, we deduced that fPAMMPs may be used to extract DNA from cells or tissues. To verify this deduction, we first tried to extract gDNA from *Escherichia coli* BL21 and DH5α by lysing these bacteria with NaOH. Because fPAMMPs can remain stable and cannot be dissolved in acid or bases, we speculated that fPAMMPs may also bind DNA in NaOH lysis. The results indicated that fPAMMPs can extract DNA via a simple procedure (Figure 3A-b). Because it was verified that fPAMMPs cannot be disintegrated and DNA cannot be dissociated from fPAMMPs after a long-time incubation in boiling water (Figures S2 and S4), we speculated that fPAMMP@DNA may be directly detected by PCR amplification. To verify this speculation, we tried to amplify a 165 bp fragment of the T7 RNA polymerase gene with the fPAMMPs capturing the gDNA of BL21 and DH5α (Figure 3A-b). We detected the PCR products with gel electrophoresis. The results indicated that the target DNA fragment was successfully amplified from fPAMMP@BL21 gDNA but not from fPAMMP@DH5α gDNA (Figure 3A-c). It should be noted that only BL21 has the T7 RNA polymerase gene. These results indicated that a simple DNA extraction from bacterial cells and then direct PCR amplification can be achieved using fPAMMPs.

To further explore if this method is effective to other kinds of samples, we next applied the fPAMMP-based DNA extraction and direct PCR amplification method to a variety of samples including animal tissue, human cell, tissue, and blood, and plant leaf tissue. As a result, from fPAMMP@gDNA of mouse liver tissue, a 165 bp fragment of the GAPDH gene (house-keeping gene) and a 165 bp fragment of the RELA gene were successfully amplified (Figure 3B-a). From fPAMMP@gDNA of human samples (cell, tissue, and blood), five STRs (a 120–140 bp fragment of D11S4951, a 201–229 bp fragment of D11S4957, a 218–253 bp fragment of GATA193H05, a 215–231 bp fragment of D2S2951, and a 174–202 bp fragment of D6S2421), and a 138 bp fragment of the human GAPDH gene (house-keeping gene) were successfully amplified (Figure 3B-b). Especially, from fPAMMP@gDNA of the human blood (plasma) sample, which contains cell-free DNA (cfDNA), a 193 bp fragment of the TERT promotor was successfully amplified (Figure 3B-c). From fPAMMP@gDNA of plant tissue, a 165 bp fragment of the NOS gene and a 151 bp fragment of the zSSllb gene (house-keeping gene) were successfully amplified (Figure 3B-d). These results showed that the fPAMMP-based DNA rapid extraction and direct PCR amplification method can be widely applied to various common samples, including bacteria, cultured human cells, animal and plant tissues, and human plasma.

**Further Optimization of DNA Extraction with fPAMMPs.** To further shorten the extraction time, we investigated different fPAMMP incubating times. The NaOH
Lysates of bacteria were incubated with fPAMMPs for 20, 10, 5, 2, and 1 min. The results showed that DNA was efficiently captured on fPAMMP at various incubation times (Figure 4A-a). The target DNA, T7 RNA polymerase gene, could be efficiently amplified by PCR even with the fPAMMP being incubated only for 1 min (Figure 4A-b), indicating that DNA in NaOH lysate could be rapidly absorbed on fPAMMPs. In fact, the incubating time could be even shortened to 30 s without affecting PCR amplification (Figure 4B-a). At this condition, enough DNA for PCR amplification was still captured by fPAMMPs, although the amount of DNA captured on fPAMMPs was significantly decreased (Figure 4B-b).
Ultimately, DNA could be rapidly captured on fPAMMPs by gently inverting the tube 5–8 times after fPAMMPs were added to NaOH lysate, which spent only about 15 s (Figure 4B-c). With this very rapid extraction, the captured DNA was also enough for PCR detection (Figure 4B-d). However, with the decrease in incubation time, the amount of DNA captured on fPAMMPs was also decreased (Figure 4A-a,B-a,B-c). The whole process only needed a single NaOH solution and took about 30 s. Using this rapid protocol, different amounts of cells were used to check the sensitivity of this DNA extraction method. The results indicated that this NaOH/fPAMMP-based DNA extraction method has high sensitivity (Figure 4C-a). It was found that two target DNA fragments, 16S rDNA and T7 RNA polymerase gene, could be specifically amplified from fPAMMP@DNA extracted with various amounts of cells (Figure 4C-b,c). Moreover, the PCR products could be directly sequenced by Sanger sequencing (Figure 5S). Importantly, we found that the fPAMMP@DNA could be kept at different conditions (−80, −20, and −4 °C) for a long time (from 1 week to 1 month) without affecting the PCR detection of target genes (16S rDNA and T7 RNA polymerase gene; Figure 4D).

**DNA Extraction with fPAMMPs/PAMMPs and qPCR Detection.** Although the fPAMMP@DNA can be detected by normal PCR, it is time-consuming for detecting PCR products with agarose gel electrophoresis. To further widen the application of fPAMMP@DNA, we next explore whether fPAMMP@DNA can be detected by qPCR. Concerning the potential interference of fPAMMPs’ autofluorescence on qPCR detection, we eradicated the fPAMMPs’ autofluorescence with NaBH₄. The results indicated that the NaBH₄ treatment removed the green and red VF (Figure 5A). However, this
treatment did not affect the dispensability, morphology, and size of fPAMMPs (Figure 5A,B). The fPAMMPs without autofluorescence were then called as PAMMPs. The NIRF imaging revealed that the NIRF of fPAMMPs was also removed by the NaBH₄ treatment (Figure 5C). In mechanism, the NaBH₄ treatment also did not change the charge of fPAMMPs. Therefore, the PAMMPs could be also used to extract DNA as fPAMMPs. The PAMMPs were then used to capture DNA, and the PAMMP@DNA was detected with qPCR. The results revealed that DNA could still be effectively extracted with PAMMP (Figure 5D-a). The normal PCR detection revealed that the target DNA (16S rDNA) could be successfully amplified from PAMMP@DNA (Figure 5D-b). Importantly, the target gene (T7 RNA polymerase gene) could be quantitatively detected from PAMMP@DNA using qPCR (Figure 6A). Nevertheless, we finally found that the target gene (T7 RNA polymerase gene) could be also sensitively detected from fPAMMP@DNA by qPCR, without a significant effect from autofluorescence (Figure 6B).

Figure 6. QPCR detection of the T7 RNA polymerase gene with PAMMP@DNA and fPAMMP@DNA. (A, B) QPCR detection with (A) PAMMP@DNA and (B) fPAMMP@DNA. The amplification plots and melt curves of standards and samples were provided. The copy numbers of different samples were calculated with the standard curve and provided as numbers on the standard curve. (1) PAMMP/fPAMMP@BL21 DNA extracted with 50 μL of BL21 culture (start culture), (2) PAMMP/fPAMMP@BL21 DNA extracted with 50 μL of 10 time-diluted start culture, (3) PAMMP/fPAMMP@BL21 DNA extracted with 50 μL of 100 time-diluted start culture, (4) PAMMP/fPAMMP@DH5α DNA extracted with 50 μL of DH5α culture, and (5) PAMMP/fPAMMP.
Compared with the existing DNA extraction methods, our method has significant advantages over the traditional and current extraction methods. In the traditional method, DNA was purified with phenol/chloroform extraction. This method is time-consuming and uses harmful reagents such as phenol and chloroform. Currently, DNA extraction is usually carried out with a similar mechanism and process, in which several solutions with complicated components are needed, such as lysis, binding, washing, and elution buffers. Many components of these buffers are not amiable to the environment and operator. In the current DNA extraction, spin columns or magnetic beads are used to capture DNA. DNA is extracted by using a similar procedure consisted of lysis, binding, washing, and elution. This procedure usually spends at least half an hour and often needs at least four times of solution and tube transfers. However, in our method, except plant tissue, only one solution (0.4 M NaOH), one tube, and an easy two-step operation are needed. No tube transfer is needed in our method. This greatly simplifies reagents, equipment, and operation. In addition, fPAMMP@DNA can be stored at variant conditions (4, 20, and −80 °C) for a long time (tested to 1 month) without affecting PCR detection. Finally, our method is more cost-effective than the current DNA extraction methods.

**CONCLUSIONS**

In this study, we synthesized a new type of autofluorescent polycrylamide microsphere (fPAMMPs) that has excellent autofluorescence. We synthesized low-yield fPAMMPs of different sizes by in situ polymerization and high-yield fPAMMPs of small size by inverse microemulsion polymerization. The fabricated fPAMMPs had strong and stable autofluorescence of both VF and NIRF. The fabricated fPAMMPs also had high stability in various stringent conditions such as base, acid, and high temperature. Finally, the fabricated fPAMMPs had high positive charges, which could be used to effectively capture various biomolecules such as streptavidin and DNA. Based on these features, we developed a new method to extract DNA from various samples with fPAMMPs in a few minutes (Scheme 1). Furthermore, the fPAMMP@DNA could be directly detected by both normal and quantitative PCR (Scheme 1). Moreover, the fPAMMPs without autofluorescence (PAMMPs) could be easily obtained and also be used to efficiently extract DNA for qPCR detection. To sum up, this study has prepared new fPAMMPs and PAMMPs, developed a new rapid DNA extraction technology, and demonstrated their applications in PCR detection.

**EXPERIMENTAL SECTION**

**Materials.** Acrylamide (AM) and N-(3-aminopropyl)-methacrylamide hydrochloride (APMA) were purchased from Sigma-Aldrich (MO, USA). Glutaraldehyde (GTA), Span 80, and NaOH were acquired from Sinopharm Chemical Reagent (Shanghai, China). Mineral oil, ammonium persulfate (APS), \( \text{N,N',N'-tetramethylethene-1,2-diamine (TEMED,} \) and \( \text{N,N'-methylenebis(acrylamide (MBA) \) were obtained from Biosharp (Hefei, China).} \) e-Poly-\( \text{l-lysine (e-PL) \) was purchased from Shanghai Shifeng Biological Technology (Shanghai, China). Premix PrimerSTAR HS (2×); code no.: R040A) was purchased from Takara. The GoTag Probe qPCR Master Mix (2×) was purchased from Promega. Oligonucleotides were manufactured by Sangon Biotech (Shanghai) Co., Ltd.

**Synthesis of fPAMMPs via Spot Polymerization.** fPAMMPs were prepared as follows: 264 mg of AM, 80 mg of MBA, and 12 mg of APMA were dissolved in 1 mL of deionized (DI) water under ultrasound to obtain the uniform acrylamide monomer liquid. The mix solution contained 80 μL of acrylamide monomer liquid, 80 μL of 10% e-PL, and 5 μL of 20% APS. A 0.5 μL aliquot of the mix solution was pipetted on a plate with polyethylene to form microspheres, which was then covered by the mineral oil containing 0.4% TEMED. Microspheres were polymerized at 37 °C for 1 min and washed three times with deionized water after removing the mineral oil. PAMMPs with different particle sizes can be obtained by changing the drop volume of the mixed solution. A BioDot AD1500 aspirate/dispense platform was used to spot 200, 100, 50, 30, 20, and 10 nL aliquots of the mix solution. The PAMMP solutions were added with 25% glutaraldehyde for a final concentration of 0.1% and incubated at 37 °C for 30 min. The fPAMMPs were washed three times with deionized water to remove excess glutaraldehyde.

**Synthesis of fPAMMPs via Modified Inverse Microemulsion Polymerization.** According to the literature, fPAMMPs were synthesized by modified inverse microemulsion polymerization. A typical synthesis is as follows: 1 mL of Span 80 and 70 mL of hexane were added to a 250 mL three-neck flask equipped with a magnetic stirrer and a nitrogen inlet. The mixture was stirred under nitrogen purging until the surfactant was uniformly dispersed. At the same time, 264 mg of acrylamide, 25 mg of APMA, and 80 mg of MBA were dissolved in 1 mL of DI water under ultrasound to obtain the uniform acrylamide monomer liquid, which was then mixed with 1 mL of 10% e-PL and 80 μL of 20% APS. The solution was added to n-hexane, and the mixture was stirred continuously for 2 h in a nitrogen atmosphere at 380 rpm. Finally, 280 μL of TEMED was added to initiate the reaction, and the mixture was stirred for 2 h. The microspheres were collected and washed alternately with DI water or absolute alcohol three times. The beads finally washed with DI water.
and resuspended in DI water. The bead solution was added with 25% glutaraldehyde for a final concentration of 0.1% and incubated at 37 °C for 4 h. The prepared fPAMMPs were washed five times with DI water to remove excess glutaraldehyde. The fPAMMPs were finally resuspended in 10 mL of water and stored at room temperature in the dark.

**Characterization of fPAMMPs.** The size and morphology of the fPAMMPs were evaluated with a scanning electron microscope (SEM). The VF and its stability were detected and photographed with a fluorescence microscope (IXS1 with a DP71 camera; Olympus). Fluorescence emission spectra were detected with a Hitachi F-7000 fluorescence spectrometer (Hitachi High-Technologies). The NIRF at 720 and 820 nm emission wavelengths was detected with an NIFR imager, the Odyssey infrared imaging system (LI-COR Bioscience). To study the porosity, 1 mL of fPAMMPs was mixed with 200 μL of IRDye 800CW streptavidin and incubated for 4 h. The beads were washed six times with deionized water to remove the excess IRDye 800CW streptavidin. The eluate was retained and detected by the Odyssey infrared imaging system. For checking the stability of fPAMMPs, 200 μL of fPAMMPs was incubated with an equal volume of 0.4 M NaOH or HCl at room temperature for 30 and 60 min, respectively. Moreover, 200 μL of fPAMMPs was also incubated at 95 °C for 30 and 60 min. Then, the VF and NIRF were detected with a microscope and NIRF imager.

**Reduction of fPAMMPs.** One milliliter of fPAMMPs was added with 50 μL of NaBH₄ solution (1% w/v). The mixture was incubated at room temperature overnight. After washing three times with DI water, the fPAMMPs without fluorescence (named as PAMMPs) were obtained. Then, the VF and NIRF were detected with a microscope and NIRF imager. PAMMPs were also characterized as fPAMMPs.

**DNA Extraction with fPAMMPs and PAMMPs.**

**Bacteria.** *E. coli* BL21 and DH5α from glycerol stock were streaked onto a Luria–Bertani (LB) agar plate and incubated for 16 h at 37 °C. A well-isolated single colony of *E. coli* cells was inoculated into a tube containing 2 mL of LB broth and incubated at 37 °C for 8 h with vigorous shaking at 220 rpm. The culture was then centrifuged at 12,000 rpm (5415D, Eppendorf, GER) for 5 min, and the supernatant was discarded. The precipitate obtained was resuspended in 200 μL of DI water. The fPAMMP@DNA was washed three times with wash buffer and resuspended in 200 μL of 0.4 M NaOH and transferred into a 1.5 mL tube. Cells were lysed by gently inverting the tube several times. Then, 100 μL of fPAMMPs was added to above various NaOH-lysed sample and incubated in a rotator for 40 min. Then, 100 μL of 0.4 M NaOH was added and transferred into a 1.5 mL tube. The prepared fPAMMPs were added and incubated in a rotator for 20 min. The fPAMMP@DNA was washed three times with DI water and resuspended in 50 μL of DI water.

**Plant.** For gDNA extraction from plant tissue, 10–20 mg of leaf tissue was ground in a 1.5 mL tube with a glass pestle in the presence of 200 μL of cell lysis buffer (20 mM Tris, 25 mM NaCl, 2.5 mM EDTA, and 0.05% SDS). Then, 100 μL of fPAMMPs was added and incubated in a rotator for 20 min. The fPAMMP@DNA was incubated in a rotator for 20 min. The fPAMMP@DNA was washed three times with wash buffer (10 mM Tris, pH 8.0, and 0.1% Tween 20%). The fPAMMP@DNA was resuspended in 50 μL of DI water.

**PCR Amplification with fPAMMP/PAMMP@DNA.**

**Bacteria.** The T7 RNA polymerase gene and 16S rRNA were detected with fPAMMP@DNA of *E. coli* BL21 and DH5α. The PCR reaction was carried out in a 50 μL volume containing 1 μL of fPAMMP@DNA, 0.5 μM each primer (T7 RNA Pol-F and R or 16S rDNA-27-F and 16S rDNA-1492-R; Table S1), and 1× Premix PrimerSTAR HS. The PCR program was as follows: (i) 98 °C for 3 min, (ii) 35 cycles at 98 °C for 10 s and 68 °C for 40 s, and (iii) 72 °C for 3 min. The results of the DNA extraction and the amplification reaction were visualized by 1.2% agarose gel electrophoresis. The copy number of fPAMMP@BL21 DNA was detected by qPCR using 2× Fast SYBR Green Master Mix (Applied Biosystems), according to the manufacturer’s instructions. A series of dilutions of a free T7 RNA polymerase gene fragment was used as standard samples to draw the standard curve. The PCR reaction was carried out in a 20 μL volume containing 1 μL of sample (fPAMMP/PAMMP@BL21/DH5α DNA) or standard sample, 0.25 μM each primer (T7 RNA Pol-F and R; Table S1), and 1× Fast SYBR Green Master Mix. The qPCR programs were run on a real-time PCR machine, StepOnePlus (Applied Biosystems). Each qPCR detection was performed in at least three technical replicates. Melting curve analysis was extracted DNA from 50 μL of BL21 bacterial culture, 50 μL of 10 time-diluted 50 μL BL21 bacterial culture, 50 μL of 100 time-diluted 50 μL BL21 bacterial culture, and 50 μL of DH5α bacterial culture.

**Mouse.** For genomic DNA (gDNA) extraction from animal tissues, mouse liver tissue was ground in liquid nitrogen and then dropped into 200 μL of 0.4 M NaOH. After the addition of 100 μL of fPAMMPs, the mixture was incubated in a rotator for 20 min. The fPAMMP@DNA was washed three times with DI water and resuspended in 50 μL of DI water.

**Human.** (1) When gDNA was extracted from human cell lines, HL-7702 cells were cultured in DMEM containing 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 mg/mL streptomycin in a 5% CO₂ humidification incubator at 37 °C. Cells were seeded in a 24-well plate at a density of 5 × 10⁴ cells/well and cultivated for 24 h. Cells were collected by trypsinization and resuspended in 200 μL of PBS. Cells were resuspended in 200 μL of 0.4 M NaOH and lysed by gently inverting the tube several times. (2) When gDNA was extracted from human tissues, esophageal cancer tissue was ground in a mortar with liquid nitrogen. The tissue was then added with 200 μL of 0.4 M NaOH and transferred into a 1.5 mL tube. Cells were lysed by gently inverting the tube several times. One hundred microliters of fPAMMPs was added to above various NaOH-lysed sample solutions. The mixture was incubated in a rotator for 20 min. The fPAMMP@DNA was washed three times with DI water and resuspended in 50 μL of DI water.
performed. Data analysis was performed using the Applied Biosystems StepOne software v2.3, and the copy number was calculated according to the standard curve.

Mouse. The genes GAPDH and RELA were detected with fPAMMP@DNA. The PCR reaction was carried out in a 50 μL volume containing 3 μL of fPAMMP@DNA, 0.5 μM each primer (mGAPDH-F and R or RELA-F and R; Table S1), and 1× Premix PrimerSTAR HS. The PCR program was as follows: (i) 95 °C for 3 min; (ii) 35 cycles at 95 °C for 15 s, 58 °C for 30 s, and 72 °C for 30 s; and (iii) 72 °C for 3 min.

Human. Five short tandem repeat (STR) and TERT genes were detected with fPAMMP@DNA. The PCR reaction was carried out in a 50 μL volume containing 3 μL of fPAMMP@DNA, 0.5 μM each primer (hGAPDH-F and R, TERT-F and R, D11S4951-F and R, D11S4957-F and R, GATA193H05-F and R, D2S2951-F and R, or D6S2421-F and R; Table S1), and 1× Premix PrimerSTAR HS. For STR and GAPDH, the PCR program was as follows: (i) 95 °C for 3 min; (ii) 35 cycles at 95 °C for 15 s, 55 °C for 30 s, and 72 °C for 30 s; and (iii) 72 °C for 3 min. For the TERT gene, the PCR program was as follows: (i) 98 °C for 3 min; (ii) 35 cycles at 98 °C for 15 s and 68 °C for 45 s; and (iii) 72 °C for 2 min.

Plant. Two transgenic genes, NOS and zSSllb, were detected with fPAMMP@DNA. The PCR reaction was carried out in a 50 μL volume containing 3 μL of fPAMMP@DNA, 0.5 μM each primer (zSSllb-F and R or NOS-F and R; Table S1), and 1× GoTaq Probe qPCR Master Mix. The PCR program was as follows: (i) 95 °C for 5 min; (ii) 35 cycles at 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 40 s; and (iii) 72 °C for 7 min.

Associated Content

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c01181.

Table S1, PCR primers; Figure S1, fluorescence images of fPAMMPs after being irradiated for different times with excitation light; Figure S2, detection of stability of fPAMMP in various treatments; Figure S3, NIRF images of fPAMMP@iDR dye 800CW streptavidin after keeping at room temperature for variant times; Figure S4, electrophoretic detection of eluted fPAMMP@DNA; and Figure S5, PCR amplification of target DNA for sequencing (PDF).

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Author Contributions

J.W. (the first author) and S.Z. contributed equally in this work.

Author Contributions

J.W. (the first author) prepared all fPAMMPs/PAMMPs and performed all DNA extraction and PCR/qPCR detection with fPAMMPs/PAMMPs. J.W. (the first author) wrote the manuscript. S.Z. prepared all detected samples including bacteria, cultured human cells, human blood plasma, and mouse and human tissues. Y.X. prepared and tested plant tissues before they were applied to the fPAMMP experiment, provided the plant cell lysis solution and washing solution, and also gave helpful discussion about plant DNA extraction with fPAMMPs. Z.L. gave beneficial discussion and suggestion in this study and also reviewed and edited the manuscript. J.W. (the corresponding author) conceptualized, supervised and funded the study. J.W. (the corresponding author) also wrote the paper.

Notes

The authors declare no competing financial interest.

Acknowledgments

This work was supported by the grants from the National Natural Science Foundation of China (grant 61971122).

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