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Nanoparticles Affect PCR Primarily via Surface Interactions with PCR Components: Using Amino-Modified Silica-Coated Magnetic Nanoparticles as a Main Model

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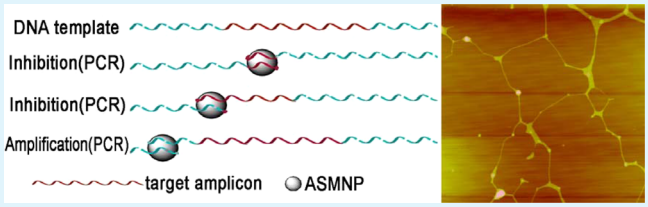
Supporting Information

ABSTRACT: Nanomaterials have been widely reported to affect the polymerase chain reaction (PCR). However, many studies in which these effects were observed were not comprehensive, and many of the proposed mechanisms have been primarily speculative. In this work, we used amino-modified silica-coated magnetic nanoparticles (ASMNPs, which can be collected very easily using an external magnetic field) as a model and compared them with gold nanoparticles (AuNPs, which have been studied extensively) to reveal the mechanisms by which nanoparticles affect PCR. We found that nanoparticles affect PCR primarily by binding to PCR components: (1) inhibition, (2) specifity, and (3) efficiency and yield of PCR are impacted. (1) Excess nanomaterials inhibit PCR by adsorbing to DNA polymerase, Mg^{2+}, oligonucleotide primers, or DNA templates. Nanoparticle surface-active groups are particularly important to this effect. (2, a) Nanomaterials do not inhibit nonspecific amplification products caused by false priming as previously surmised. It was shown that relatively low concentrations of nanoparticles inhibited the amplification of long amplicons, and increasing the amount of nanoparticles inhibited the amplification of short amplicons. This concentration phenomenon appears to be the result of the formation of "joints" upon the adsorption of ASMNPs to DNA templates. (b) Nanomaterials are able to inhibit nonspecific amplification products due to incomplete amplification by preferably adsorbing single-stranded incomplete amplification products. (3) Some types of nanomaterials, such as AuNPs, enhance the efficiency and yield of PCR because these types of nanoparticles can adsorb to single-stranded DNA more strongly than to double-stranded DNA. This behavior assists in the rapid and thorough denaturation of double-stranded DNA templates. Therefore, the interaction between the surface of nanoparticles and PCR components is sufficient to explain most of the effects of nanoparticles on PCR.



KEYWORDS: *nanomaterials, polymerase chain reaction, effect, amino-modified silica-coated magnetic nanoparticles, gold nanoparticles*

1. INTRODUCTION

The polymerase chain reaction (PCR) is a powerful tool for the in vitro enzymatic amplification of a specific segment of DNA (it is able to exponentially amplify a single molecule of DNA segment more than a billion-fold in a few hours).[1] It has been widely applied in the fields of medical diagnosis,[2,3] food safety,[4,5] archeological studies,[6] and basic research. Additives are sometimes used to enhance the efficiency, specificity or sensitivity of PCR, such as dimethyl sulfoxide (to improve PCR product yield),[7] formamide (to enhance PCR specificity),[8] or single-stranded DNA-binding protein (SSB, to increase the PCR amplification efficiency and specificity).[9] Given the rapid development of nanotechnology in the past decade, many researchers have attempted to use nanomaterials as additives to optimize PCR. Numerous studies have reported that nanomaterials, including gold nanoparticles (AuNPs),[10] silver nanoparticles,[11] carbon nanotubes,[12] titanium dioxide,[13] cadmium-telluride (CdTe) quantum dots (QDs),[14,15] carbon nanoparticles,[16] dendrimers,[17] and β-cyclodextrins-capped platinum nanoparticles[18] enhanced the specificity of PCR. Furthermore, some nanomaterials, such as AuNPs,[19,20] C_{60}-fullerene,[21] β-cyclodextrin-capped platinum nanoparticles,[18] and graphene nanoflakes,[22] affected the efficiency of PCR. In addition, some nanomaterials, such as AuNPs,[19] ZnO,[23] and carbon nanotubes,[24] appeared to improve the yield and sensitivity of PCR.

Many possible mechanisms have been proposed to account for the effect of nanoparticles on PCR. The main speculations include the following: (1) the effects were the results of binding between the nanomaterial and the DNA polymerase;[25−27] (2) the function of nanomaterials was similar to that of SSB, which

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selectively bind single-stranded DNA (ssDNA) and minimize mispairing between primers and templates;[28] and (3) nanomaterials improved the thermal transfer efficiency in the aqueous solution.[13,19] However, these conclusions can not completely explain all of the effects of nanomaterials on PCR.

PCR occurs in a complex mixture of buffer, substrates, and enzyme in a small volume (10−50 μL). By definition, nanoparticles are extremely small particles, and they always present at very low concentrations in the PCR system. Therefore, it is difficult to precisely determine the nature of the interaction between the nanoparticles and components of the PCR mixture and the effect of these interactions on PCR. Herein, we used amino-modified silica-coated magnetic nanoparticles (ASMNPs) as additives to explore the effects of nanomaterials on PCR. ASMNPs are superparamagnetic; thus, they can be separated easily by an external magnetic field, even when very small quantities are employed in a very small volume. Using ASMNPs as a main model nanoparticle, we demonstrated that most of the effects of nanoparticles on PCR were due to the binding between the surface of nanoparticles and PCR components: (1) Excess nanomaterials inhibited PCR by adsorbing to several PCR components, including DNA polymerase, Mg^{2+}, oligonucleotide primers, and DNA templates. (2) Some nanomaterials inhibited nonspecific amplification products due to incomplete amplification by preferably adsorbing to single-stranded incomplete amplification products, while they were not able to inhibit nonspecific amplification products due to false priming (longer amplifications products were more readily inhibited only because nanoparticles bound to DNA templates, irrespective of specificity). (3) Some nanomaterials that strongly bound to ssDNA positively affected PCR, such as increasing efficiency and yield.

2. MATERIALS AND METHODS

2.1. Nanoparticles. The detailed methods for the preparation and characterization of ASMNPs, silica-coated magnetic nanoparticles (SMNPs) and AuNPs are presented in the Supporting Information. ASMNPs and silica-coated magnetic nanoparticles (SMNPs) were approximately 77 nm in diameter, amino-modified silica nanoparticles and silica nanoparticles were approximately 38 nm in diameter, AuNPs were approximately 13 nm in diameter, and CdTe QDs were approximately 3.8 nm in diameter. Titanium oxide nanoparticles (approximately 25 nm) and carbon nanoparticles (approximately 30 nm) were purchased from Aladdin-Reagent Co., Ltd. (Shanghai, China). The working solution of ASMNPs was prepared by resuspending the nanoparticles in deionized sterile water (1 mg/mL) after repeated washing.

2.2. DNA Templates, Blocking Solution, and Primers. Lambda DNA was purchased from Thermo Fisher Scientific (USA). *Salmonella* genomic DNA and *Staphylococcus aurous* genomic DNA were isolated from *Salmonella enterica* serovar Enteritidis (ATCC13076) and *Staphylococcus aurous* (ATCC13565) using the CTAB method.[29] To prepare a solution to block the surface of the nanoparticles, a 10% solution of skim milk powder in TE buffer (Tris-hydrochloride buffer, pH 8.0, containing 1.0 mmol/L EDTA) was hydrolyzed with protease K (Roche, USA) at 52 °C for 1 h followed by boiling for 10 min, and the solution was then centrifuged (10 000 rpm) to remove denatured protein. Other than P283−F/R,[10] oligonucleotide primers were designed using the Primer 5.0 program (Premier Biosoft International, Palo Alto, CA). All primers were synthesized by Shanghai Biotech Corporation (Shanghai, China). The primer sequences are presented in Table 1.

2.3. Conventional PCR Amplification. PCR mixtures (25 μL, Thermo, USA) contained forward primers and reverse primers (0.2 μmol/L each) and deoxyribonucleoside triphosphates (0.1 mol/L each). *Taq* DNA polymerase (1.0 U, Thermo, USA) was used to amplify DNA fragments. The reaction conditions were 5 min at 94 °C for denaturation, followed by 35 cycles of 30 s at 94 °C, 30 s at 52 °C, and 40 s at 72 °C. This protocol was followed by an additional extension step at 72 °C for 8 min. DNA amplifications were carried out in a Mastercycler Personal 5332 Thermocycler (Eppendorf, Germany). The PCR products were analyzed by agarose gel (2.0%) electrophoresis with ethidium bromide staining and visualized using a GIS202 Electrophoresis Image Analysis System (Tanon Science & Technology Co., Ltd., Shanghai, China).

Table 1. Oligonucleotide Primers Employed in PCR and RT-PCR

primer name	amplicon size (bp)	sequence
primers used for lambda DNA		
P283-F	283	GGCTTCGGTCCCTTCTGT
P283-R		CACCACCTGTTCAAACTCTGC
primers used for *Salmonella* and *Staphylococcus aureus* genomic DNA		
QS95-F	191	TCAATAATCGCAGTATCCAGTAATG
QS95-R		GAAGAGATTTTAGCGCAGTGTAG
St1-F	203	CTATTTGCTGTATTAGGTGGCG
St1-R		CCGTAAAGACTTCCGACTAACC
St4-F	236	CGTTACATAGTCAGGCTTATTCGC
St4-R		ATGATTTCACAGTTGTCGCACC
FS21-F	522	TGGCTATCCGGTCGATACTC
FS21-R		TCTCCTTAATCGGCAAAACG
S206-F	206	AAAGATAGCCCTGGGAAATACG
S206-R		TCGGTCGCCACGATAAGA
S417−F	417	GAGGTCACGCACCATCACAAT
S417-R		ATACGGCACCACCGCATAG
S643−F	643	TGACTTCAACTCGCATACCT
S643-R		TGCTTCTGATGGCGTTTAG
S1187−F	1187	AGCTCCCGGAGTTTCTCCC
S1187-R		ACGCTCTTTCGTCTGGCATTA

2.4. Real-Time PCR (RT-PCR) Amplification. RT-PCR was carried out in 25 μL reactions containing 1 μL of DNA templates, 5 pmol of each primer, and 12.5 μL of 2× SYBR Green PCR master mix (TaKaRa, Dalian, China). The following PCR thermocycling procedure was employed: 2 min at 95 °C followed by 40 cycles of 15 s at 95 °C, 15 s at 60 °C, and 20 s at 72 °C. Amplifications were carried out in Mastercycler ep realplex (Eppendorf, Germany).

2.5. Melting Curve Analysis. At the end of the RT-PCR, melting curves were obtained to study the effect of nanoparticles on the apparent T_m. The reaction was maintained at 95 °C for 15 s, and then the temperature was decreased to 60 °C and held for 15 s, followed by heating slowly to 95 °C for 20 min. The fluorescence data were recorded and analyzed using Realplex software (Eppendorf, Germany).

2.6. PCR in the Presence of ASMNPs. When the magnetic nanoparticles were added to the PCR system, a defined volume of nanoparticle working solution was transferred into the PCR tube in advance, the magnetic nanoparticles were collected under an external magnetic field, the supernatant was discarded, and the PCR components were added to the nanoparticle pellets.

3. RESULTS AND DISCUSSION

3.1. Mechanism by Which Nanomaterials Inhibit PCR.

3.1.1. Nanoparticles Inhibit PCR Primarily Because of Their Surface Properties. As observed for other nanoparticles,[26] the inhibition of PCR by ASMNPs was concentration-dependent (Figure 1A). The amplification of the PCR products appeared to be completely inhibited when the amount of ASMNPs exceeded 20 μg. However, PCR amplification was recovered when small amounts of blocking solution were added to the PCR mixture containing 20 μg of ASMNPs (Nevertheless, higher amounts of blocking solution once again inhibited PCR

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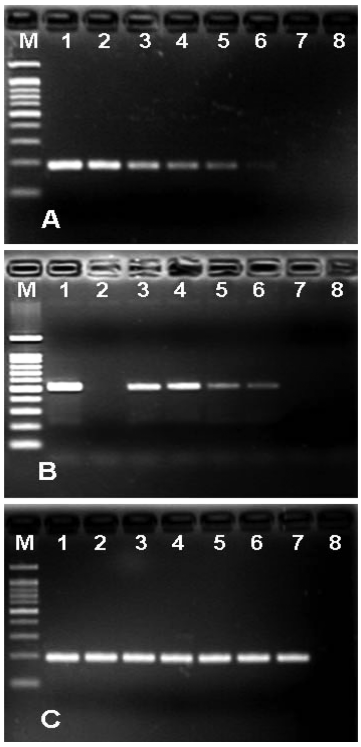

Figure 1. Inhibition of PCR by ASMNPs (primers, QS95-F/-R; DNA templates, 38 ng of *Salmonella* genomic). The effects of different amounts of ASMNPs on PCR: (A) M, markers; lanes 1−7, 0, 2.5, 5, 10, 15, 20, and 25 μg ASMNPs per assay; lane 8, NC (negative control without DNA templates and additives). The effects of different amounts of blocking solution on PCR with 20 μg of ASMNPs; (B) M, markers; lane 1, PC (positive control without ASMNPs); lane 2−7, 0, 1, 2, 3, 4, and 5 μL of blocking solution per assay; lane 8, NC. The effect of different amounts of blocked ASMNPs (weighed as bare ASMNPs) on PCR (C): M, markers; lanes 1−7, 0, 2.5, 5, 10, 15, 20, and 25 μg of blocked ASMNPs; lane 8, NC.

amplification, Figure 1B). This effect could be attributed to the ability of bare ASMNPs to adsorb to PCR components, which inhibited the PCR amplification; and the blocking solution prevented from the adsorption of bare ASMNPs and PCR components. To further confirm whether the surface of nanoparticles played an important role in adsorbing to PCR components, we prepared blocked ASMNPs (ASMNPs/blocking solution: 1 mg/1 mL; 60 °C for 30 min) and compared the effects of blocked and bare ASMNPs on PCR. The results demonstrated that the blocked ASMNPs (Figure 1C) indeed eliminated the inhibition of PCR. Wan et al.[30] compared three different gold nanoparticles and observed that PCR inhibition was related to the total particle surface area, irrespective of particle size. This phenomenon is consistent with our conclusion that nanomaterials affect PCR mainly via their surfaces. To explore more details about the inhibitory effect on PCR caused by the surface of ASMNPs, we used nonamino-modified nanoparticles as controls. A comparison of Figure 2A and Figure 2B shows that PCR was more sensitive to inhibition caused by ASMNPs than by SMNPs. Similarly, a comparison of silica nanoparticles (nonmagnetic) with amino-modified silica nanoparticles (nonmagnetic) demonstrates that the amino modification of the nanoparticles enhances the inhibition of PCR (Figures 2C and D). This finding suggests that some active groups, such as amino groups, play important roles in the inhibition of PCR. This effect could be due to the

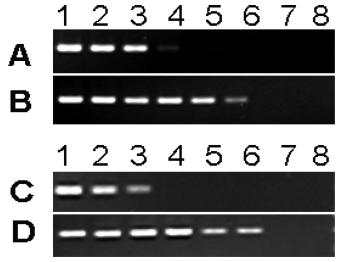

Figure 2. Inhibition of PCR by amino-modified and nonamino-modified nanoparticles. PCR was carried out using 3.8 ng of *Salmonella* genomic DNA and the primers QS95-F/-R in the presence of amino-modified silica-coated magnetic nanoparticles (A); silica-coated magnetic nanoparticles (B); amino-modified silica nanoparticles (C); and silica nanoparticles (D). Lanes 1−7: 0, 2.5, 5, 10, 15, 20, and 25 μg of nanoparticles. Lane 8: NC.

ability of nanoparticles to adsorb more PCR components via these active groups. Cao et al. studied the effects of several types of nanoparticles, including composite nanoparticles, and also observed that surface groups were important factors in the effect of nanoparticles on PCR.[17,31−33] For example, they observed that PCR was considerably more sensitive to amino-modified nanoparticles than carboxyl-terminated nanoparticles.

3.1.2. Several PCR Components Are Able to Be Adsorbed by ASMNPs. While some researchers noted that excess nanomaterials inhibited PCR amplification, this effect was previously only attributed to the interaction of the nanomaterials with thermostable DNA polymerase,[26] and other components of the PCR assay mixture were not examined. In the current study, ASMNPs, which are superparamagnetic, were used to easily identify the components of PCR that were adsorbed by nanomaterials.

We conducted a series of experiments to determine if the binding of DNA polymerase and other PCR components to the ASMNPs contributed to the inhibition of PCR. First, the binding between ASMNPs and DNA polymerase was evaluated. Twenty micrograms of ASMNPs were added to the PCR mixture, which was then incubated at 94 °C for 5 min, followed by 5 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 3 min (to simulate PCR). The ASMNPs were then magnetically separated, and the supernatant was thermocycled (35 cycles) to complete the PCR. In a concurrent PCR assay, an additional 1 U of DNA polymerase was added after the removal of the ASMNPs (the additional DNA polymerase was also subjected to the same 5 cycles of PCR in advance). Figure 3A, lane 3 shows that PCR remained inhibited even if ASMNPs were removed from the solution prior to the complete thermocycling. This persistent inhibition could be due to the removal of critical assay components that had adsorbed to the ASMNPs. The addition of 1 U of DNA polymerase after the removal of the ASMNPs only partially overcame the inhibition of the PCR (Figure 3A, lane 4). Therefore, DNA polymerase indeed adsorbed to ASMNPs, but other components of PCR were also adsorbed, which contributed to the inhibition of PCR.

An additional set of experiments was conducted to evaluate the effect of adding other PCR components to the ASMNP-treated supernatants. After 5 cycles of simulated PCR and the magnetic separation of ASMNPs, extra Mg^{2+}, dNTPs, oligonucleotide primers or DNA templates were added to the supernatant (in each case, DNA polymerase was not added until the start of the full PCR). Figure 3B shows that the

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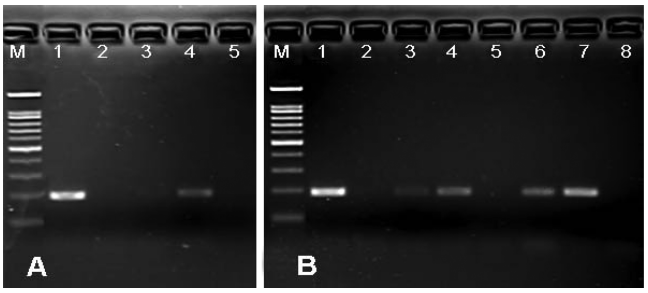

Figure 3. Adsorption of PCR components by ASMNPs affects PCR (PCR template, 3.8 ng of *Salmonella* genomic DNA; primers, QS95-F/-R). The adsorption of DNA polymerase to ASMNPs (A). Lane 1: PC (no added ASMNPs; template DNA was also treated with 5-cycles of "simulated" PCR prior to the standard thermal cycling). Lane 2: With 20 μg of ASMNPs. Lane 3: Supernatant of PCR mixture after mixing with 20 μg of ASMNPs and removal of ASMNPs. Lane 4: Lane 3 with an additional 1 U of *Taq* DNA polymerase. Lane 5: NC. The adsorption of other PCR components to ASMNPs (B). Lane 1: PC. Lane 2: With 20 μg of ASMNPs. Lane 3: Supernatant of PCR mixture after mixing with 20 μg of ASMNPs and removal of ASMNPs. Lanes 4–7: Lane 3 with an additional 1.5 μL of $MgCl_2$ solution (25 mmol/L), 1.0 μL of dNTPs solution (2.5 mmol/L each), 1.0 μL of primers solution (5 pmol each), and 1.0 μL of *Salmonella* genomic DNA (3.8 ng). Lane 8: NC.

addition of other PCR components, except for the dNTPs, also partially restored PCR amplification. These results indicate that Mg^{2+}, oligonucleotide primers and DNA templates could also adsorb to ASMNPs. Silica nanoparticles have previously been reported to be able to adsorb Mg^{2+} because their surfaces contained anionic groups; and ASMNPs strongly adsorbed Mg^{2+} by forming a complex with the amino groups.[34,35] The addition of primers or template DNA increased the amount of PCR product (Figure 3B, lanes 6 and 7, respectively), suggesting that the adsorption of nucleic acids by ASMNPs is also an important factor in the observed inhibition of PCR. Nevertheless, this experiment did not demonstrate a restoration of PCR production amplification upon the addition of dNTPs (Figure 3B, lane 5). This lack of restoration could be due to the inability of ASMNPs to adsorb dNTPs or a large excess of nucleotides in PCR mixtures. Whatever the reasons, the result indicated dNTPs was not one of the factors related to the inhibition of PCR.

Furthermore, to directly demonstrate that ASMNPs are able to adsorb PCR components, we mixed ASMNPs with bovine serum albumin (BSA), dNTPs, oligonucleotide primers, and template DNA in 1× PCR buffer, respectively. The mixtures were incubated at 94 °C for 5 min, and the ASMNPs and any bound molecules were magnetically separated. The amount of each component in solution was determined by UV spectroscopy or RT-PCR before and after magnetic treatment and separation. Because the commercial reagent and PCR system contained very little DNA polymerase, we used BSA as a substitute to explore the adsorption of proteins to ASMNPs. Figure 4a shows the UV absorption spectra of BSA in 1 x PCR buffer before and after magnetic treatment and separation. A calibration curve ($y = 0.2888x - 0.0075$, $R^2 = 0.9987$) was established based on the absorption values of a set of standard BSA samples of known concentration at 280 nm. Using this curve, we determined that 4.28 μg of BSA adsorbed to 20 μg of

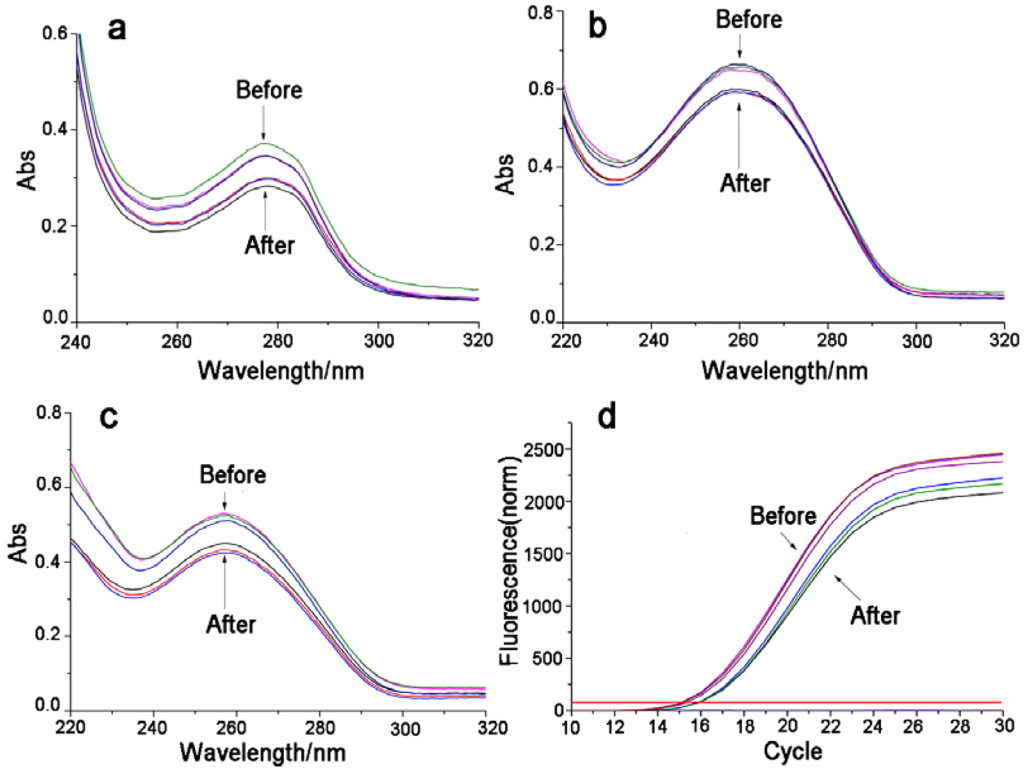

Figure 4. Adsorption of macromolecules to ASMNPs. UV absorbance spectra of macromolecules in 1× PCR buffer before and after magnetic treatment and separation: BSA (2 mg/mL, a); dNTPs (0.4 μmol/L each, b); and oligonucleotide primers QS95-F/-R (100 μmol/L total, c). RT-PCR profiles are shown for assays using 2 μL of a *Salmonella* genomic DNA solution (100 μL of 1× PCR buffer containing 28 ng of *Salmonella* genomic DNA before and after magnetic treatment and separation) as templates (d).

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ASMNPs. Because a typical commercial preparation of Taq DNA polymerase has a specific activity of 80 000 U/mg of protein, and a typical PCR assay containing 1 U of Taq polymerase only contains approximately 12.5 ng of protein,[29] thus, ASMNPs likely affected the PCR amplification by adsorbing DNA polymerase. Similarly, calibration curves (dNTPs $y = 1.1286x + 0.0122$, $R^2 = 0.9999$; oligonucleotide primers $y = 0.0275x - 0.0432$, $R^2 = 1$) were established based on the absorption values of a set of standard dNTP and oligonucleotide primers samples of known concentration at 260 nm. On the basis of these curves and the absorption spectra of dNTPs and oligonucleotide primers before and after magnetic treatment and separation (Figures 4B and C, primers QS95-R/F), we determined that 20 μg of ASMNPs could adsorb 0.11 nmol of dNTPs and 6 pmol of oligonucleotide primers. The dNTPs and oligonucleotide primers adsorbed to ASMNPs, but only 4.4% of the dNTPs in the system adsorbed to 20 μg of ASMNPs, while 60% of oligonucleotide primers in the system adsorbed to the same amount of ASMNPs. These results coincide with the gel electrophoresis results in Figure 3. The adsorption of dNTPs did not inhibit PCR amplification, because most of the dNTPs remained in solution, while the adsorption of oligonucleotide primers inhibited PCR amplification because a large amount of oligonucleotide primers adsorbed to the ASMNPs. Figure 4D shows the results of RT-PCR using a DNA template of 2 μL of a solution of *Salmonella* genomic DNA before and after treatment with ASMNPs (mixing at 94 °C for 5 min) and magnetic separation. A calibration curve ($y = -3.458x + 40.126$, $R^2 = 0.9995$) was established based on the Ct values of RT-PCR using a set of standard *Salmonella* genomic DNA samples of known concentration as DNA templates. Using this curve, we determined that 20 μg of ASMNPs adsorbed 2.284 ng of *Salmonella* genomic DNA in 1x PCR buffer. In summary, the adsorption of Mg^{2+}, DNA polymerase, oligonucleotide primers, and DNA templates to nanomaterials could all be potential causes of the inhibition of PCR amplification.

3.2. Effect of Nanoparticles on PCR Specificity. As mentioned in the introduction, many types of nanomaterials have been hypothesized to enhance the specificity of PCR. However, the designs of some experiments were nonsystematic and consequently these experiments could confound the reported conclusions. In fact, the nonspecific amplifications of PCR included two main cases: (1) nonspecific amplification products present single bands in stained agarose gels (caused by false priming) and (2) nonspecific amplification products present in a diffuse smear of bands (typically attributed to incomplete amplification). These two cases need to be investigated, respectively.

3.2.1. Nanomaterials Do Not Inhibit the Nonspecific Amplification Products of PCR Caused by False Priming. First, the effect of nanoparticles on nonspecific amplifications caused by false priming was investigated. To ensure a systematic and rigorous experiment, we selected three pairs of primers (St1-R/F, St4-R/-F, and FS21-R/-F) from approximately 50 pairs of primers that were used to identify and detect several common pathogens in our lab, such as *Salmonella*, *Staphylococcus aureus*, and *Listeria monocytogenes*. The three pairs of primers result in three types of nonspecific PCR products: (1) nonspecific amplification products are longer than the target amplicons, (2) nonspecific amplification products are longer and shorter than the target amplicons, and (3) nonspecific amplification products are shorter than the target amplicons. The target amplification product of the PCR with the primer pairs St1-r/-f using *Staphylococcus aureus* genomic DNA as templates was 203 bp, while a second "nonspecific" amplification product of approximately 700 bp was produced when *Salmonella* genomic DNA was added to the *S. aureus* genomic DNA templates. Figure 5A shows that

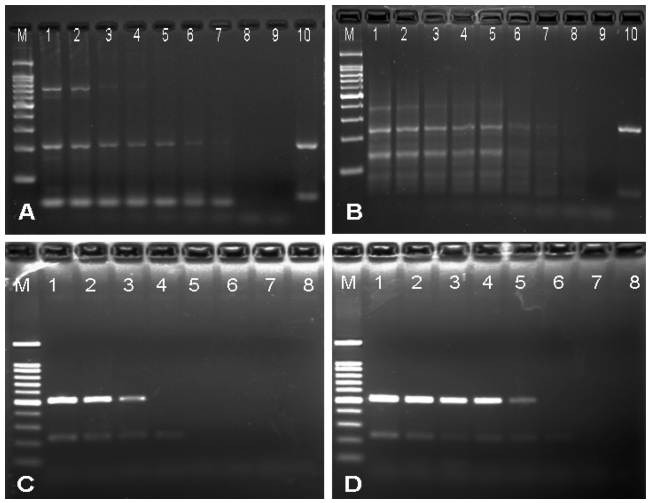

Figure 5. Effect of ASMNPs and AuNPs on the amplification of nontarget PCR products caused by false priming. PCR was conducted using the primers st1-F/-R in the presence of varying amounts of ASMNPs (A). M: Markers. Lanes 1−8: 0, 2.5, 5, 10, 15, 20, 25, and 30 μg of ASMNPs (DNA templates, 0.1 ng of *Staphylococcus aureus* genomic DNA and 20 ng of *Salmonella* genomic DNA). Lane 9: NC. Lane 10: Positive control using only 0.1 ng of *Staphylococcus aureus* genomic DNA as DNA templates. PCR was conducted using the primers st4-F/-R in the presence of varying amounts of ASMNPs (B). M: Markers. Lanes 1−8: 0, 2.5, 5, 10, 15, 20, 25, and 30 μg of ASMNPs (DNA templates, 0.1 ng of *Staphylococcus aureus* genomic DNA and 20 ng of human genomic DNA). Lane 9: NC. Lane 10: Positive control only using 0.1 ng of *Staphylococcus aureus* genomic DNA as DNA templates. PCR was conducted using the primers FS21-F/-R in the presence of varying amounts of ASMNPs (C). M: Markers. Lanes 1−7:0, 5, 10, 15, 20, 25, and 30 μg of ASMNPs (DNA templates, 0.1 ng of *Salmonella* genomic DNA). Lane 8: NC. PCR was conducted using the primers FS21-F/-R in the presence of varying amounts of AuNPs (D). M: Markers. Lanes 1−7: 0, 1.3, 2.6, 3.9, 5.2, 6.5, and 7.8 pmol of AuNPs (DNA templates, 0.1 ng of *Salmonella* genomic DNA). Lane 8: NC.

increasing the amount of ASMNPs preferentially inhibited the amplification of the longer nonspecific PCR product. In this case, ASMNPs seemingly enhanced the specificity of PCR, as previously reported.[14,28] However, in the second case, we determined that this was not an accurate conclusion. When the *S. aureus* genomic DNA template was mixed with human genomic DNA, the PCR (primers St4-r/-f) generated two nonspecific amplification products (approximately 140 and 390 bp) in addition to the 236 bp *S. aureus* target product. Figure 5B shows that increasing the amount ASMNPs inhibited the longer nonspecific products (approximately 390 bp), while the shorter nonspecific products (approximately 140 bp) persisted. This result suggests that increasing the amount of ASMNPs inhibited all amplification products, but the amplification of longer PCR products was inhibited first. To confirm this conclusion, we added ASMNPs to a PCR (using FS21-r/-f as primers and *Salmonella* genomic DNA as template) that only yielded shorter nonspecific products (approximately 230 bp).

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ACS Appl. Mater. Interfaces 2015, 7, 13142−13153

Figure 5C shows that ASMNPs indeed preferentially interfered with the amplification of longer PCR products, even though the long product was the target products. These experiments clearly demonstrated that the amplification of longer products was inhibited first, irrespective of specificity. Similarly, AuNPs, which have been widely reported to enhance the specificity of PCR,[10,20,26] also showed the same manner (Figure 5D).

To further investigate the relationship between PCR inhibition and the length of the amplification products, a quadruple PCR (primers, S206-F/-R, S417-F/-R, S643-F/-R, S1187-F/-R) was developed to amplify four DNA sequences of *Salmonella* of different lengths: 206 bp, 417 bp, 643 bp, and 1187 bp. Various amounts of ASMNPs or AuNPs were added to the quadruple PCR system. Increasing amounts of ASMNPs (Figure 6A) and AuNPs (Figure 6B) indeed inhibited the amplification of longer products first, then inhibited the amplification of shorter products, and finally inhibited the amplification of all PCR products at the higher concentrations tested. This inhibition effect appeared to not be related to the performance of the oligonucleotide primers. Although the yield of the longest products with primers S1200-F/-R was the highest, its amplification was inhibited first. While previous researchers[14,28] concluded that nanomaterials enhanced the specificity of PCR caused by false priming, their conclusions could be confounded by the fact that all of the nonspecific PCR products they observed in these studies were larger than the target amplicons. Vu et al.[25] also reported that gold nanoparticles did not enhance the specificity of PCR, but they concluded that AuNPs favored the amplification of shorter products. Our results suggested that all PCR amplifications were inhibited at high concentration of ASMNPs. The amplification of shorter amplicons was not favored but merely less sensitive to this inhibition. In addition, some researchers reported that the specificity of PCR with some types of nanomaterials, such as AuNPs or quantum dots, was not influenced at very low annealing temperatures.[10,28] In fact, the mechanizem could also be that longer nonspecific amplicons were preferentially inhibited by an appropriate amount of nanomaterials.

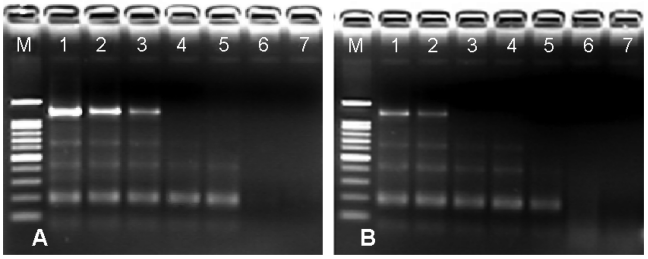

Figure 6. Inhibition of quadruple PCR caused by varying concentrations of ASMNPs (A) and AuNPs (B). Primers: S206-F/-R, S417-F/-R, S643-F/-R, and S1187-F/-R. DNA templates: *Salmonella* genomic DNA. (A) M: markers. Lanes 1−6: 0, 5, 10, 15, 20, and 25 μg ASMNPs. Lane 7: NC. (B) M: Markers. Lanes 1−6: 0, 1.3, 2.6, 3.9, 5.2, and 6.5 pmol of AuNPs. Lane 7: NC.

Nevertheless, many types of nanomaterials have been hypothesized to enhance the specificity of PCR amplification prior to our present study, now only ASMNPs and AuNPs had been tested. Therefore, we selected another three representative types of nanomaterials (titanium dioxide nanoparticles, CdTe nanoparticles, and carbon nanoparticles) to determine if the preferential inhibition of the amplification of long PCR

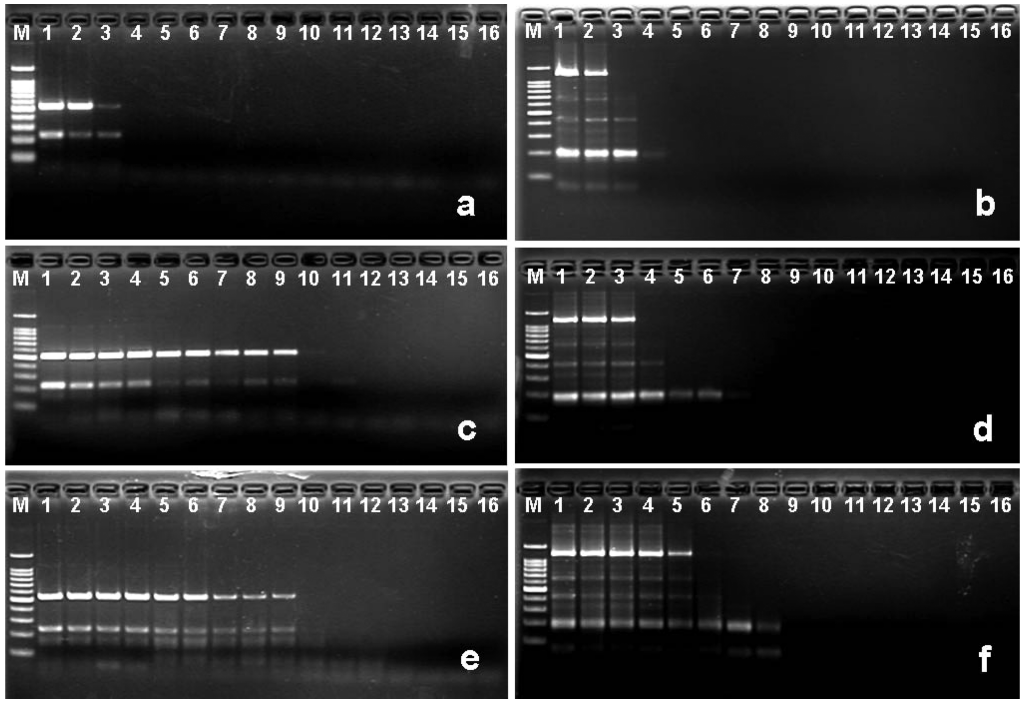

Figure 7. Effect of carbon nanoparticles (a and b), titanium dioxide nanoparticles (c and d), and CdTe quantum dot nanoparticles (e and f) on the amplification of short nonspecific amplicons (a, c, and e) using primers FS21-F/-R and quadruple PCR (b, d, and f) using primers S206-F/-R, S417-F/-R, S643-F/-R, and S1187-F/-R (DNA templates, *Salmonella* genomic DNA). (a and b) M: Markers. Lanes 1−15: 0, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.5, 3.0, and 3.5 μg of carbon nanoparticles. Lane 16: NC. (c and d) M: Markers. Lanes 1−15: 0, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.5, 3.0, and 3.5 μg of titanium dioxide. Lane 16: NC. (e and f) M: Markers. Lanes 1−15: 0, 0.02, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.8, 2.0, 10, and 20 nmol of CdTe quantum dots. Lane 16: NC.

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ACS Appl. Mater. Interfaces 2015, 7, 13142−13153

products was a phenomenon common to other types of nanomaterials. In fact, similar to ASMNPs and AuNPs, these nanomaterials also did not inhibit nonspecific products that were shorter than the target amplicons (Figure 7A, C, and E) but inhibit the amplification of longer PCR products followed by shorter products and finally completely inhibited PCR as the concentration of nanoparticles increased (Figure 7B, D, and F). These results, which were obtained using five representative nanomaterials (including several classes of nanomaterials: metal oxide nanoparticles, metal nanoparticles, semiconductor nanoparticles, and nonconductive nanoparticles), suggest that earlier claims that these nanoparticles enhanced the specificity of PCR were not accurate.

3.2.2. Mechanism by Which Nanoparticles Inhibit the Amplification of Amplicons of a Given Length. We have shown that the inhibitory effect was related to the binding between nanomaterials and DNA polymerase, Mg^{2+}, primers, and DNA templates. Thus, we next investigated these factors to identify the mechanism by which nanoparticles specifically inhibit the amplification of amplicons of a certain length.

3.2.2.1. Formation of ASMNPs:Taq DNA Polymerase Complexes Inhibits PCR Amplification and Is Not Related to Amplicon Length. Previously, the effects of nanomaterials on PCR, positive or negative, were all attributed to the binding between nanomaterials and DNA polymerase.[25−27] Although our previous results also suggested that ASMNPs could adsorb proteins, such as hydrolyzed skim milk, BSA, and even Taq DNA polymerase, the ability of the binding between ASMNPs and DNA polymerase to positively affect PCR, such as enhancing its specificity, was unclear. We mixed DNA polymerase with ASMNPs (1 mg: 200 U), and after incubation, the ASMNP:polymerase complexes were separated by a magnet. The complexes were then blocked with skim milk and washed. Various amounts of the ASMNP:polymerase complexes were added to the PCR system without free *Taq* DNA polymerase. The results (Figure 8A and B) show that no

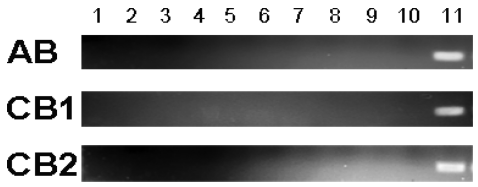

Figure 8. Inhibition of DNA polymerase by adsorption to ASMNPs (primers, QS95-F/-R; DNA templates, *Salmonella* genomic DNA). ASMNPs:polymerase complexes were prepared by passive adsorption binding (AB); covalent binding using glutaraldehyde as a coupling agent (CB1); covalent binding using EDC as a coupling agent (CB2). The amount of ASMNPs:polymerase complexes: lane 1−10, 5, 10, 20, 30, 40, 50, 60, 70, 80, or 160 μg; lane 11, lane 5 with an additional 1 U of *Taq* DNA polymerase.

amplification products were produced at any levels of the complexes tested. That is, the binding of the polymerase by ASMNPs did not positively affect PCR but inhibited PCR amplification. In addition, we also covalently linked ASMNPs to DNA polymerase using glutaraldehyde or 1-ethyl-3-(3-dimethyllaminopropyl) carbodiimide hydrochloride (EDC) as a coupling agent, as described previously.[36,37] The detailed coupling procedures are presented in the Supporting Information. The results (Figures 8 CB1 and CB2) were the same as those observed for the passive absorptive binding between *Taq* DNA polymerase and ASMNPs. In all cases, PCR with the ASMNPs:*Taq* DNA polymerase complexes did not produce amplicons. This lack of products was possibly due to steric hindrance (e.g., blocking of the DNA polymerase active site) or changes in enzyme conformation, which affected enzyme activity or substrate binding when *Taq* DNA polymerase was adsorbed to ASMNPs. Therefore, we should consider other factors to determine the mechanism of special inhibitory effect related to the length of amplicons.

3.2.2.1. Specific Inhibition of Amplicons of a Certain Length Is Due to the Binding between DNA Templates and ASMNPs. DNA, including oligonucleotide primers and DNA templates, has been demonstrated to be able to adsorb to ASMNPs. Oligonucleotide primers are short ssDNA, and the binding of primers to ASMNPs would likely result in the inhibition of PCR. Therefore, the binding of DNA templates and ASMNPs is likely responsible to the specific inhibition of amplicons of a certain length. ASMNPs are zero-dimensional nanomaterials, and DNA is a linear macromolecule. Once DNA molecules adsorb to ASMNPs, some DNA strands could bind to, or even wind around the particles, which would form a large number of "joints". If the "joints" contain all or part of the target amplicon's sequence (Figure 9A, case 1 and 2), the amplification would fail to initiate or terminate prematurely. If the nanoparticle does not bind to the target sequence (Figure 9A, case 3), PCR would not be inhibited. When the concentration of ASMNPs remains constant, the likelihood of the binding between part of or the entire amplicon and nanoparticles positively correlates with the length of the amplicon. This relationship reasonably explains why the inhibitory effect of ASMNPs on PCR is related to the amplicon length. To test the above speculation directly, the complexes formed by 30 μg of ASMNPs and 1 ng of *Salmonella* genomic DNA were characterized by AFM. The images (Figure 11B−C) show that "joints" indeed formed when the ASMNPs bound to large DNA fragments; free DNA was relaxed, while the DNA fragments between two "joints" were straightened by ASMNPs. The lengths of DNA fragments between two "joints" ranged from approximately 200 to 1200 nm (approximately 588 bp to approximately 3529 bp). Although we could not determine the amount of target amplicon sequences that were made inaccessible in these "joints", the binding of ASMNPs and the target sequences clearly interfered with amplification, suggesting that a target amplicon yielding a longer product was more likely to be bound by ASMNPs than a sequence yielding shorter amplicons. In addition, AuNPs:DNA complexes and carbon nanopowder:DNA complexes have previously been characterized[16,38] by AFM, yielding images similar to those presented here. Coincidentally, carbon nanopowder and AuNPs have also been reported to be able to enhance the "specificity" of PCR.[10,16]

3.2.3. Some Nanomaterials Inhibit Nonspecific Amplification Products Caused by Incomplete Amplification. In addition to false priming, nonspecific products could also be caused by incomplete amplification. In this case, the incomplete products appear as a diffuse smear of bands in stained agarose gels. We used multiple rounds of PCR, a common model, to explore the effects and mechanism. First, we amplified a 283 bp sequence using lambda DNA as a template, as previously described.[10] In the next round of PCR, the amplification products were employed as the templates. We observed that the nonspecific products (a slight diffuse smear of bands) began to appear in the third-round of PCR (Figure S7, Supporting Information); thus, the fourth round of PCR was used to

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ACS Appl. Mater. Interfaces 2015, 7, 13142−13153

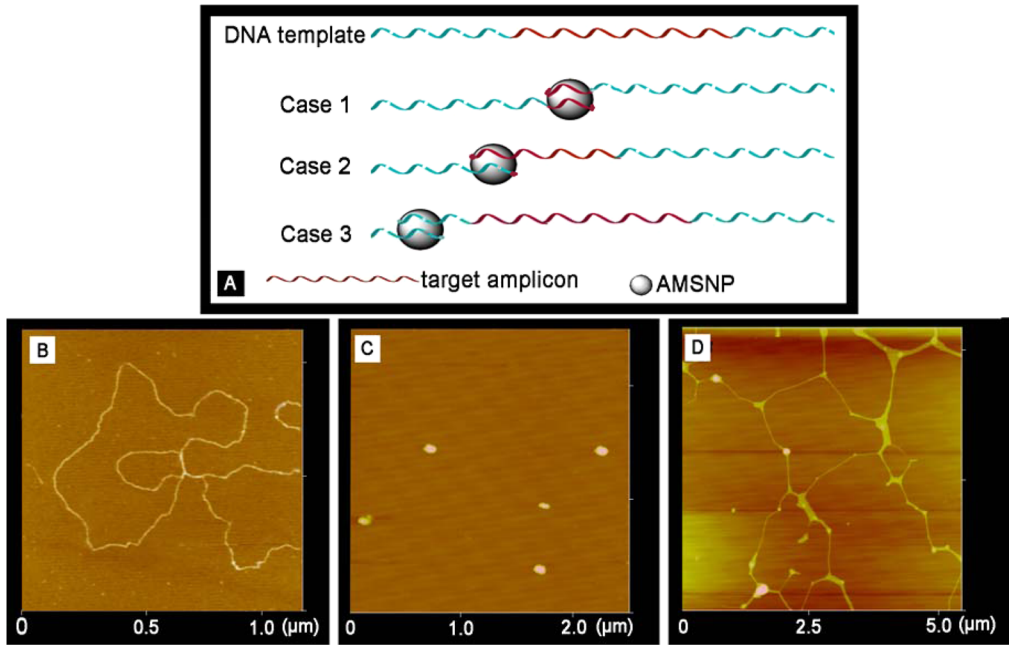

Figure 9. Binding of ASMNPs to DNA. Schematic diagram of possible binding situations between DNA templates and ASMNP (A), AFM images of *Salmonella* genomic DNA (B), ASMNPs (C), and ASMNPs:DNA complexes (D).

determine whether nanoparticles would enhance the amplification specificity. Figure 10A shows that the specificity of PCR positively correlated with the amount of ASMNPs up to 15 μg, but exceeding this limit significantly inhibited the amplification. The effect of AuNPs was also tested (Figure 5B), and the results were similar to those reported previously:[10] the appropriate amount of AuNPs was beneficial to the specificity.

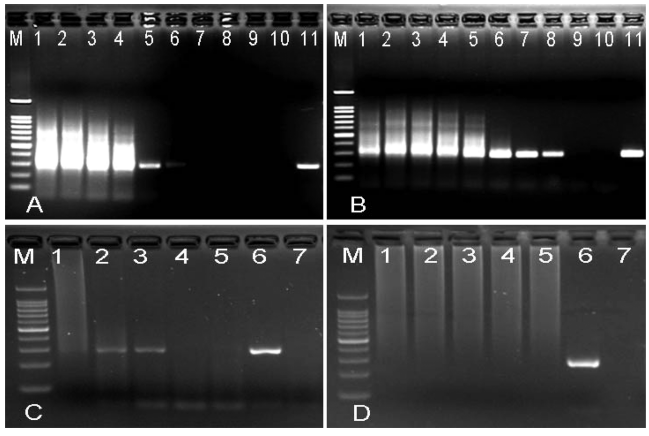

Figure 10. Effect of different amounts of ASMNPs (A) and AuNPs (B) on the specificity of the amplification of multiple-round PCR products [PCR template, 1 μL of the products of the third round of PCR (1:1000 dilution); primers, P283-F/-R]. (A) M: Markers. Lanes 1−9: 0, 2.5, 5, 10, 15, 20, 25, 30, and 35 μg of ASMNPs. Lane 10: NC. Lane 11: PC (DNA templates, lambda DNA). (B) M: Markers. Lanes 1−9: 0, 1.3, 2.6, 3.9, 5.2, 6.5, 7.8, 9.1, and 10.4 pmol of AuNPs. Lane 10: NC. Lane 11: PC (template, lambda DNA). The result of PCR using 1 μL of supernatant as DNA template after magnetic treatment and separation (C and D). M: Markers. Lanes 1−5: 0, 20, 40, 80, and 100 μg of ASMNPs. Lane 6: PC (DNA templates, lambda DNA). Lane 7: NC. (C) The mixtures (ASMNPs and 100 μL of 100-fold diluted products of third round PCR) were heated from room temperature (RT) to 90 °C; (D) the mixtures were kept at RT for 10 min.

The similar effects of ASMNPs and AuNPs on the specificity of amplification of multiple-round PCR products indicated that the enhancement of PCR specificity was not related to the type of material used to prepare the nanoparticles.

3.2.4. Mechanism by Which Nanomaterials Inhibit Nonspecific Amplification Products Caused by Incomplete Amplification. Luo et al.[39] observed via denaturing polyacrylamide gel electrophoresis that the nonspecific products of multiple-round PCR consisted of a large number of single-stranded oligonucleotides. Most single-stranded PCR products were smaller than single-stranded target amplicons, but these oligonucleotides cross-hybridized with each other to form multistranded aggregates that appeared as a diffuse smear of bands in stained agarose gels. This explanation seems quite plausible. In rare cases, the elongation of the DNA strands is terminated prematurely during PCR. Although the amount of incomplete amplification products is usually low for one round of PCR, the amount increases significantly after multiple amplifications. This phenomenon and explanation seem to contradict our previous conclusion that the amplification of the longer products was inhibited first, followed by the inhibition of shorter amplicons as the amount of nanoparticles increased, irrespective of specificity. In fact, we attributed these two phenomena to different mechanisms. Before the fourth round of PCR, we mixed different amounts of ASMNPs with 100 μL of 100-fold diluted products from the third round of PCR and heated the mixtures from room temperature to 90 °C. After magnetic separation, 1 μL of supernatant was used as a DNA template for PCR. Unexpectedly, the diffuse smear of bands disappeared, and the target products were enriched (Figure 10C). Hence, nanoparticles enhanced the specificity of PCR not during but at the beginning of the amplification reaction. We also mixed the products of the third round of PCR with ASMNPs at room temperature for 10 min, separated the ASMNPs and complexed DNA, and used 1 μL of supernatant as a template for PCR. For this experiment, ASMNPs did not enhance the specificity (Figure 10D). The temperature clearly

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ACS Appl. Mater. Interfaces 2015, 7, 13142−13153

needs to be increased in the presence of the ASMNPs to enhance the specificity of PCR.

Next, to explore how the temperature increase affected the adsorption between ASMNPs and DNA, 20 μg of ASMNPs were mixed with 100 pg of *Salmonella* genomic DNA in 500 μL of 1× PCR buffer, followed by vortexing for 10 min at various temperatures (30−90 °C). The complexes were then blocked using blocking solution and washed three times with 1× PCR buffer. Finally, the pellets containing magnetic nanoparticles and bound DNA were collected and used as templates for PCR. At lower temperatures (30−50 °C), the amount of amplification products was small (Figure 11A). That is, the

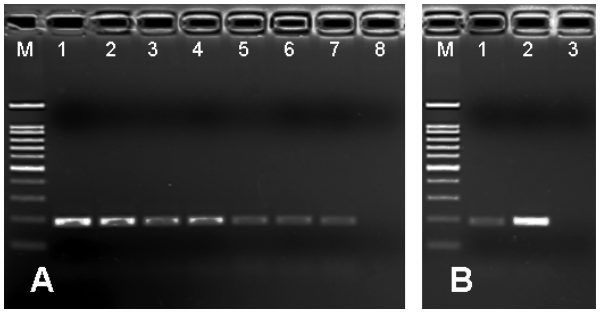

Figure 11. Effect of temperature on the capacity of ASMNPs to adsorb *Salmonella* genomic DNA (A). M: Markers. Lanes 1−7 temperatures: 90, 80, 70, 60, 50, 40, and 30 °C. Lane 8: NC. Comparison of the adsorption capacity of ASMNPs for dsDNA and ssDNA at room temperature (B). M: Markers. Lane 1: dsDNA (*Salmonella* genomic DNA). Lane 2: ssDNA (heat denatured *Salmonella* genomic DNA). Lane 3: NC.

capacity of ASMNPs to adsorb DNA was relatively low at these temperatures. At higher temperatures (60−90 °C), the adsorption of DNA by ASMNPs significantly increased, as demonstrated by increasing in the yield of amplification products. Genomic DNA maintained a double-stranded structure at low temperatures, and more genomic DNA became single-stranded as the temperature increased. ASMNPs possibly possess a much higher binding affinity for ssDNA than for dsDNA, which was reflected by the greater amount of PCR products observed for ASMNP:DNA complexes generated at higher temperatures. To further determine if this was the truth, *Salmonella* genomic DNA was denatured by heating before being mixed with ASMNPs. The PCR results (Figure 11B) show that considerably more PCR products were generated when ASMNP:DNA complexes formed with heat-denatured ssDNA than with double-stranded genomic DNA. This suggests that ASMNPs indeed have a higher affinity for ssDNA. Similarly, several researchers have reported that AuNPs bound ssDNA much more strongly than dsDNA.[40−43] The inhibition of nonspecific products of multiple-round PCR was caused by the absorption of ASMNPs and the incomplete amplification products. The incomplete amplification products were shorter than the target products. Thus, they were denatured preferentially to full-length PCR products, and they also preferentially adsorbed to ASMNPs; thus, the incomplete amplification products did not be amplified again and again. In addition to multiple-round PCR, the long PCR system[12] is also prone to premature termination, the mechanism that nanoparticles enhance the specificity of long PCR is possibly the same as multiple-round PCR. Furthermore, Mi et al.[27] reported that AuNPs inactivated the DNA polymerase at low temperatures, resembling an antibody-based hot-start PCR, and the amplification of nonspecific products was reduced in the presence of AuNPs. They speculated that the phenomenon was caused by the binding of DNA polymerase and nanoparticles. Once again, based on the results presented here, an appropriate amount of AuNPs might have allowed the incomplete amplification products to be adsorbed before PCR.

3.3. Mechanism by Which Nanomaterials Affect PCR Efficiency and Yield. Li et al.[19] reported that AuNPs enhanced the efficiency of PCR: when the reaction time (including denaturation time, extension time, and annealing time) was shortened, the yields of PCR products in the presence of AuNPs were the same or higher than those in the absence of the AuNPs. We concluded that nanoparticles affected PCR efficiency by facilitating DNA melting via strongly binding between ssDNA and nanoparticles as shown in section 3.2.4. To further prove this speculation, we explored the effect of nanoparticles on the melting temperatures (Tm) of PCR products. A section of *Salmonella* genomic DNA was amplified by RT-PCR with SYBR Green I dye using the primers QS95-F/-R. Then, various amounts of AuNPs were added to the PCR solution, and the melting curves were determined. The apparent T_m negatively correlated with the amount of added (Figure 12A), which was consistent with previously reported results.[44] ASMNPs also decreased the T_m (Figure 12B). These results further suggest the strong binding between ssDNA and nanoparticles prevents ssDNA from reannealing to dsDNA. Our inference was consistent with a previous report[44] by Lou et al., who suggested that AuNPs improve PCR efficiency by facilitating the dissociation of dsDNA, but they attributed this effect to the surface interaction between AuNPs and the products in a manner similar to SSB. During DNA replication, dsDNA is dissociated by DNA helicase, and SSB binds to the single-stranded regions. After a complementary strand is synthesized, SSB will break away from original site and bind

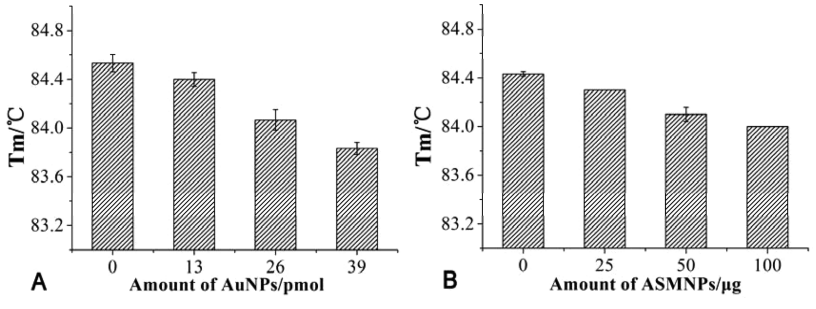

Figure 12. Effect of AuNPs (A) and ASMNPs (B) on DNA melting.

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to a new single-stranded region.[9] However, nanoparticles have not been shown to be able to dynamically dissociate from dsDNA and bind to ssDNA in a manner analogous to that of SSB. In addition, as presented in section 3.2.2 of this study, once nanoparticles bind to amplicons (or PCR products), amplification is inhibited. In summary, we suggest that the key effect of nanoparticles on PCR efficiency is during the initial denaturation, which is directly related to the availability of ssDNA substrates because the original amplicon sequences located in the whole DNA templates are used as main templates in the first cycles. When denaturation is not efficient in the beginning, many original DNA templates, such as genomic DNA, will not become single stranded, and most importantly, amplicon sequences located in dsDNA templates will not dissociate. In this case, the amount of amplification products will be drastically reduced because PCR amplification is exponential. The addition of nanoparticles facilitates the denaturation of original DNA templates and improves the efficiency and speed of this process; thus, the short PCR denaturation step does not affect the reaction efficiency or the net amount of the products. The single-stranded amplicon sequences located in the DNA templates must also remain free (not bound to nanoparticles), except to facilitate the dissociation of DNA templates as much as possible. Therefore, the amount of nanoparticles should be optimized to maximize the amplification efficiency. Yuan et al. reported that after the modification of AuNPs with poly(diallyldimethylammonium) chloride (PDDA), a substantially smaller amount of the modified AuNPs was needed to enhance the efficiency of PCR.[20] Cationic conjugated polyelectrolytes interacts with ssDNA more strongly than dsDNA;[45,46] thus, the improved property of PDDA-AuNPs is consistent with our conclusion: the enhanced efficiency and yields of PCR are because of the strong binding between these modified nanomaterials and ssDNA. In addition, the two research groups used CdTe QDs as additives to affect PCR. Wang et al.[28] reported that the nanoparticles did not affect the efficiency of PCR, but Liang et al.[14] reported that nanoparticles enhanced the PCR efficiency. The contradictory results could have been due to the considerable difference in length between the original DNA templates used in these two studies. Wang et al. used plasmid pSK (3,757 bp[47]), while Liang et al. used *Triticum aestivum* genomic DNA (the whole genome is approximately 4.8−5.7 Gb[48]) as their DNA template. Because the plasmid pSK is much smaller, it would readily denature, even in the absence of the nanoparticles; thus, the added CdTe QDs minimally affected the efficiency of PCR. Our above assumptions are also supported by previous reports,[49,50] which demonstrated that the interactions between QDs and ssDNA were stronger than those between QDs and dsDNA. More directly, Algar et al. reported that QDs hastened the melting transition and altered the melting temperature of dsDNA via hydrogen-bonding interactions between ssDNA and QDs capped with mercaptoacetic acid ligands.[41] Similar to our conclusions, they determined that the interactions between ssDNA and QDs helped "pull" the duplex apart by "grabbing" segments of the duplex, which prevented the duplex from reforming.

However, ASMNPs did not enhance the efficiency or yield of PCR, irrespective of their concentration (data not shown). We propose the following reasons for this observation: in addition to promoting the denaturation of dsDNA, ASMNPs also adsorb polymerase, Mg^{2+}, primers, and DNA templates to inhibit the amplification of PCR; thus, the negative effects may have outweighed the positive effects. In summary, we speculate that the nanomaterials that enhance the efficiency and yield of PCR should have a strong binding capacity for ssDNA and a low binding capacity for other PCR components.

Most of the effects of nanomaterials on PCR are due to the interaction between the surface of nanoparticles and PCR components. The results of PCR clearly depend on the adsorption preference for individual PCR components. Even for a given type of nanomaterial, the amount of nanoparticles used will need to be optimized based on the composition of the PCR mixture (i.e., different amounts of DNA templates and DNA polymerase) to obtain the desired effect. In addition, several different mechanisms play roles in the effect of nanoparticles on PCR; thus, we should consider several factors.

4. CONCLUSION

On the basis of the experimental evidence presented, we observed that the surface of nanomaterials played an important role in their effect on PCR: (1) The inhibition of PCR was primarily because of the nanomaterial surface, especially the surface active groups. Not only DNA polymerase but also Mg^{2+}, oligonucleotide primers, and DNA templates adsorbed to nanomaterials, which might inhibit PCR. (2) Nanomaterials did not inhibit nonspecific amplification products caused by false priming. Our experimental evidence suggested that longer amplicons were more readily inhibited at lower concentrations of nanomaterials, and the amplification of shorter amplicons was inhibited at higher concentrations of nanomaterials. The so-called enhanced specificity reported by others was an artifactual conclusion based on the inhibition of the amplification of longer nonspecific amplicons. This specific inhibitory effect related to the amplicon size was caused by the binding of nanomaterials and DNA templates. However, when the nonspecific amplification products were the result of incomplete amplification, nanomaterials could enhance the specificity of PCR by preferentially adsorbing the single-stranded incomplete products. (3) The efficiency and yield of PCR was able to be enhanced by adding certain types of nanomaterials, such as AuNPs, because these nanomaterials could strongly adsorb ssDNA, facilitating the more rapid and thorough denaturation of the dsDNA templates. In summary, the surface of nanoparticles significantly affects PCR, and the effect on PCR depends on the surface properties of the particles. Understanding the physical properties of nanoparticles and the mechanisms by which they affect PCR will facilitate a more rational approach for optimizing nanoparticles for the enhancement of PCR.

■ ASSOCIATED CONTENT

Supporting Information

Preparation and characterization of several types of nanoparticles, preparation of ASMNPs:DNA polymerase complexes by covalent methods, error-prone multiple rounds of DNA amplification, and figures. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/am508842v.

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Notes

The authors declare no competing financial interest.

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