Nanomaterials to Enhance Polymerase Chain Reaction

Subjects: Biochemical Research Methods

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Polymerase Chain Reaction (PCR) is one of the most common technologies used to produce millions of copies of targeted nucleic acid in vitro and has become an indispensable technique in molecular biology. However, it suffers from low efficiency and specificity problems, false positive results, and so on. Although many conditions can be optimized to increase PCR yield, such as the magnesium ion concentration, the DNA polymerases, the number of cycles, and so on, they are not all-purpose and the optimization can be case dependent. Nano-sized materials offer a possible solution to improve both the quality and productivity of PCR. Nanoparticles (NPs) have attracted significant attention and gradually penetrated the field of life sciences because of their unique chemical and physical properties, such as their large surface area and small size effect, which have greatly promoted developments in life science and technology. Additionally, PCR technology assisted by NPs (NanoPCR) such as gold NPs (Au NPs), quantum dots (QDs), and carbon nanotubes (CNTs), etc., have been developed to significantly improve the specificity, efficiency, and sensitivity of PCR and to accelerate the PCR reaction process.

Keywords: NanoPCR ; nanomaterials ; specificity ; efficiency ; mechanisms

1. Introduction

Polymerase chain reaction (PCR) technology, first proposed by Mullis in the United States in 1983 and invented in 1985, is a molecular biology technique used to amplify specific DNA fragments and is regarded as a unique DNA replication in vitro. The most prominent feature of PCR is that it can significantly increase the trace amount of DNA. So far, it has been widely used in many different fields, such as medical diagnosis [1], food safety [2], archaeological research [3], basic bioresearch, etc. However, the development of PCR is subject to certain limits owing to low specificity, efficiency, and sensitivity. Although some important parameters in PCR have been optimized to improve its specificity and efficiency, including polymerase concentration, annealing temperature, cycle number, template type, primer design, and magnesium ion concentration, the effect is still not satisfactory [4]. Nanotechnology, therefore, has been applied to improve the performance of PCR. At present, many nanomaterials have been successfully used to enhance the specificity and efficiency of PCR, such as Au NPs [5], QDs [6], CNTs [7], graphene oxide (GO) and reduced GO (rGO) [8], partial metal oxidation materials [9][10] (e.g., titanium dioxide, zinc oxide) and other composite materials like macromolecule polymer doped with Au NPs [11], amino-modified semiconductor magnetic NPs [12], and so on.

Nanomaterials are composed of particles with at least one external dimension less than 100 nm, and they have been widely used in electronics, aerospace, military, chemical, biomedical, and healthcare products [13]. In PCR, nanomaterials are added to the reaction system that mainly contains primers, enzymes, and templates, due to the characteristics of nanomaterials and their role in PCR. Here, numerous NPs were divided into three categories according to the effects of nanoparticles in PCR. The first type of nanomaterial, such as Au NPs, GO, carbon nanopowder (CNP), etc., has good thermal conductivity, which could speed up the process and shorten the reaction time of the original reaction procedure, therefore enhancing the efficiency of PCR. The second one, which includes CNTs, magnetic NPs, polymer-modified silica, QDs, etc., may interact with the surface of nanomaterials via van der Waals forces among the reaction system components, or provide many binding sites to fix polymerases, so that the added template and material form a competitive relationship, and thus enhance the specificity of PCR. The third type includes polymer-modified gold, GO, CNTs, ZnO with amino groups, etc., which have a positive surface charge and attract negatively charged nucleotide chains (templates and primers) containing phosphate groups, thereby enhancing the specificity of PCR. Of course, the enhancement of each type of NP described here in PCR is not just a cause, it is the main enhancement mechanism. Some NPs create catalytic activity or similar to ssDNA-binding proteins (SSB), which have the characteristics of selective adsorption of ssDNA. This is often caused by a variety of factors.

2. Utilizing Different Nanomaterials to Enhance PCR Effects

2.1. Metal Nanomaterials

2.1.1. Au NPs

Au NPs are the most well-studied nanoparticles, and their interesting chemical and photophysical properties make them an integral part of nanoscience and ideal for biological and other commercial laboratories, which contain non-toxic, good biocompatibility, and unique chemical and optoelectronic properties. For example, Au NPs are characterized by adjustable size and physical size, catalytic activity, high surface volume ratio, high stability, easy synthesis and surface modification, and strong light absorption and scattering properties [5][14]. **Figure 1** shows transmission electron microscopy (TEM) images of Au NPs in different morphologies [15][16].

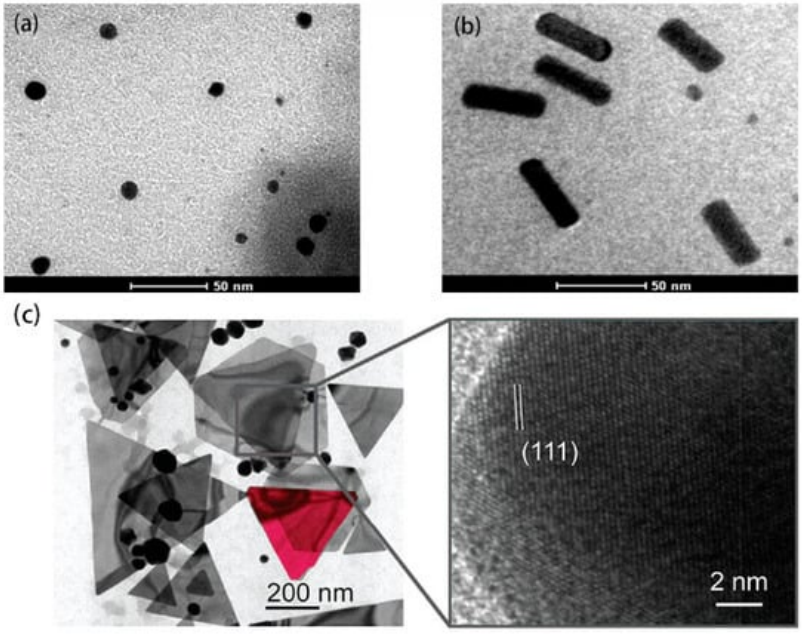

Figure 1. TEM images of (**a**) spherical, (**b**) rod (Reproduced with permission from Ref. [15], Copyright 2022, Elsevier), and (**c**) **triangular** Au NPs. (Reproduced with permission from Ref. [16], Copyright 2020, American Chemical Society).

Li et al. for the first time reported the optimization of Au NPs on PCR. The Au NPs with a particle size of about 13 nm were proved to dramatically improve the efficiency of PCR. Compared with the reagent without Au NPs, the amplification yield of PCR reagent with Au NPs increased at least 10^4 to 10^6-fold with shortened PCR time in testing different sizes of the DNA fragments [17]. Subsequently, Pan et al. studied the interaction mechanism of Au NPs and DNA polymerase in the PCR system. It was found that Au NPs could optimize the PCR amplification strategy and inhibit the nonspecific amplification of PCR. The amplification limit of detection (LOD) increased approximately seven-fold [18]. In 2008, Binh et al. found that the effect of Au NPs was not to increase PCR specificity but to favor smaller products over more oversized products, regardless of specificity. Such an effect could be duplicated simply by reducing polymerase concentration but be reversed by increasing polymerase concentration or adding BSA as a competitive displacer [19]. The study of the interaction between Au NPs and DNA polymerase indicated that the addition of DNA polymerase could eliminate the inhibitory effects of the excess Au NPs, and in the reverse, Au NPs could eliminate the inhibitory effects of the excess DNA polymerase [20]. Moreover, Au NPs have been proven to be able to simultaneously enhance both PCR efficiency and specificity by improving the thermal conductivity of the PCR solution [21]. Afterwards, Lou et al. generally summarized the three effects of Au NPs on PCR: (1) Au NPs adsorbed polymerase; (2) Au NPs decreased the melting temperatures (Tm) of both complementary and mismatched primers and increased the Tm difference between them; and (3) Au NPs facilitated the dissociation of the PCR products in the denaturing step [22]. Mandal et al. proposed that the enhancement of PCR yield by Au NPs with a particle size of 11 nm could be attributed to the greater affinity and thermodynamic stability of Au NPs for Taq DNA polymerase compared to the primer or DNA template [23].

2.1.2. Ag NPs

At present, the use of metallic silver, silver nitrate, and silver sulfadiazine to treat burns, wounds, and bacterial infections has significantly declined because of the emergence of antibiotics. With the tremendous impetus of nanotechnology gains, nano-sized silver (Ag NPs) shows dramatically diverse chemical, physical, and optical properties and has high optical

tunability, large absorption cross sections, and scattering properties [16]. **Figure 2**a,b are the TEM images of triangular NPs and spherical Ag NPs, respectively.

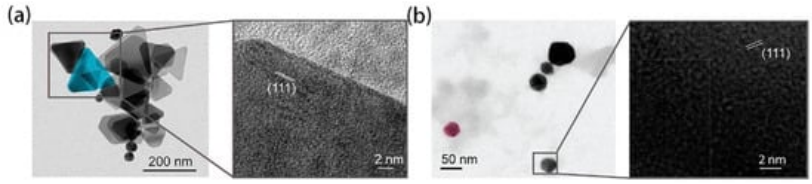

Figure 2. (**a**) Triangular and (**b**) spherical Ag NPs with lattice orientation zoomed image. (Reproduced with permission from Ref. [16], Copyright 2020, American Chemical Society).

Wang et al. found that the presence of Ag NPs could significantly retain the specificity of long PCR products after three rounds of repeated amplification [24]. Liu et al. studied the effect of Ag NPs on DNA synthesis in PCR where Ag NPs over a certain size and concentration significantly inhibited PCR amplification [25]. Recently, Kadu et al. reported the effect of the shape of Ag NPs on photothermal properties and PCR efficiency. Triangular Ag NPs were able to increase PCR efficiency [16].

2.2. Carbon-Based Nanomaterials

2.2.1. CNTs

CNTs are greatly advantageous because of high electron transport without electronic scattering and electronic conductivity; thus, they provide high-performance sensing transistors. More specifically, CNTs possess a high aspect ratio (the ratio of lateral size to thickness), large specific surface area (SWCNT > 1600 m^2/g, MWCNT > 430 m^2/g), as well as good mechanical and electrical (~5000 s/cm) properties [26][27]. The SEM and TEM images of SWCNTs and MWCNTs are shown in **Figure 3**.

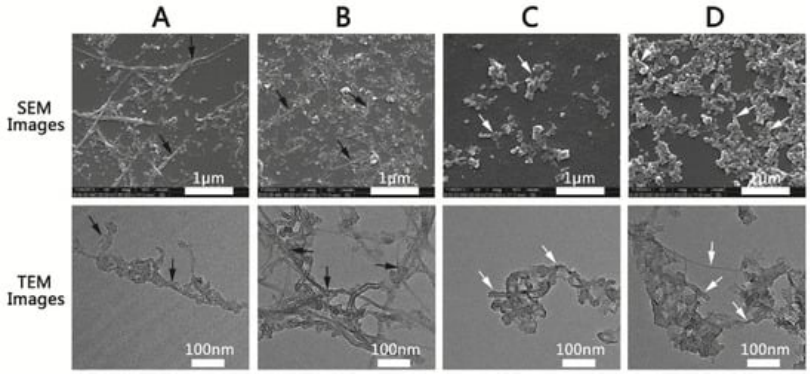

Figure 3. The SEM and TEM images of (**A**) unmodified SWCNTs, (**B**) modified SWCNTs, (**C**) unmodified MWCNTs, and (**D**) modified MWCNTs. The black and white arrows refer to SWCNTs and MWCNTs, respectively. (Reproduced with permission from Ref. [27], Copyright 2018, Japanese Society for Dental Materials and Devices).

Cui et al. first reported the positive effect of CNTs on PCR amplification. The addition of single-walled CNTs (SWCNTs) into the reaction liquid increased the amount of PCR product at a SWCNT concentration of 3 μg μL^{-1}, but reversed at SWCNT concentrations higher than 3 μg μL^{-1} [7]. The beneficial effect of both SWCNTs and multiwalled CNTs (MWCNTs) was also reported to enhance the specificity and total efficiency of long PCR (14 kb). The hydroxylic and carboxylic CNTs had similar enhancing effects as well. Moreover, various functional groups and polymer-modified CNTs also played an even more substantial role in enhancing PCR amplification [28]. The PEI-modified MWCNTs with different surface charge polarities as a novel class of enhancers were successfully used to improve the specificity and efficiency of PCR. Positively charged PEI-modified MWCNTs (CNT/PEI) significantly enhanced the specificity and efficiency of PCR at an optimum concentration as low as 0.39 mg L^{-1}, whereas neutral CNT/PEI modified with acetic anhydride (CNT/PEI.Ac) had no such effect. Although the negatively charged CNT/PEI modified with succinic anhydride (CNT/PEI.SAH) could enhance the PCR, the optimum concentration required (630 mg L^{-1}) was over three orders of magnitude higher than that of the positively charged CNT/PEI [29]. On the other hand, the amine functionalized MWCNT (NH_2-MWCNT) dispersion enhanced total PCR efficiency up to 70% after being sonicated, centrifuged, and filtered, while NH_2-MWCNTs inhibited the reaction significantly at similar concentrations without being filtered [30]. The study of three kinds of CNTs containing pristine, amine-functionalized, and carboxyl-functionalized SCNTs showed that both the pristine and the amine-

functionalized SCNTs could enhance the final amplification yields of the samples. However, the carboxylated SCNTs displayed an inhibitory action in all samples [31].

2.2.2. CNP

CNP has high specific surface area, strong adsorption, and high electrochemical capacity. As seen in **Figure 4**a, CNP has two broad peaks at 2θ of 25° and 43.8°, respectively. The diffraction peaks correspond to the planes (002) and (101) of graphite, indicating either a high degree of graphitization or a high degree of crystallinity, which can increase the thermal conductivity of CNP nanofluids due to the amorphous particles scatter phonon. This is probably the main reason why it enhances PCR. **Figure 4**b,c show the SEM photographs of the morphology of CNP. Clearly, the CNP is irregular, and the particles tend to aggregate with the diameter of CNP around 60 nm [32].

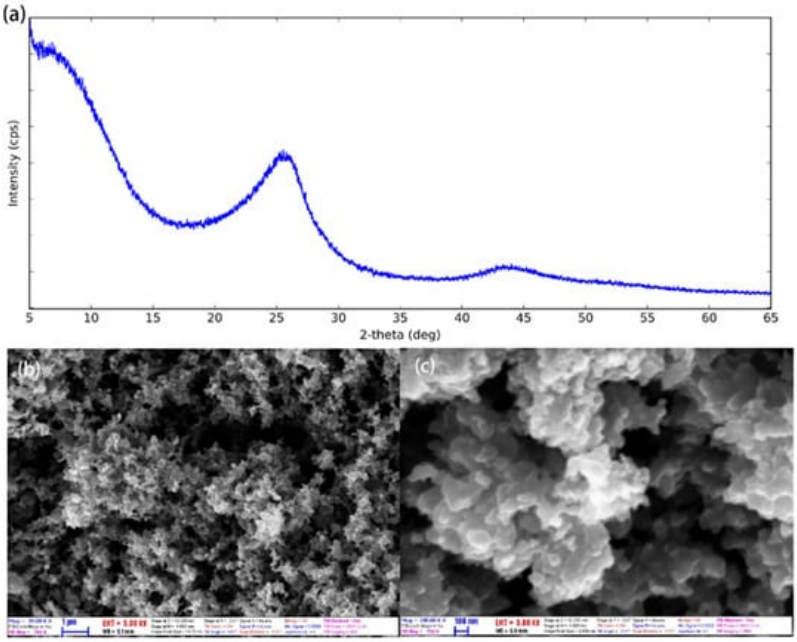

Figure 4. (**a**) XRD pattern of CNP at the following XRD conditions: X-ray: 40 kV, 30 mA. Scan speed: 3.0 degree/min. (**b**,**c**) SEM images of CNP with magnification ×20,000 and ×100,000. (Reproduced with permission from Ref. [32], Copyright 2021, MDPI).

Over ten years ago, carbon nanopowder was proven for the first time to have a beneficial effect on enhancing the efficiency of PCR amplification in a repeated PCR and a long PCR system. For the repeated PCR, the addition of a certain amount of CNP could obtain the target products even in sixth-round amplification with high specificity dependent on the concentration of CNP. The CNP significantly improved the amplification efficiency for long PCR reactions [33].

2.2.3. Graphene

Graphene, known as a 2D crystal of sp^2-hybridized carbon atoms arranged in six-membered rings, has an extensive theoretical specific area, unparalleled thermal and electricity conductivity, and fascinating electronic properties such as an ambipolar electric field effect along with ballistic conduction of charge carriers [34]. However, during the preparation of GO, the oxygen-containing functional groups are usually introduced on the surface of graphene, and these heteroatoms will combine with adjacent carbon atoms through covalent bonds or weak van der Waals forces, resulting in a sharp decrease in thermal conductivity due to high-density defects caused to graphene [35]. Therefore, the thermal conductivity better enhances the properties of rGO than GO in PCR.

In the study of graphene-enhanced PCR, Jia et al. found that the specificity of the PCR amplification could be improved by adding GO at concentrations from 12 $mg{\cdot}mL^{-1}$ to 60 $mg{\cdot}mL^{-1}$. However, GO did not affect the PCR when the GO concentration was lower than 12 $mg{\cdot}mL^{-1}$, while it exhibited an inhibitory effect at concentrations higher than 70 $mg{\cdot}mL^{-1}$. This study first demonstrated that rGO could significantly improve PCR specificity. It was then concluded that rGO was superior to GO in enhancing specificity [36]. Wang et al. further demonstrated that 1 $\mu g{\cdot}mL^{-1}$ of GO effectively enhanced the specificity of the error-prone multi-round PCR [8]. In addition to conventional graphene, Abdul et al. explored the effect of graphene nanoflakes (GNFs) on PCR and found that 0.01% (*w*/*w*) GNFs provided an unambiguous 10-fold enhancement in the PCR yield. In addition, the thickness of the GNFs had a significant impact on the yield of PCR products. The 8 nm-thick GNFs increased the yield higher than other sizes [37]. Recently, Zhong et al. discussed the

effects of GO through surface modification on PCR. The zwitterionic polymer-modified GO was found to be superior to other GO derivatives, with different charges enhancing the specificity of PCR [38].

2.3. Oxide Nanomaterials

2.3.1. TiO_2

TiO_2 has been known as one of the cheapest and most widely-available types of NPs utilized for thermal conductivity enhancement [39]. Murshed et al. [40] demonstrated that TiO_2 NPs have wonderful physical and chemical stability. It has been found that their particle size, shape, and volume fraction are the most critical factors that contribute to enhanced thermal conductivity.

Both size and concentration of TiO_2 NPs affects PCR. It was found that TiO_2 NPs inhibited DNA synthesis in vitro more severely than the TiO_2 particles in microscale at the equivalent concentration and the inhibition effect of TiO_2 NPs was concentration-dependent in the dark [9]. About a decade ago, Rak et al. observed that TiO_2 NPs with ~25 nm diameter caused significant enhancement of PCR efficiency for various types of templates. The optimal concentration was determined to be 0.4 nM, resulting in up to a seven-fold increase in the amount of PCR product. As much as a 50% reduction in overall reaction time was also achieved by utilizing TiO_2 NPs without compromising the PCR yield [41]. Upon the addition of TiO_2 NPs with a particle size of 7 nm to the ordinary PCR, RT-qPCR, and RT-PCR (reverse transcription PCR), the effects of TiO_2 NPs were investigated. The results indicated that 0.2 nM TiO_2 NPs could achieve target amplification at a very low template concentration in an ordinary PCR system. Furthermore, relative to the larger TiO_2 particles (25 nm) used in a previous study, the smaller TiO_2 particles (7 nm) used in this study increased the yield of PCR by three-fold or more [42].

2.3.2. ZnO

ZnO has been widely studied because it is non-toxic and easy to synthesize. Up to now, powdery ZnO in various morphologies, including nanowires, nanoflowers, and spherical and hierarchical structures have been successfully prepared and used to study their photocatalytic properties [43]. ZnO nanoflowers and their composites have been effectively used for PCR [44]. The XRD patterns and SEM images of ZnO nanoflowers are shown in **Figure 5**. The diffraction peaks are exactly the same as the standard card in the ZnO powder diffraction file (PDF) #36-1451. This clearly shows that the synthesized ZnO nanoflowers are of high purity. The SEM images show that the synthesized ZnO nanoflowers are self-assembling and clearly depict the nanopetal-like structure that emerges from the center of the flower. The synthesized ZnO nanoflowers are clear, uncrowded, and well dispersed, with an average diameter of about 1–2 μm [44].

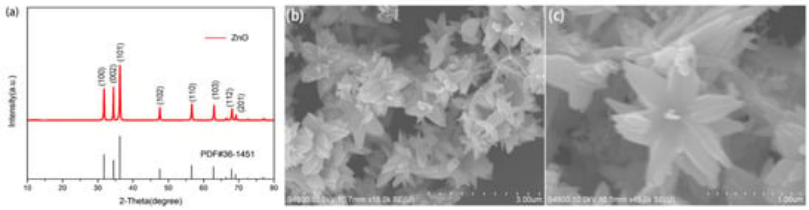

Figure 5. (**a**) XRD patterns of ZnO nanoflowers. SEM images of ZnO: (**b**) low magnification with a diameter of 3.00 μm, (**c**) high magnification with a diameter of 1.00 μm. (Reproduced with permission from Ref. [44], Copyright 2020, MDPI).

The tetrapod-shaped SiO_2-coated ZnO nanostructure with amino groups on the surface was first discovered to have a positive effect on PCR and could increase the yield of PCR amplification [10]. The incorporation of the ZnO nanoflowers in PCR led to a drastic improvement in the efficiency and yield of the ZnO nanoflower-assisted PCR, and reduced the time to perform the PCR assay [44].

2.3.3. Fe_3O_4

Magnetic NPs like Fe_3O_4 are characteristic of good magnetization and super-para-magnetism. Compared with other nanomaterials, the surface of magnetic NPs is more able to be functionalized.

For instance, Fe_3O_4 nanomaterials have been found to be able to improve the sensitivity of PCR with a detection limit reaching 4.26 mol·L^{-1}. Kambli et al. compared the PCR efficiency enhanced by three transition metal NPs in the form of stable colloidal suspensions at varying concentrations. The AFM images of three nanoparticles are shown in **Figure 6**. Compared to the citrate stabilized Ag NPs (25 nm, 45%) and Au NPs (15.19 nm, 134%), the highest amplification

efficiency of 190% was received using the ammonium salt of oleic acid-coated Fe_3O_4 NPs with an average size of 33 nm at a concentration of 7.2 × 10^{-3} nM in a conventional PCR system [45].

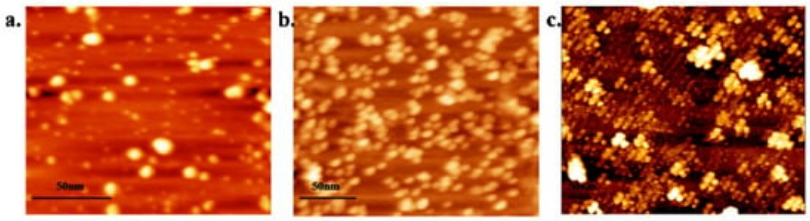

Figure 6. AFM images (**a**) Ag, (**b**) Au and (**c**) magnetite NPs, respectively. (Reproduced with permission. from Ref. [45], Copyright 2016, Elsevier).

Ozalp et al. synthesized magnetic core-silica shell NPs for easy one-step fixation of Taq polymerase directly from crude extract formulations. The magnetic properties of the pellets facilitate rapid purification to eliminate inhibitory elements present in the crude extract during Taq polymerase isolation. They found that at room temperature, after one month, the common Taq enzyme lost about 50% of the cationic activity of the amplification product, while the Taq-silica hybrid retained its original activity for about five months. Additionally, by recovering the Taq polymerase immobilized on the magnetic silica nanoparticles, repeated PCR was performed, and it was found that the immobilized enzymes still retained their original activity after four cycles, although their activity decreased to 45% after seven cycles [46]. Recently, Yajima et al. successfully developed photo-cross-linkable probe-modified magnetic particles (PPMPs) for sequence-specific recovery of target nucleic acids using optical cross-linkable artificial nucleic acid probes and magnetic particles. PPMPs were prepared by adding biotin to the end of the photo-cross-linkable probe following affinity binding with streptavidin-coated magnetic beads. Nucleic acid probes modified with photo-cross-linked artificial nucleic acids can hybridize to the nucleic acid of interest in a sequence-specific manner and then firmly capture the nucleic acid of interest by covalent bonding mediated by UV irradiation. Then, the target nucleic acid is detected by trapping the target-bound probe on the surface of the magnetic particles and subjecting these collected magnetic particles to PCR so as to improve the sensitivity of the PCR detection (**Figure 7**) [47].

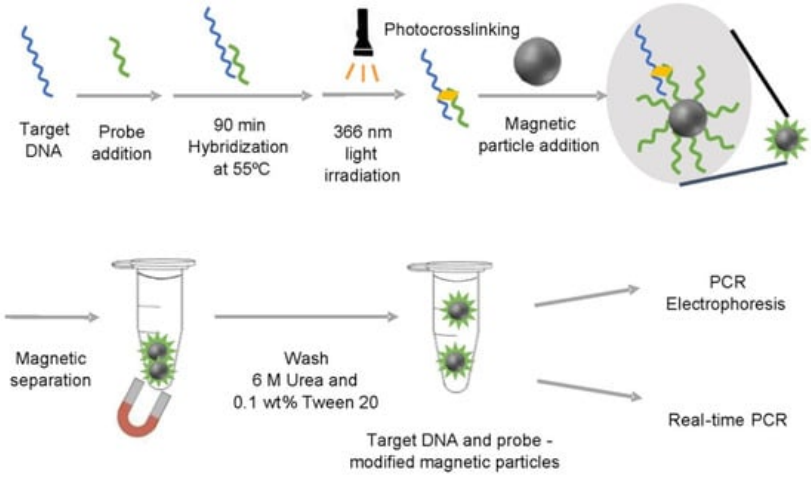

Figure 7. Recovery of target nucleic acids using photo-cross-linkable artificial nucleic acid probes. (Reproduced with permission from Ref. [47], Copyright 2022, American Chemical Society).

2.3.4. MgO

MgO nanomaterials have unique properties such as being highly stable with good dispensability and less toxic effects. For example, Narang et al. introduced MgO NPs to a PCR system and caused significant improvement in PCR efficiency [48].

2.3.5. SiO_2

SiO_2 nanomaterials with well-defined morphology and porosity were first prepared and characterized by Stober. Carbonized polydopamine silica (C-PDA silica) were synthesized and employed to increase PCR efficiency (**Figure 8**). As compared with the effects of SiO_2 NPs and PDA silica on PCR, C-PDA silica exhibited about 1.5 and 1.2 times higher efficiency. As a result, C-PDA silica can not only reduce the PCR cycle but also increase the final quantity of the PCR product [49].

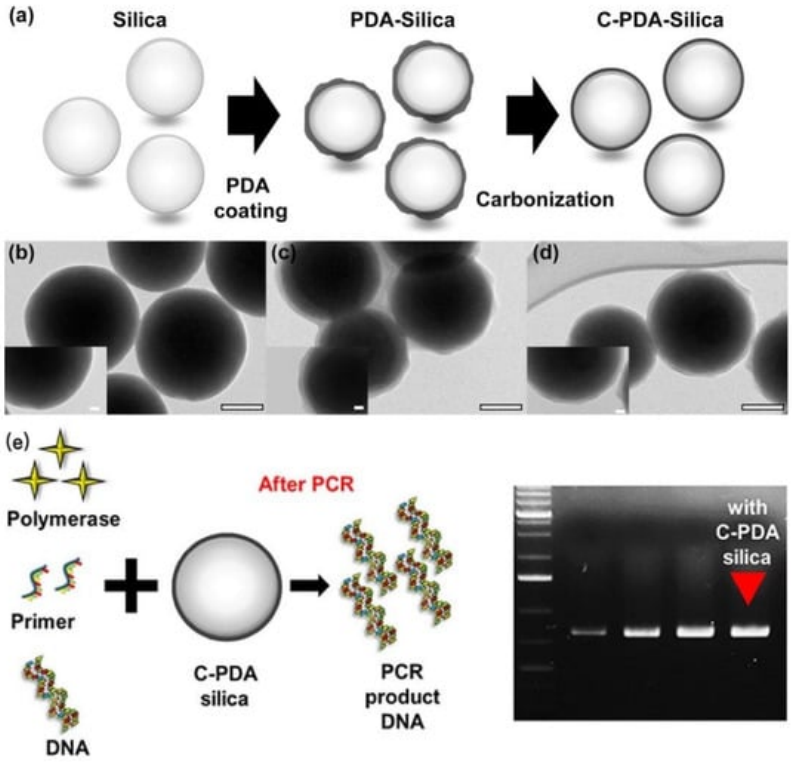

Figure 8. (**a**) Schematic illustration of the synthesis process of silica, PDA silica, and C-PDA silica and (**b**) their corresponding TEM images. All scale bars in (**b**–**d**) are 100 nm for the main panels and 20 nm for the insets, respectively. (**e**) C-PDA silica was employed to increase the PCR efficiency. (Reproduced with permission from Ref. [49], Copyright 2015, American Chemical Society).

2.4. Fluorescent Nanomaterials

2.4.1. QDs

QDs, as a new kind of fluorescent material, possess many excellent characteristics such as size-tunable emission, wide absorbance bands, narrow symmetric emission bands, high photostability, etc.

In 2009, Wang et al. [6] first found that CdTe QDs could increase the specificity of the PCR at different annealing temperatures with DNA templates of different lengths. Also, CdTe QDs were reported to accelerate PCR speed [50]. Then a Pfu polymerase based multi-round PCR technique was developed through being assisted by CdTe QDs, and the specificity could be retained even in ninth-round amplification [51]. The stacking of the primers on graphene QDs(GQDs) could improve the sensitivity and specificity of PCR by improving the efficiency of base-pairing between the primer and the template. By increasing polymerase activity, GQDs could improve the yield of PCR, where GQDs are tuned through chelating magnesium ions with their peripheral carboxylic groups [52].

2.4.2. Up-Conversion Nanomaterials

Photon up-conversion is the phenomenon where high-energy photons are emitted upon the excitation of low-energy photons (**Figure 9**). Nucleic acid detection based on up-conversion NPs (UCNPs) displays a high signal-to-noise ratio and no photobleaching and has been widely applied. For example, Wang et al. [53] demonstrated that the addition of UCNPs to the reaction mixtures at appropriate concentrations could improve PCR specificity.

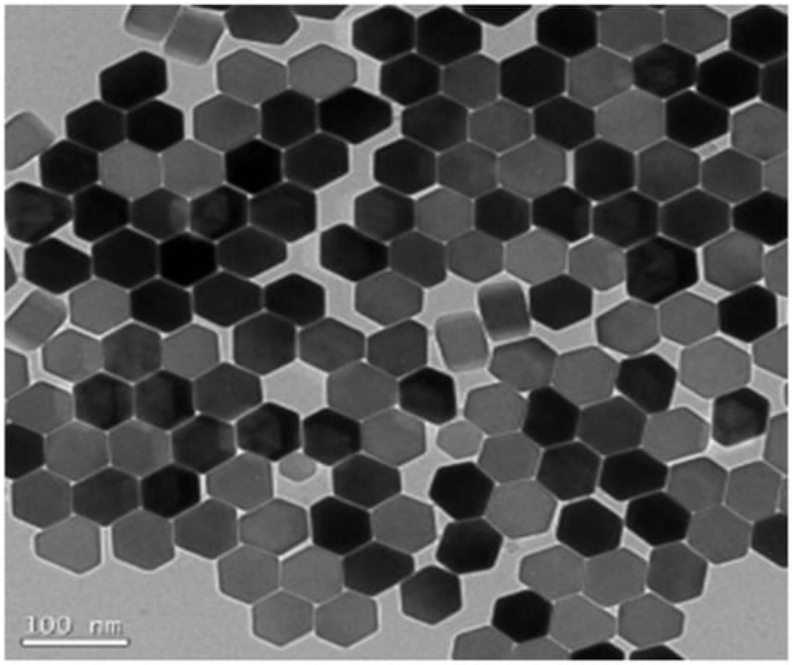

Figure 9. TEM image of 70 nm bare UCNPs. (Reproduced with permission from Ref. [53], Copyright 2013, Public Library of Science).

2.5. Others

2.5.1. Hybrid Nanocomposites

In the past decade, the application of composite nanomaterials in PCR has emerged to optimize the disadvantages of nanoparticles such as easy aggregation, poor adsorption capacity, poor thermal conductivity, etc., through surface modification or compounding of multiple NPs for the purpose of improving the characteristic properties of nanoparticles.

Although Au NPs have been used maturely in PCR, it has been found that surface-modified Au NPs have also had a strong enhancement effect on PCR in recent years. In addition, some Au modified complexes have further specific effects on PCR. Chen et al. synthesized the dendrimer-entrapped Au NPs (Au DENPs) using amine-terminated G5 dendrimers as templates and different compositions as additives to investigate their effects on the specificity and efficiency of PCR amplification. It was found that the optimum concentration of Au DENPs could be reduced to as low as 0.37 nM, much lower than that of NH_2-G5 dendrimers without Au NPs entrapped [54]. One year later, using poly (diallyl dimethylammonium) chloride (PDDA) as novel PCR enhancers, Yuan et al. verified the improvement of three kinds of Au NPs modified with different surface charge polarities in the efficiency and specificity of an error-prone two-round PCR system. The optimum concentrations of positively charged PDDA-Au NPs were different and as low as 1.54 pM, while the negatively charged Na_3Ct-Au NPs were over three orders of magnitude higher than the positive ones [11]. Additionally, polyethylene glycol (PEG)-modified polyethylenimine (PEI)-entrapped Au NPs (PEG-Au PENPs) showed potential capacity to enhance the specificity and efficiency in both two-round PCR and GC-rich PCR. As the proportion of gold content increased, the optimum concentration of the modified Au NPs decreased (**Figure 10**) [55].

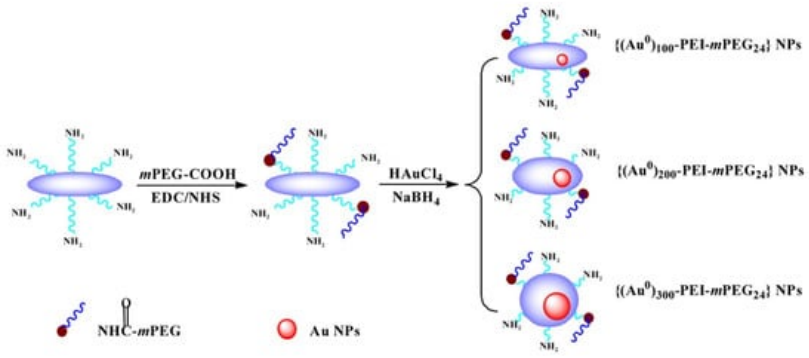

Figure 10. Schematic illustration of the synthesis of PEG-Au PENPs. (Reproduced with permission from Ref. [55], Copyright 2016, American Chemical Society).

Some nanomaterials can be effectively applied to PCR, but there are always some limitations. For example, despite the merits and capabilities of GO, a severe agglomeration level leads to a limited surface area, which may impede PCR

performance. The modification of GO with Au NPs overcomes these challenges as hybrid nanomaterials maintain the beneficial features of both precursor materials and provide advantages unique to the hybrid material through the combination of functional components (**Figure 11**). Jeong et al. [56] synthesized an Au NP and GO hybrid composite and applied it as a PCR enhancer.

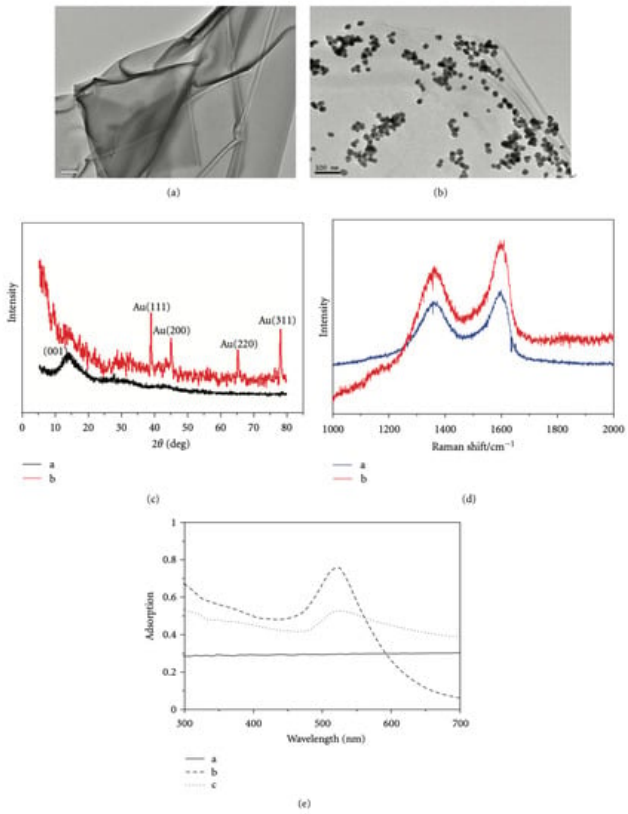

Figure 11. (**a**,**b**) TEM images of the GO and GO-Au composites, respectively. (**c**) XRD images of the GO and GO-Au composites. (**d**) Raman spectra of the GO and GO-Au composites. (**e**) UV-vis absorption spectra of the GO, Au NPs and GO-Au composites from a to c. (Reproduced with permission from Ref. [57], Copyright 2012, Hindawi).

Dao Van et al. [58] successfully synthesized Fe_3O_4/SiO_2 NPs consisting of a 10–15 nm core and a 2–5 nm thick silica shell. The Fe_3O_4/SiO_2 NPs were found to be more efficient at purifying DNA from HBV and EBV than using commercial Fe_3O_4/SiO_2 particles, as indicated by (i) brighter PCR amplification bands for HBV and EBV viruses and (ii) higher sensitivity for PCR-based EBV loading detection.

2.5.2. Other NPs

Metal-organic frameworks (MOFs) are special organic–inorganic hybrid porous solids with extraordinarily high surface areas, tunable pore sizes, adjustable internal surface properties, and an extraordinary degree of variable structures. These features endow MOFs with potential gas or liquid adsorption/storage applications, such as drug delivery, polymerization, catalysis, and biosensors. Recently, Sun et al. used UiO-66 and ZIF-8 to optimize the error-prone two-round PCR and found that both UiO-66 and ZIF-8 not only enhanced the sensitivity and efficiency of the first-round PCR but also increased the specificity and efficiency of the second-round PCR. Moreover, both MOFs could widen the annealing temperature range of the second-round PCR [59]. Also, Rasheed et al. developed a hexagonal boron nitride (hBN) NP-based PCR assay for the rapid detection of Acanthamoeba to amplify DNA from low amoeba cell density. As low as 1×10^{-4} (wt%) was determined as the optimum concentration of hBN NPs, which increased Acanthamoeba DNA yield up to ~16%. Further, it was able to reduce PCR temperature, which led to a ~2.0-fold increase in Acanthamoeba DNA yield at an improved PCR specificity at 46.2 °C low annealing temperature. hBN nanoparticles further reduced standard PCR step time by 10 min and cycles by 8 min, thus enhancing Acanthamoeba detection rapidly [60].

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