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REVIEW

Development of Nano-Polymerase Chain Reaction and Its Application

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Abstract: Polymerase chain reaction (PCR) has become one of the powerful techniques since its invention in 1980s. Nevertheless, PCR technique was still frequently impaired by its low specificity, poor sensitivity, false positive results, etc. Recently, nanomaterials including metal nanoparticles, carbon nanomaterials, quantum dots and nano metal oxide have been added into PCR solution to improve both quality and productivity of PCR. Nanoparticle assisted PCR (NanoPCR) has received considerable attention due to its unprecedented sensitivity, selectivity and efficiency. In this view, we firstly summarized the mainly used nanoparticles in NanoPCR, including gold, quantum dots, carbon tube, graphene and metallic oxide. And then, we discussed the possible mechanisms for highly improved sensitivity and selectivity, as well as the recent applications of NanoPCR.

Key Words: NanoPCR; Nanomaterials; Specificity; Amplification efficiency; Hot-start; Review

1 Introduction

Polymerase chain reaction (PCR), a technique that can accelerate specific DNA amplification in vitro[1], was first proposed by American scientist Mullis in 1985. PCR technology can be used in gene cloning[2], DNA sequencing[3], gene analysis[4], gene chip[5] and forensic science[6], etc. Owing to the exponential amplification of PCR, large amounts of DNA fragments can be obtained for DNA sequencing and genetic analysis. Nevertheless, PCR technique is still frequently impaired by its low specificity, sensitivity and false negative results, especially in GC-rich fragments[7]. Therefore, it is very important to develop new technologies to improve the amplification efficiency and specificity of PCR[8]. Many PCR enhancers have been found such as dimethyl sulfoxide (DMSO), glycerol, formamide and betaine, etc[9–11]. Although these methods can improve the specificity and efficiency of PCR, there are still some problems to solve.

In recent years, nanoparticles have attracted great attention and gradually penetrate into the field of life sciences because of their unique chemical and physical properties, such as large surface area and small size effect, which greatly promoted the development of life science and technology. Also, the nanoparticles assisted PCR technology (NanoPCR) based on the nanoparticles such as gold nanoparticles (AuNPs)[12], quantum dots[13], carbon nanotubes[14], etc, has been developed to significantly improve specificity, amplification efficiency and sensitivity of PCR, and accelerate PCR reaction process. This technology has become a current research hotspot. In this review, we mainly summarized the mechanism and application of PCR amplification technology based on various nanoparticles.

2 Effects of nanoparticles on PCR amplification system

Nanoparticles possess many special properties, such as good surface effect, quantum effect, etc. However, different nanoparticles have different characteristics in water-solublility, biocompatiblity and heat stability, etc[15], hence, the effects of different nanoparticles on the PCR are diverse.

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2.1 Influence of AuNPs on PCR system

Li *et al*[12] prepared a PCR synergist based on 10-nm AuNPs using sodium citrate as stabilizer. Upon addition of 0.4–0.8 nM AuNPs, the specificity of two re-amplification systems was improved, and the amplification of nonspecific fragments was obviously inhibited (Fig.1). However, AuNPs exceeding 1.0 nM could inhibit PCR amplification. In addition, the specificity of PCR amplification was still very high, even at lower annealing temperatures (25–40 °C). Hence, AuNPs broadened the annealing temperature of PCR. Subsequently, Pan *et al*[16] used the multi-round PCR as the research system and found that target band was still observed strongly after the addition of AuNPs in the sixth round of amplification. However, for the general PCR system (without AuNPs), the target band could not be obtained at the fourth round, which further confirmed that AuNPs enhanced the specificity of PCR amplification. Vu *et al*[17] used multiple PCR to study the effect of AuNPs on PCR amplification. They believed that AuNPs could not directly increase the specificity of PCR, but contribute to the amplification of the small fragments. In addition, it was believed that the effect of AuNPs was mainly due to its good surface effect rather than heat conduction effect, and there was a stronger adsorption between AuNPs with DNA polymerase, which led to a decrease of the actual concentration of polymerase in the system and promoted the expansion of small fragments.

Li *et al*[18] studied the effect of 13-nm AuNPs at concentration of 0.7 nM on PCR amplification. It was found that AuNPs could significantly increase the efficiency of PCR amplification and shorten the reaction time. Investigation of different PCR systems including different DNA polymerase and different lengths of DNA fragments showed that the sensitivity could be increased by 5–10 times for conventional PCR, and at least 10^4 times for real-time quantitative PCR. The author considered that the effect of AuNPs was attributed to its good thermal conductivity. On the basis of this, Yang *et al*[19] found that the addition of AuNPs could promote the amplification of high-GC template GNAS1 promoter region (about 84% GC). Girilal *et al*[20] synthesized AuNPs with the cell-free extract from Bacillus stearothermophilus using biosynthesis method. The synthesized AuNPs had good heat conduction effect and thermal stability, and could shorten the reaction time of PCR and improve its specificity and efficiency.

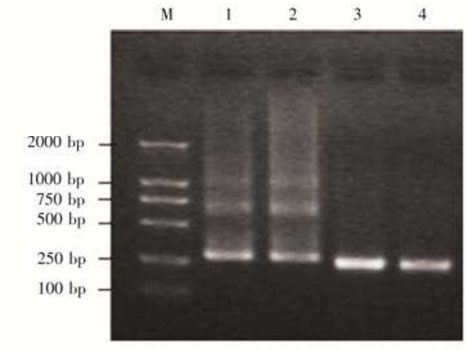

Fig.1 Effect of gold nanoparticles (10 nm AuNPs, 0.4 nM) on the specificity of polymerase chain reaction (PCR). PCR was performed by employing a 283-bp target sequence from a λ-DNA template, and PCR products were analyzed by agarose gel electrophores (1.5%). Lane M is marker; lanes 1 and 2 show the results of PCR performed in the absence of AuNPs; lanes 3 and 4 show the results of PCR performed with AuNPs. bp = base pair[12]

Because the interaction between different surface-modified AuNPs and PCR components is different, the impact of AuNPs on PCR is also different. Cao *et al*[21] found that polyamidoamine (PAMAM) dendrimers (G5-NH2) could increase the specificity of PCR. Then Chen *et al*[22] encapsulated amino-terminal polyamide amines (PAMAM) dendrimers (G5-NH_2) with gold (Au-DENPs) and applied it in PCR. The results showed that Au-DENPs could effectively improve the specificity and efficiency of PCR. When the molar ratio of Au atom to G5.NH2 was 100:1, the detection limit was 0.37 nM. The author thought that macromolecules containing AuNPs could maintain 3D spherical morphology, which facilitated the interaction with PCR components. They also studied the effect of different terminal polyamide amine (PAMAM) modified AuNPs on PCR[23], and found that amino and hydroxyl-modified AuNPs was more effective, and the required concentration was minimum.

2.2 Influence of quantum dots on the system

Quantum dots (QDs), as novel fluorescent inorganic nanomaterials, have attracted great attention and been widely used in monomolecular detection, cell imaging, tumor target, etc.[24] due to their adjustable emission wavelength, high luminous efficiency and narrow emission peak. In addition, QDs have good physical and chemical properties and biocompatibility, and are also used in PCR amplification, such as thiacetic acid modified CdTe and thioglycolic acid modified CdSe and CdSe/ZnS[25–27].

Wang *et al*[25] investigated the effect of carboxyl-modified CdTe QDs on specificity of PCR system at different annealing temperatures and using different length template DNA. By comparing the results of AuNPs, it was found that QDs could significantly improve the specificity of PCR system. But when the concentration of CdTe quantum dots exceeded the optimal concentration, an inhibiting effect would be observed. Especially for short DNA fragments, the effect of QDs was more significant. Similar to AuNPs, QDs had a strong specific amplification effect on PCR system in the annealing temperature range of 30–45 °C.

Ma *et al*[26] modified QDs with thioacetic acid and found that the yield and specificity of the PCR system were