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Magnetic particles for *in vitro* molecular diagnosis: From sample preparation to integration into microsystems

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ABSTRACT

Colloidal magnetic particles (MPs) have been developed in association with molecular diagnosis for several decades. MPs have the great advantage of easy manipulation using a magnet. In nucleic acid detection, these particles can act as a capture support for rapid and simple biomolecule separation. The surfaces of MPs can be modified by coating with various polymer materials to provide functionalization for different applications. The use of MPs enhances the sensitivity and specificity of detection due to the specific activity on the surface of the particles. Practical applications of MPs demonstrate greater efficiency than conventional methods. Beyond traditional detection, MPs have been successfully adopted as a smart carrier in microfluidic and lab-on-a-chip biosensors. The versatility of MPs has enabled their integration into small single detection units. MPs-based biosensors can facilitate rapid and highly sensitive detection of very small amounts of a sample. In this review, the application of MPs to the detection of nucleic acids, from sample preparation to analytical readout systems, is described. State-of-the-art integrated microsystems containing microfluidic and lab-on-a-chip biosensors for the nucleic acid detection are also addressed.

Contents

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1. Introduction

In recent decades, miscellaneous colloidal particles have been used extensively in various applications of biomedicine, e.g., biological imaging, targeted drug delivery, medical therapy, and biomedical diagnosis [1–3]. Among these colloidal particles, colloidal magnetic particles (MPs) have been the most extensively used in biomedical applications since they can be manipulated using a magnet. MPs can play important roles in the quantification of biomolecules due to their various advantages, e.g., biocompatibility, high dispersion, and high surface to volume ratio [4–6]. Several detection methods can be applied by using MPs with various types of terminal functionalization [7,8]. Modification with polymer coating materials and additional biological components has been described for specific diverse applications.

Since the discovery of circulating nucleic acids, including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) in plasma and serum, they have been the most important targets for molecular diagnosis of various diseases [9]. The presence of these nucleic acids due to pathogens or genetic mutations can differentiate patients from the healthy population. However, the detection of nucleic acids must have high sensitivity to recognize very low amounts of the target in the sample. In some diseases, highly sensitive and specific quantification of target molecules is a key factor in accurate and precise diagnosis [10,11]. The development of polymerase chain reaction (PCR), a molecular technique that can amplify a few copies of a DNA sequence to millions of copies, has expanded the horizon of molecular diagnosis. PCR has become the basis for latter developments, e.g., real-time PCR (RT-PCR), loop-mediated isothermal amplification (LAMP), rolling circle amplification (RCA), and circle-to-circle amplification (C2CA), which improved many aspects of PCR [12–14]. Although PCR has been enhanced its usability through minor procedural changes throughout the past, the post-amplification readout system of PCR products using gel electrophoresis is complex, time consuming and may exhibit non-specific and contaminated products [15]. While the sensitivity of RT-PCR represents the most advanced PCR-based application to date, the real-time analysis using fluorescent DNA intercalating dyes requires optimization of the conditions and consideration of non-specific binding between the dyes and amplified double-stranded DNA. Herein, the development of colloidal MPs has advanced several applications in the analysis of biomolecules for better specificity, e.g., bioseparation and specific-tagged particles [6,16]. In addition to specificity, highly sensitive detection is also achieved in applications based on individual particles, e.g., fluorescence microarrays [17], functionalized biosensors [18], and microfluidic systems [19].

In this review, we focused on the application of colloidal MPs in molecular detection, especially in PCR-based methods and their related procedures. The review sheds light on the state-of-the-art techniques for two main applications. The first is applications of MPs in sample preparation and extraction of target molecules using particle-based separation methods. The second is the molecular detection and readout system with functionalized MPs, including magneto immuno-PCR, microfluidic biosensors, and lab-on-a-chip microsystems. Synthesis and functionalization of colloidal particles in molecular detection were also discussed.

2. Colloidal MPs in sample preparation

Genetic materials that contain hereditary nucleic acid sequences, including DNA and RNA, have become the major target of PCR-based detection for various diseases. In PCR-based detection, extraction and purification of target DNA/RNA are required to eliminate unwanted PCR inhibitors and residual contamination in the sample. Conventional methods to extract and purify nucleic acids are dependent on treatment with a chemical solution, e.g., phenol-chloroform and spin column chromatography with centrifugation [20]. However, these conventional methods require a large volume of sample and lose some of the extracted DNA in the washing step. Colloidal particles have been applied to the sample to enhance the efficiency of extraction and purification methods. Spherical particles are suitable for molecular biology applications because they provide several advanced properties [6,7]. Colloidal particles have been implemented for extraction, purification, and pre-concentration *via* rapid and simple processes [21–23]. Depending on the purpose of the application, several types of material have been applied in the synthesis of colloidal particles.

MPs are also of great interest in sample preparation as a separating agent. Iron oxides, including magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3), have attracted substantial attention due to their biocompatibility, low toxicity, and simple preparation. Fe_3O_4 MPs can adsorb DNA sequences without modification [24,25]; however, negatively charged MPs may reduce the binding capacity to DNA and slightly increase inhomogeneous aggregation [26]. To enhance the efficiency and diversity of MPs, coating substances onto the particle surface has revealed a new dimension of the particles for a variety of applications.

2.1. Coating with aminated polymer

Coating MPs with a polymer can increase their stability, protect their surfaces from oxidation and reduce aggregation in the absence of a magnetic force [27]. The coating of MPs with aminosilane compounds, e.g., [3-(2-aminoethylamino)propyl]trimethoxysilane (AEEA), resulted in higher DNA binding and releasing capacities [28]. Silanization of AEEA was subsequently modified with dendrimer, i.e., polyamidoamine, which increased the cationic characteristics, reduced agglomeration and provided a high surface area for DNA binding [29]. Moreover, the efficiency of DNA binding and releasing was directly related to the generation of the dendrimer coating (Fig. 1) [30].

2.2. Coating with carboxylated polymer

MPs modified with a carboxylic functionalized surface were reported to adsorb DNA under a high concentration of sodium chloride [23,31–33]. DNA interacts with the carboxyl group on the surface of MPs through hydrogen bonding [31,34]. The concentration of sodium chloride also promoted salting out of the DNA containing hydrophobic nitrogen bases from the aqueous phase to the particles. Functionalization of the carboxyl groups was also performed using several compounds, e.g., methacrylic acid, phosphonic acid and dimercaptosuccinic acid [35–37]. Another interesting modification was the covalent grafting of poly(maleic anhydride-*alt*-methyl vinyl ether), which provided rapid functionalization of the carboxyl groups onto the cationic MPs [38,39].

2.3. Covalent coupling

The separation of nucleic acids using MPs was enhanced by immobilization of specific oligonucleotide sequences on the activated surfaces of the particles, primarily achieved by surface modification with specific biomolecules that can be covalently bound *via* activation of a crosslinking agent, e.g., glutaraldehyde and carbodiimide [40–42]. Amino-functionalized Fe_3O_4 MPs were immobilized on oligonucleotide sequences with 5′-amino modification in the presence of glutaraldehyde solution in sodium chloride-sodium citrate buffer (SSC) (Fig. 2). The immobilized MPs were able to capture DNA from various samples by hybridiza-