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Preparation and Characterization of Super-paramagnetic Nano-beads for DNA Isolation

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Abstract: Unique coupling reagent, bis-(2-hydroxyethyl methacrylate) phosphate was used to prepare coated and functionalized superparamagnetic nanobeads, leading to a simple, effective method for coating the nanobeads. With this method, the thickness of the coating layer and the functional group contents on the nano-beads could be controlled by changing the quantity of the coated monomers. The nanobeads were characterized by means of transmission electron microscopy (TEM) and Fourier transformation infrared spectroscopy (FTIR). The carboxyl-modified magnetic nano-beads were employed to streamline the protocol of isolation of genomic DNA from the human whole blood.

Keyword: Coating, superparamagnetic nano-beads, coupling reagent, DNA isolation.

The magnetic beads used for bioseparation are usually coated with polymer layers and modified with functional groups[1~3]. Many kinds of natural polymers[4], synthetic ones, such as polyelectrolytes[5], and non-ionic polymers[6] were used for coating particles. Two strategies were adopted: One is to coat the magnetite cores directly without use of coupling agent[5,7]. However, in these cases, self-assembling of polymer particles always takes place and the size distribution is rather wide. Another one is to use coupling agents, such as the silanizing reagent, but it is not very suitable for Fe_3O_4 crystals due to its weak interaction with Fe_3O_4 particle[8]. So finding out novel coupling agents applicable to Fe_3O_4 crystals is with great interests.

In this study, a new method was developed to coat magnetic beads by introducing unique coupling reagent, bis-(2-hydroxyethyl methacrylate) phosphate so that the monomers participated only in the surface polymerization process without forming separate nuclei[9]. The thickness of the coating layer, the size and density of the coated nanobeads could be controlled by changing the quantity of the coated monomers. The nanobeads were characterized by TEM and FTIR. The carboxyl-modified magnetic nanobeads were employed to simplify the protocol of genomic DNA extraction from the

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human whole blood within a few minutes.

Experimental

$FeCl_3 \cdot 6H_2O$ (4.73 g) and $FeCl_2 \cdot 4H_2O$ (1.99 g) were dissolved in 55 mL of deionized water. 100 mL solution of 4 mol/L NaOH was added to a flask equipped with a stirrer. The temperature was maintained at 63 ℃. The mixture of Fe^{2+}/Fe^{3+} was rapidly injected to the flask. After 30 min the nanocrystals were separated by applying magnetic field and washed with deionized water. Nanocrystals 3.27 g were added to a flask containing 500 mL of toluene and 0.81 g sodium lauryl benzene sulfonate. The nanocrystals were well dispersed into toluene by ultrasound for 0.5 h and followed by violent agitation. A mixture of 0.15 g initiator benzoylperoxide, 1.2 mL monomer 2-hydroxyethyl methacrylate, 0.8 mL cross-linking trimethyrolpropanetriacrylate, 0.4 mL coupling agent bis-(2-hydroxyethyl methacrylate) phosphate and 0.6 mL functionization agent methacrylic acid were added into the flask. The reaction temperature was kept at 78 ℃ for 12 h under nitrogen atmosphere.

To a 1.5 mL Eppendorf tube containing 300 μL acid citrate dextrose anticoagulated blood was added 200 μL cell lysis solution. The mixture was agitated gently by vortex and incubated at room temperature (RT) for 3 min to lyse the leucocytes. Afterward, 50 μL of nano-beads suspended in Tris-EDTA buffer (15 μg/μL, pH 6.5) was added. Then isopropyl alcohol 200 μL was added to the suspension. After standing for 5 min the supernatant was discarded. The magnetic nano-bead-DNA conjugates were immobilized on a magnetic stand, and washed twice with 100 μL of 70% ethanol solution. Ethanol was evaporated at RT, 50 μL solution of Tris-EDTA (pH 8.0) was added to the conjugates and incubated at RT for 10 min to elute DNA.

Results and Discussion

Coating and functionalization of superparamagnetic nanocrystals

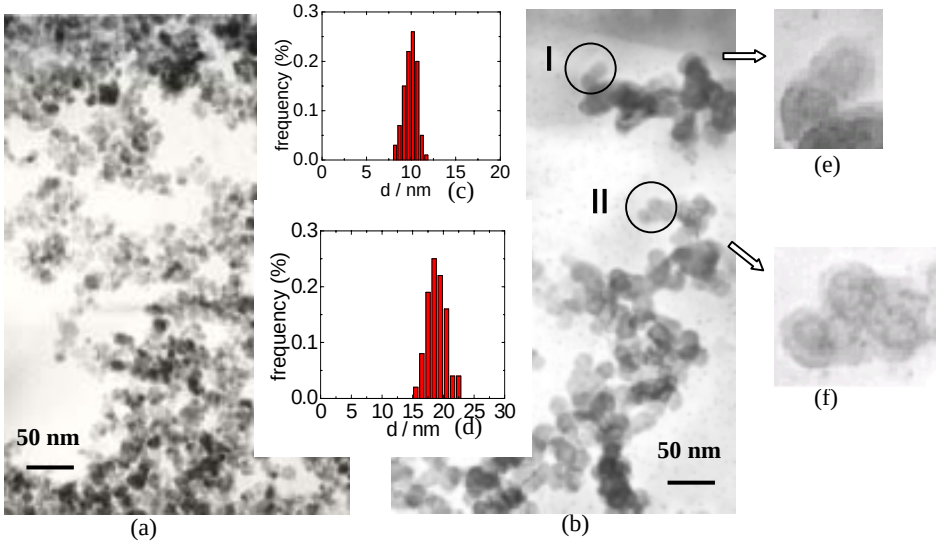
Because the surface polarity of the Fe_3O_4 crystals is very strong and yet the polarity of the polymer is rather weak. It is difficult that the polymerization reaction takes place just on the surface of the magnetic nanocrystals, without forming polymer particles with wide size distribution. To solve this problem an efficient coupling agent, bis-(2-hydroxyethyl methacrylate) phosphate, was added to the monomers. One end of the coupling agent is a phosphoryl group with strong polarity which is capable to bind to ferric oxide strongly, and the other end is a double bond allowing the copolymerization with the monomers. Through the above-mentioned procedure the monomers eventually adsorbed onto the surface of the nano-beads and polymerized *in situ* to form a layer of polymer network. Upon the completion of the coating the resulted nanocrystals were separated by a magnetic field. By introducing above-mentioned functional reagent, the processes of coating and functionalization were all completed in one step. After coating the mean diameter of the nanoparticles was increased to 18.5±3.2 nm with excellent uniformity as shown in **Figure 1**. Compared with the wide size distribution (50-500 nm) of the beads made from directly coating method[5], the size distribution of our coated

beads was narrowed and the uniformity was improved remarkably.

FT-IR Spectra of the superparamagnetic nano-beads

The peaks of 1728 and 1155 cm^{-1} in the IR spectrum of Fe_2O_3 coated nanobeads are absorption bands of carbonyl groups from methacrylic acid and 2-hydroxyethyl methacrylate. The band at 3456 cm^{-1} might be contributed by the hydroxyl group from methacrylic acid.

Figure 1 TEM images of naked & coated beads

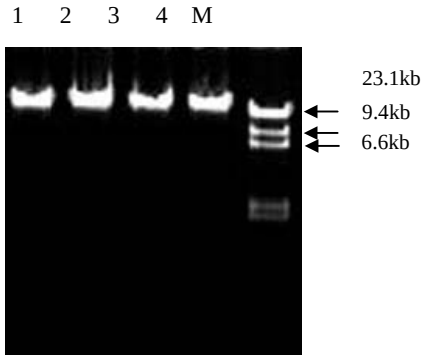


(a) Fe_3O_4 magnetic nanobeads; (b) coated Fe_3O_4 magnetic nanobeads; (c) The histogram of the size distribution of the naked beads, in which data were statistically calculated from the particle size in **Figure 1a**; (d) and the histogram of the size distribution of coated magnetic beads, in which data were statistically calculated from the particle size in **Figure 1b**; (e) Magnified picture for round Ⅰ from **Figure 1b**; (f) Magnified picture for round Ⅱ from **Figure 1b**.

Isolation of genomic dna using coated nano-beads

By using our beads the protocol of DNA isolation was shortened to 15-30 min. Genomic DNA isolated from 300 μL human whole blood was examined by agarose gel electrophoresis and the results were shown in **Figure 2**. When the phenol/chloroform method was used to extract genomic DNA from 300 μL whole blood, it required 150 min to accomplish and yield was around 5.46 μg with an OD260/OD280 ratio of 1.87. However, using 50 μL magnetic nano-beads suspension it took only 30 min to yield approximately 2.87 μg DNA with an OD260/OD280 ratio 1.85. Compared to the phenol/chloroform method, the nano-bead method could extract genomic DNA with lower yield but comparable purity.

Figure 2 Agarose gel electrophoresis of genomic DNA



Lanes 1,2: 5 μL DNA by using phenol/chloroform method; 3,4: 5 μL DNA by using our method; Lane M: 1 μL λ phage DNA/Hind III digest marker

Conclusion

In the current report magnetic nanocrystals were coated and then functionalized with carboxyl groups to obtain the expected 18.5±3.2 nm superparamagnetic nanoparticles. Isolation of genomic DNA from blood was carried out in one step by the application of carboxyl-functionalized nano-beads. The nano-beads method is suitable for fast extraction of genomic DNA without involving the conventional centrifugation procedure and the use of hazardous agents. It is promising for enabling automated high throughput isolation of genomic DNA from blood samples while the yield may be compromised.

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