올리고뉴클리오티드를 결합 분자로 활용한 콜로이드 조합체의 제조

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Preparation of Colloidal Assembly Using Oligonucleotide as Linker molecule

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Introduction

Self-assembled structure of monodisperse colloidal particles can be used for various applications such as photonic crystals and chemical sensing materials [1, 2]. Typical building blocks of self-assembly are polystyrene or polymethylmethacrylate latex spheres and silica suspension with narrow size distribution. Several methods have been developed to prepare self-assembled structure of these building blocks such as colloidal crystallization of microspheres in aqueous droplet [3]. The drawback of this method is that the crystallization process is difficult to control the particle periodicity and interparticle distances. The utilization of DNA as molecular linker can allow us to overcome these drawbacks. In 1996, Mirkin group has succeeded to prepare the controlled assembly of gold nanoparticles by using the adsorption of thiol-terminated complementary oligonucleotides as linker molecules [4]. They used the hybridization reaction of oligonucleotides with complementary base sequences to assemble gold colloid to macroscopic aggregate. However, only few articles presented the preparation of colloidal assembly of monodisperse polymeric microspheres by sequence specific hybridization of oligonucleotides [5]. Here we describe a method for assembling monodisperse polystyrene microspheres by using oligonucleotide as linker molecule. Polystyrene building blocks were synthesized by soapless emulsion polymerization, and the hybridization of these building blocks was verified by combining two different sized microspheres adsorbed with oligonucleotides.

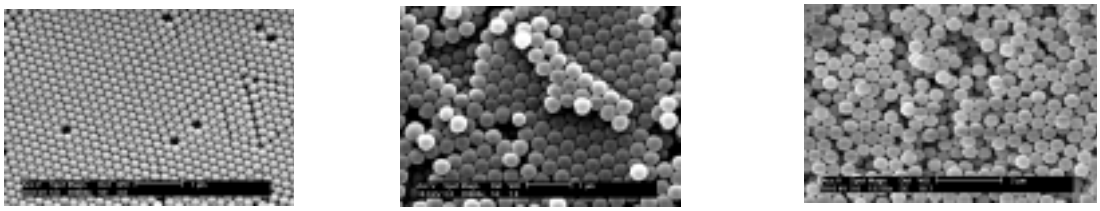
Experimental

Synthesis of uniform latex particles. Monodisperse carboxylated polystyrene nanoparticles used in this study was prepared by soapless emulsion polymerization using potassium persulfate as initiator and sodium hydrogen carbonate as buffer [6]. The polystyrene latex spheres with three different diameters were synthesized by varying the amount of comonomer, acrylic acid. Table 1 contains the amount of comonomer and diameters of polystyrene beads prepared in the present study. As noted, the size of microsphere could be controlled by varying the concentration of acrylic acid. The prepared latices were stable for several months without precipitation. The scanning electron moscroscopy (SEM) images of the prepared polystyrene beads with 217, 413, and 500 nm diameter are shown in figure 1. Particle size was measured by both SEM image and dianamic light scattering (DLS). DLS measurements were performed with a Brookhaven instrument at a wavelength of 674 nm at 90°. The zeta potentials of the prepared latices were also measured from the electrophoretic mobility by ZETA PLUS (Brookhaven). The presence of surface carboxyl group on polystyrene latex was verified by using Fourier transform infrared spectroscopy

(FT-IR).

Table 1. Preparation Recipe and Characteristics of the Polystyrene Beads Used in the Present Study

Sample	acrylic acid (M)	diameter (nm)/polydispersity (DLS)	diameter (nm) (SEM)	Zeta potential (mV)
PS-A	0.208	236.6/(0.005)	217	-40.8
PS-B	0.083	402.8/(0.005)	413	-57.6
PS-C	0.028	508.8/(0.005)	500	-53.8



(a) PS-A (b) PS-B (c) PS-C

Figure 1. Typical SEM image of polystyrene latex spheres with diameters (a) 217 nm, (b) 413 nm, (c) 500 nm

Covelent binding of oligonucleotide on polystyrene latex and hybridization of microsphere. Table 2 contains amine-terminated oligonucleotide sequences used in the present study. The coupling of oligonucleotides to polystyrene beads was performed via carboxy-diimide chemistry by using EDC, (1-ethyl-3-[3-dimethylaminopropyl]-carbodiimide hydrochloride) as cross-linking agent [7, 8]. 39mer-sized oligo-A and oligo-B having complementary sequences was attatched to PS-B microsphere separately, and the resultant two samples were mixed together for the hybridization of PS-B latex. Similarly, oligo-C and oligo-D with complementary sequences was also adsorbed on PS-A latex for the hybridization and the resultant microsphere was mixed with latex solution containing hybridized PS-B microsphere to observe the formation of DNA-assisted colloidal assembly.

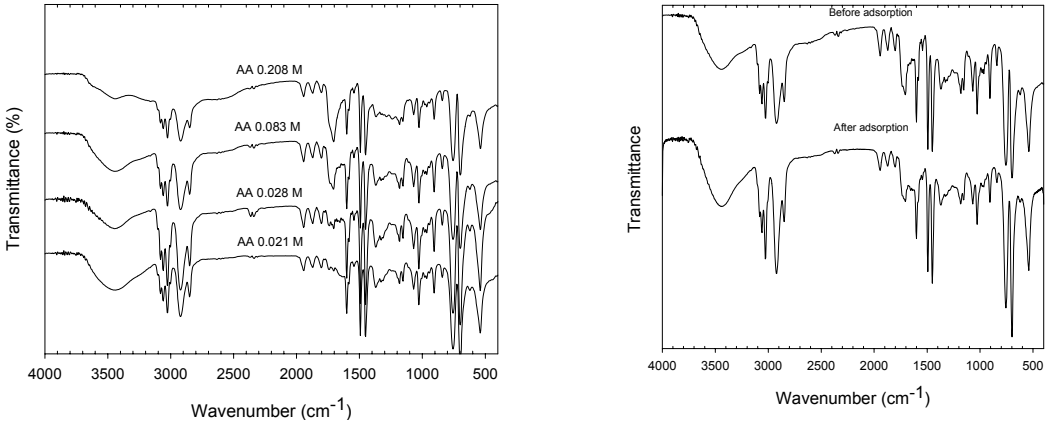
Table 2. The base sequence of oligonucleotides used in the present study

sample	sequence (5′ to 3′)	modification
oligo-A	AAA X 13	5′-amine
oligo-B	TTT X 13	5′-amine
oligo-C	CAG GTG GGA CTG GTT	3′-amine
oligo-D	AAC CAG TCC CAC CTG	3′-amine

Results and discussion

The presence of carboxyl group on the surface of polystyrene prepared via surfactant-free emulsion copolymerization of styrene and acrylic acid was verified by FT-IR. Figure 2 (a) contains the infrared spectroscopy of monodisperse carboxylated polystyrene bead A, B, and C. The sharp decrease of transmittance at 1704.9 cm^{-1}

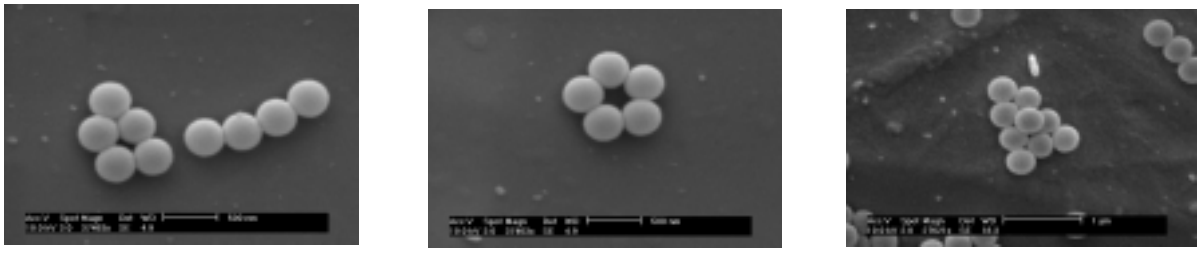
indicates the carbonyl stretching of carboxyl group. The peak intensity at 1704.9 cm^{-1} can be quantitative indication of the amount of surface carboxyl group. This intensity was increased with increasing concentration of acrylic acid during polymerization. Figure 2 (b) contains the FT-IR spectra of PS-B before and after the reaction with oligo-C. The decrease of transmittance at 1704.9 cm^{-1} after the reaction indicates that the amount carboxyl groups of polystyrene surface were decreased due to the adsorption of oligonucleotide.



(a) (b)

Figure 2. IR spectra of the polystyrene microspheres (a) with varying amount of comonomer (b) with oligonucleotide adsorption

Figure 3 contains the SEM images of hybridized clusters prepared by mixing PS-B latices attatched with oligo-A and oligo-B. These clusters had various kinds of shapes such as one-dimensional line, two dimensional pentagonal shape, and three-dimensional packed structure. However, we could observe unassembled isolated particles indicating that the yield of hybridization reaction is lower than 100 %.

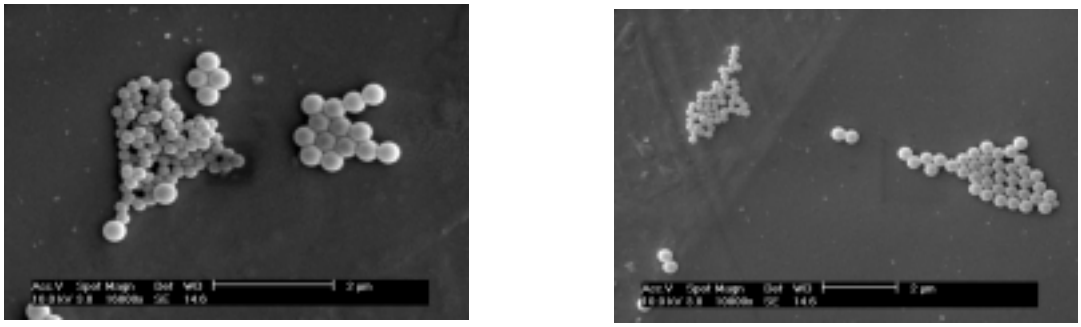


(a) (b) (c)

Figure 3. SEM images of DNA-assisted assembly of PS-B

Figure 4 contains the SEM images of the mixed sample of PS-A and PS-B. PS-A with 217 nm diameter was hybridized with 39mer-sized oligo-A and oligo-B, and PS-B with 413 nm diameter was hybridized with 15mer-sized oligo-C and oligo-D. Since the oligonucleotides adsorbed on PS-A and PS-B latices have different base sequences, we can not expect the hybridization of PS-A and PS-B microsphere. As shown in figure 4, there are two kinds of

the colloidal assemblies. Each assembly is composed of microspheres with 217 and 400 nm diameter, respectively, and there is no aggregation composed of bimodal polystyrene particles. This indicates that the base sequence-specific hybridization reaction was achieved successfully.



(a) (b)

Figure 4. SEM images of DNA-assisted assembly of PS-A and PS-B

Summary and conclusion

Here, oligonucleotide was used as linker molecule to prepare colloidal assembly of polystyrene microsphere. Monodisperse carboxylated polystyrene latices were synthesized by soapless emulsion polymerization. Then, amine-terminated oligonucleitide with complementary base sequences were covalently binded to polystyrene surface via carboxy-diimide chemistry. DNA-assisted nanoparticle assembly was prepared by hybridization of oligonucleotides on the polystyrene surface. The sequence-specific hybridization reaction was confirmed by mixing bimodal polystyrene microspheres.

References

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