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Anisotropic colloidal crystal particles from microfluidics

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A B S T R A C T

Anisotropic colloidal crystal particles (CCPs) have showed their great potential in biotechnology and structural materials due to their anisotropic shapes and tunable optical property. However, their controllable generation is still a challenge. Here, a novel microfluidic approach is developed to generate anisotropic CCPs. The microfluidic device is composed of an injection capillary and a collection capillary with available size and shape. Based on the device, the anisotropic particles with non-close-packed colloidal crystal structures are achieved by photo-polymerizing droplet templates in a confined collection capillary with different shapes and sizes. Moreover, anisotropic close-packed CCPs can be made from non-close-packed CCPs through a thermal process. It is demonstrated that the anisotropic CCPs in different sizes, structural colors and shapes (rods, cuboids and disks) can be generated. These distinguishable features of resultant particles make them ideal barcodes for high-throughput bioassays. In order to prove it, DNA multiplex detection is carried out. The experimental results indicate that achieved particles have a great encoding capacity and are highly practical for multiplex coding bioassays. Therefore, we believe that the anisotropic CCPs would be highly promising barcodes in biomedical applications, including high-throughput bioassays and cell culture research where multiplexing is needed.

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1. Introduction

The self-assembly of colloidal nanoparticles provides a simple and cheap approach to create three-dimensional structure materials [1–6]. The achieved colloidal crystal materials are with long-range ordered array of the nanoparticles and thus display a structure of periodic variation in the refractive index. The structure imparts the colloidal crystals an interesting optical property of photonic band gaps (PBGs), which is of great significance for photonic crystal devices [7–9], biological and chemical sensors [10–16], tunable lasers [17] and intelligent interfacial materials [18]. A major obstacle to produce the colloidal crystal materials is lack of reliable approaches to assemble nanoparticles into well controllable shapes. Virtually most colloidal-scale particles are naturally spherical because the interfacial tension between two immiscible phases tends to minimize the surface area, leading to the formation of spherical droplets. However, non-spherically anisotropic particles are also desired due to their great potential in biotechnology, structural materials, and other fields [19–22].

Traditional methods of fabricating non-spherically anisotropic particles include template molding [23], stretching of spherical particles [24,25], self-assembly of particles [26–28] and imprint lithography technique [29]. It is usually difficult to use these methods to produce large quantities of monodisperse anisotropic particles of tunable geometries and adjustable sizes. As an alternative, microfluidic technique has emerged as a promising and versatile technique for generating non-spherically anisotropic particles due to their capabilities in manipulating fluids in controlled environments [30–35]. By confining the photo-precursor droplets in the microfluidic channels of different sizes and shapes, anisotropic particles can be generated in a flash of ultraviolet light [36]. In addition to droplet microfluidics, the anisotropic particles can also be produced by microfluidic lithography technology [37,38] which combines lithography and photo-chemistry into laminar flow microfluidics. Although many progresses have been achieved, it is still a challenge to fabricate CCPs with the feature of controllable anisotropic shapes and tunable optical properties.

In this paper, we report a novel microfluidic approach to generate a series of anisotropic CCPs. A microfluidic device was assembled by aligning an injection capillary and a collection capillary coaxially inside a square capillary. When the size of droplets was larger than the inner diameter of collection capillary, they would be squeezed into non-spherical shape. Thus, by employing collection capillaries with different sizes and shapes, the monodisperse droplet templates with corresponding geometries appeared and flowed through the capillaries. In order to introduce optical properties into the droplet templates, the dispersed phase used for

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droplet templates generation was the photo-precursor solution that contained non-close-packed colloidal nanoparticles array in the poly (ethylene glycol) diacrylate (PEG-DA) medium. By photo-polymerizing the droplet templates in situ of the confined collection capillaries, anisotropic particles with the same ordered colloidal crystal structures could be generated. These non-close-packed CCPs could be transformed into anisotropic close-packed CCPs by a simply thermal process. It was demonstrated that the anisotropic CCPs in different sizes, structural colors and shapes (rods, cuboids and disks) could be generated. As a typical application, the angle-independent rod-like CCPs were employed for multiplex biomolecule assay. Spectroscopic measurements and DNA hybridization experiments indicate that CCPs are highly practical for multiplex coding bioassays. We anticipate that this technology may find important application in multiplexed high-throughput bioassays

2. Materials and methods

2.1. Material

The material used as outer phase was hexadecane (Sigma–Aldrich Co.). The inner phase was composed of a silica nanoparticle colloidal photonic crystal aqueous solution. To prepare the inner phase, monodisperse charged silica nanoparticles were first synthesized by the Stöber method [39]. The purified silica nanoparticles were dispersed in the pre-gel solution composed of 10% (v/v) poly (ethylene glycol) diacrylate (PEG-DA, MW 700, Sigma–Aldrich Co.) and 1% (v/v) photoinitiator (2-hydroxy-2-methylpropiophenone, Sigma–Aldrich Co.), and subsequently were shaken with an ion-exchange resin (Bio-Rad AG501-X8(D)) until strong diffraction became visually evident (20 min). After removing the resin by centrifugation, the colloidal crystal aqueous solution was achieved and sonicated for 30 min before use.

2.2. Methods

2.2.1. Microfluidics

The capillary microfluidic device consisted of coaxially assembled glass capillaries with different cross-sectional shapes on glass slides. To construct the microfluidic device for fabricating rod-shaped particles, a round capillary with an outer diameter/inner diameter of 1/0.58 mm (World Precision Instrument, Inc., Shanghai, China), tapered to achieve an orifice diameter of approximately 100 μm, was used for the inner tube. Another round capillary with an outer diameter of 1 mm and an inner diameter of 200 μm, was used as collection tube and coated with a hydrophobic reagent (trimethoxy (octadecyl) silane, Sigma–Aldrich Co.). The above capillaries were then coaxially assembled in a square capillary with a side length of 1.05 mm (AIT Glass, Rockaway, NJ, USA). A transparent epoxy resin was used to seal the tubes where required.

To fabricate the cuboid-shaped particles, typically the orifice diameter of inner tube was adjusted to 200 μm, while the collection tube was changed into a square capillary with an inner diameter of 500 μm. For the disk-shaped particles, flat capillaries with an inner width/height of 1/0.2 mm, were used as inner tube and collection tube instead of round capillaries. By heating and pulling, the inner flat tube had a tapered tip with an inner width diameter of about 50 μm. All the collection tubes were treated with the hydrophobic reagent.

In this work, inner phase and outer phase were pumped into the capillary microfluidic device using syringe pumps (PHD 2000 series, Harvard, Plymouth Meeting, PA, USA). By controlling the flow rates, droplet templates were stably generated in the collection tube and in situ photo-polymerized by UV-light (365 nm, 100w).

Finally, the particles were thoroughly washed with hexane, ethanol and pure water, respectively.

2.2.2. Thermal processing

Representative non-close-packed colloidal crystal hydrogel particles were first dried at 65 °C for 6 h, and then sintered in the muffle at a rate of 5–800 °C for hold time of 3 h. After natural cooling to ambient temperature, the particles were taken out.

2.2.3. Biomolecule assay

For further study of the anisotropic CCPs in biomolecule assays, oligonucleotides (Table 1) were bought from Invitrogen Biotechnology Co., Ltd. Hybridization buffer (750 mmol L^{-1} NaCl, 150 mmol L^{-1} sodium citrate, pH 7.4) and wash buffer (PBS, 10 mmol L^{-1} phosphate sodium buffer solution, pH 7.4, 100 mmol L^{-1} NaCl) were obtained from Shanghai Your Sun Biological Technology Co., Ltd.

For the immobilization of probes, the particles were decorated with 5% (v/v) (3-Glycidyloxypropyl) trimethoxysilane (GPTMS) in toluene solution for 24 h, washed respectively by toluene, ethyl alcohol and pure water for three times, and then incubated with the amino-functionalized probe DNA solution (10 μM) at 4 °C overnight. In order to block residual reactive sites, the particles were immersed in 5% (w/v) bovine serum albumin (BSA) at room temperature for 1 h. All reagents were measured by 2 μL per bead. After being washed with wash buffer, the particles were used as carriers for DNA sequence detection in microwells. For multiplexed assays, rod-like particles in three colors were immobilized with oligonucleotide probes, named probe A, probe B, and probe C, correspondingly. The particles were incubated in hybridization buffer with 10 μM AMC-labeled target DNAs under continuous shaking at 37 °C for 1 h. Then, the particles were rinsed away three times with wash buffer in turns to remove unbound target DNA. Finally, the fluorescence spectra and images were taken after the reaction.

2.2.4. Characterization

Photographs of photonic crystal hydrogel particles were taken by an optical microscope (OLYMPUS BX51) equipped with a cool CCD camera (Media-Cybernetics Evolution MP 5.0). Reflection spectra were recorded by an optical microscope equipped with a fiber optic spectrometer (Ocean Optics, USB2000-FLG). Fluorescence spectra were recorded by a microscope (OLYMPUS IX71) equipped with a fiber optic spectrometer (Ocean Optics, QE65000). The microstructures were characterized by a scanning electron microscope (SEM, HITACHI, S-3000N).

3. Results and discussion

3.1. Preparation of colloidal nanoparticles solution as dispersed phase

To introduce optical properties into the anisotropic particles, we conducted the fabrication by using a PEG-DA aqueous solution containing 1% (v/v) photoinitiator and charged monodisperse silica nanoparticles as dispersed phase. When the concentration of

Table 1
Sequences of oligonucleotides used in this work.

Base sequence	Name
5′-NH$_2$-C6-GCG GCC TTA ATC ATT TCG CTT TCA GAA CTG-3′	Probe DNA A
5′-NH$_2$-C6-TGATCG CGG TGT CAG TTC TTT-3′	Probe DNA B
5′-NH$_2$-C6-GTGGAA TTG AGC AGC GTT GGT-3′	Probe DNA C
5′-AMC-CAG TTC TGA AAG CGA AAT GAT GAA GGC CGC-3′	Target DNA A
5′-AMC-AAA GAA CTG ACA CCG CGA TCA-3′	Target DNA B
5′-AMC-ACC AAC GCT GCT CAA TTC CAC-3′	Target DNA C