Photonic Crystal Based Centrifugal Microfluidic Biosensors

Seung-Kon Leea, b, Tae Jung Parkb, Soyoung Kimb, Gi-Ra Yic, Sang Yup Leeb, Seung-Man Yang*a, b
aNational Creative Research Initiative Center for Integrated Optofluidic Systems and bDepartment of Chemical and Biomolecular Engineering, Korea Advanced Institute of Science and Technology, Daejeon 305-701, Korea; cKorea Basic Science Institute, Seoul, 136-713, Korea

ABSTRACT

In this paper, we report a fast and facile method for fabricating colloidal photonic crystals inside microchannels of radially symmetric microfluidic chips. As the suspension of monodisperse silica or polystyrene latex spheres was driven to flow through the channels under the centrifugal force, the colloidal spheres were quickly assembled into face centered cubic arrangement which had photonic stop bands. The optical reflectance spectrum was modulated by the refractive-index mismatch between the colloidal particles and the solvent filled in the interstices between the particles. Therefore, the present microfluidic chips with built-in colloidal photonic crystals can be used as in-situ optofluidic microsensors for high throughput screening, light filters and biosensors in integrated adaptive optical devices.

Keywords: photonic crystals, centrifugal microfluidics, colloidal crystals, band-gap tuning, biosensor

1. INTRODUCTION

Colloidal crystals self-organized inside microchannels are of great importance for various applications including microfluidic mixing,1 microscale separation of biological materials,2, 3 chemical and bio sensors,4 macroporous matrices of catalysts5 and microfluidic control of light such as optofluidic waveguides.6 In addition, the multi-dimensional colloidal microstructures, which are self-assembled with different sized particles, are potentially useful for microdisplays owing to the structure-induced photonic band-gaps and microfluidic addressability.7 Recently, several research groups have tried to develop a simple way to fabricate such microstructures in microchannels. Among them, Whitesides and his colleagues materialized for the first time high quality artificial opals in microchannels by evaporating the liquid medium from colloidal suspension, and fabricated inverse opal structures by using the colloidal opals as templates.8, 9 Although their methods based on colloidal self-assembly could produce three-dimensional microstructures without machinery, they were not quite practical because it took a long process time with limited controllability compared to the conventional lithographic technique. As alternatives, vertical dip-coating of colloidal particles on patterned substrates,10 capillary force induced deposition11, 12 and microfluidic fabrication13 have been demonstrated for patterned colloidal microstructures. However, spontaneous crystallization induced by the evaporation of solvent proceeds very slowly, which is a drawback for practical applications.

Meanwhile, the centrifugal microfluidic devices have been developed for chemical and bio sensors, fluid mixers, microreactors, bio-separation, drug discovery and delivery and clinical diagnostics.14, 15 The centrifugal microfluidic units have many advantages of multiplexing, low-cost, easy control, short response time and very small usage of samples.16 As well, the rates of flow, drying and crystallization can be manipulated accurately by controlling the spin velocity, channel dimensions and surface property of the microfluidic units.17 In this paper, we describe a high-speed crystallization process to build colloidal photonic crystals inside microchannels by using centrifugal microfluidics platforms. The time required for colloidal crystallization was remarkably reduced compared to the conventional vertical coating and evaporation induced processes because the present method used strong centrifugal force. The present microfluidic devices with built-in colloidal crystals can serve as an ideal integration of microfluidics and nanophotonics because the colloidal crystals have a few photonic band gaps that can be manipulated by the refractive index mismatch between the colloidal particles and the solvent medium. By infiltrating different mobile solvents into the interstices between the colloidal particles, the refractive index mismatch can be tuned and consequently the optical reflectance peaks are shifted due to the modifications of photonic band gaps. Only small amount of samples is needed in these optofluidic devices since the volume of the microfluidic channel is nanoliter scale and the sample species can be detected immediately by optical signals associated with photonic band gaps. Therefore, microscale multiplex centrifugal chips are useful for the multi-sample diagnostics and chemical or bio sensors with infinitesimal amount of the samples. On the other hand, the fluidically modulated photonic band gaps of built-in colloidal crystals are essential for constructing...
optofluidic platforms, which require fluidic addressability and adaptive controllability. Therefore, the microfluidic chips with built-in colloidal photonic crystals have potential applications for active photonic devices such as optical waveguides, filters, switches and adaptable photonic circuits.

2. EXPERIMENTAL

2.1 Synthesis of silica and polystyrene suspensions

8.5 wt% monodisperse silica spheres were synthesized using sol-gel reaction of ethanol (HPLC grade, Merck), ammonium hydroxide (26 % Junsei), ternary distilled water and tetraethyorthosilicate (TEOS, 99.999 %, Sigma-Aldrich). The average diameter of silica particles was increased from 255 nm to 300 nm by decreasing the amount of ammonium hydroxide, and the polydispersity was kept within 5 %, as measured by dynamic light scattering (DLS) (Brookhaven Instruments). Finally, as-prepared silica colloidal suspensions were washed twice and redispersed in distilled water. 9 wt% monodisperse polystyrene (PS) spheres were synthesized by emulsifier-free emulsion polymerization. Styrene monomer (99 %, Sigma-Aldrich) was emulsified in ternary distilled water and polymerized using potassium peroxodisulfate (97%, Sigma-Aldrich) as an initiator at 70 °C. The average diameter of PS particles was 290 nm and the polydispersity estimated by DLS was less than 5 %. The surface of PS beads was modified by polyvinyl pyrrolidone (PVP, MW: 400k, Junsei) for enhancing phase stability in ethanol medium and washed with ethanol (HPLC grade, Merck) twice. In the washing process, the PS beads were precipitated by centrifugation at 7000 rpm for an hour and redispersed completely in ethanol with sonication. To remove particle aggregates, the PS suspensions were filtered with syringe filters (0.45 µm, Millipore). For specific binding of antigen of SARS corona virus, we chemically modified the surface of silica spheres by using γ-aminopropyltriethoxysilane(APS) with NH$_4$OH for 20 hours.

2.2 Fabrication of centrifugal microfluidic chips

Master patterns for the microfluidic channels were created by using conventional photolithography. A negative photoresist (SU-8 25, MicroChem) was spun onto a silicon wafer using a spin-coater. Thickness of the resulting photoresist pattern was 32 µm at a spin-rate of 1500 rpm. For the microchannels with different heights of 6, 9 and 14 µm, positive photoresist (AZ-9260, Clariant) was spun at 6000, 3000 and 1500 rpm, respectively. For all cases, the channels were 150 µm wide. Then, polydimethylsiloxane (PDMS 184-A and B, Dow Corning) microfluidic chips were fabricated by following the conventional soft-lithographic procedure. In doing this, PDMS 184-A (monomer) and 184-B (cross-linker) were mixed with a ratio of 4:1, instead of the conventional 10:1, to increase the stiffness for microfluidic channels and cells. PDMS pre-polymer (184-A) had fluidity while cross-linked PDMS became gel-like or solid-like with increasing amount of the crosslinking agent (184-B). Mata et al. reported that the PDMS molds of higher crosslinker contents had higher modulus. PDMS molds of the conventional 10:1 ratio were too flexible to avoid deformation of channels or reservoirs caused by gravity and capillary forces especially for channels with low height-to-width ratios. Indeed, the ceiling of PDMS reservoir made with 10:1 ratio was deformed and collapsed to the bottom substrate easily when the aspect ratio was smaller than 0.1. After the PDMS molds were cured at 70 °C, the intakes were made to load the colloidal suspensions into the reservoirs using a 5 mm$^2$ rectangular metal punch. We used 4-inch glass wafers of 0.5 mm in thickness (1137, Corning) as substrates after washing with acetone, isopropanol and ternary distilled water under sonication for 5 minutes in each solvent. After oxygen plasma treatment of both the PDMS and wafer surfaces, they were bonded together and annealed at 90 °C for an hour. Finally, the fabricated microfluidic chips were examined by an inverted optical microscope (TE2000, Nikon).

2.3 Experimental procedures

Figure 1 shows the planar arrangement of microfluidic units and the experimental procedure of our centrifugal microfluidic crystallization. First, colloidal suspensions were injected to the reservoirs of the centrifugal microfluidic units under the action of capillary forces, which pulled the suspensions into the reservoirs. The precedent oxygen plasma treatment made the channel surfaces hydrophilic and thereby increased the capillary forces. Since the ends of the channels were open to the ambient air, the colloidal particles were able to crystallize from the open ends of the channels in which the solvent water evaporated into air. By placing the centrifugal microfluidic chips in dry atmosphere, the colloidal suspensions began to crystallize locally near the open ends of the channels. The range of the pre-crystallized zone could be adjusted by the drying time. The crystallized zone kept the colloidal particles from escaping out of the channel against the centrifugal forces at high-speed rotation in the centrifugal crystallization process. The intakes were sealed up with thin PDMS films or polyimide tapes (Capton film, 3M) after the injection of the colloidal suspension. The PDMS films had small holes through which air could fill the reservoirs to keep the atmospheric pressure during
centrifugation and facilitate colloidal crystallization. To crystallize the colloidal particles, the microfluidic chips were rotated at 400–1600 rpm for 15–60 minutes.

Fig. 1. Schematics of the fabrication and characterization of centrifugal microfluidic platforms and arrangement of designed functional units of the centrifugal microfluidic chip. (i) Injection of suspension into the reservoir. (ii) Applying centrifugal force to the microfluidic units. (iii) Optical detection of reflectance spectra.

2.4 Characterization

Optical images of colloidal crystal patterns and cells were taken by a digital camera (EOS-20D with 100 mm 2.8 macro lens, Canon) and a CCD camera (DS-U1, Nikon) mounted on a reflection-mode microscope (L-150, Nikon). Scanning electron microscope (SEM) images were taken by an SEM system (Philips XL20SFEG). Before taking SEM images, we removed the PDMS molds from the substrate. We also observed the modulation of the optical reflectance peaks by changing the mobile phase of the built-in colloidal crystal inside the microchannels with various liquids. Decaline (Junsei), hexadacane (98%, Sigma-Aldrich), isopropanol (IPA, HPLC grade, Merck), ethanol (HPLC grade, Merck) and ternary distilled water were used to give different refractive index mismatches relative to the colloidal particles. A 150 W Xenon lamp was used as a light source to measure the reflectance data. Reflected light from the sample was collected by an objective lens (×10 LU plan, Nikon) and passed to an optical detector. The optical reflectance spectra were observed from the bottom sides of the chips because the optical observation from the glass wafer side was easier than from the top PDMS surfaces.

3. CENTRIFUGAL PROCESS

When the centrifugal microfluidic chips were rotated, two competing forces were exerted on the colloidal suspension inside the channels; namely, centrifugal and capillary forces. Centrifugal force was acting radially outward and packed the colloidal particles by sedimentation while the solvent was kept rest inside of the channels by surface tension. Centrifugal force was varied by the rotation velocity as Eq. (1).

\[ \Delta P_C = \rho \omega^2 r \Delta r \]  

In Eq. (1), \( \Delta P_C \) is the centrifugal force per unit cross-sectional area of the microfluidic channel acting on the suspension in the radial distance between \( r \) and \( r + \Delta r \), \( \rho \) is the suspension density and \( \omega \) is the angular frequency of rotation. On the contrary, capillary force \( \Delta P_S \) per unit cross-sectional area was acting on the suspension radially inward during rotation as represented in Eq. (2). \(^{19}\)

\[ \Delta P_S = a(4\gamma / D_H) + b \]  

Proc. of SPIE Vol. 6352  635207-3
Therefore, $\Delta P_S$ was governed by the surface tension $\gamma$, hydraulic diameter, $D_H$, and constants $a$ and $b$ that depended on the geometry and wettability of the channels. The hydraulic diameter $D_H$ is defined usually as four times of the ratio of area to wetted perimeter of the channel cross-section. As the rotational velocity became faster, $\Delta P_C$ was increased and balanced by $\Delta P_S$ at a certain radial frequency. When the value of $\Delta P_C$ and $\Delta P_S$ are equal, we get the linear relation of the $\omega^2$ and the $1/D_H$ as Eq. (3).

$$\omega^2 = \frac{4a\gamma}{\rho r \Delta r} \cdot \frac{1}{D_H} + \frac{b}{\rho r \Delta r}$$  \hspace{1cm} (3)$$

When the rotational velocity was increased further over the threshold value, $\Delta P_C$ exceeded $\Delta P_S$ and the suspension in the channel burst out and discharged out of the channel. It can be seen from Figure 2a that the burst angular velocity $\omega_c$ was decreased with increase in the hydraulic diameter.

![Figure 2](image)

**Fig. 2** Burst rotational frequency $\omega_c$ as a function of the channel height and the hydraulic diameter $D_H$ of the channel. (a) shows that the burst frequency decreased as the channel height increased. The white and grey bars represent the burst rotational speed of the microfluidic chips with and without the pre-crystallized zone, respectively. (b) shows that the burst centrifugal force (i.e., $\omega_c^2$) is inversely proportional to $D_H$ with or without the pre-crystallized zone. Filled and open circles represent the threshold values for the suspension to burst out in the absence and presence of the pre-crystallized zone, respectively.

As we mentioned earlier, we made the pre-crystallized zone at the open end of a channel to facilitate the crystallization of the colloidal particles. By the presence of the pre-crystallized zone, the burst angular velocity $\omega_c$ of the microfluidic chip was increased substantially as shown in Figure 2. Therefore, the centrifugal fabrication of colloidal crystal inside microchannel could be achieved at much higher speeds with the pre-crystallized zone. It can be also expected from equation (3) that the burst centrifugal force (or $\omega_c^2$) should be inversely proportional to the hydraulic diameter with or without the precrystallization zone. Indeed, our experimental result showed that $\omega_c^2$ was increased linearly with $1/D_H$ in both cases. Slightly below the burst point, the capillary force kept the liquid phase sustaining its level in the channels against the centrifugal force while the dispersed particles were settled down due to their density difference. The colloidal particles could not escape from the channels since the centrifugal force acting on the particles was not sufficient to
overcome the surface tension of the liquid which captured the particles at the open end. The colloidal particles were completely sedimented in the radial direction and crystallized into face centered cubic lattices below the burst frequency. Meanwhile, the solvent sustained its level in the microchannels because of its relatively low density. After the crystallization of the colloidal particles, the speed of revolution was increased over 4000 rpm to drain the mobile solvent from the microfluidic channels. We also checked that the residual solvent could be eliminated easily by drying in a desiccator or a vacuum oven without any noticeable damage of the microstructures. For the low surface tension solvents such as ethanol, spin bursting was useful. In case of water, drying in a vacuum oven or desiccator was much easier than spin bursting because of its high capillary force.

4. CRYSTALLIZATION STRUCTURES

The optical microscope image of the microfluidic chip reproduced in Figure 3a shows that 300 nm silica suspension was crystallized into the line patterns along the microchannels. When the refractive index of a material is modulated periodically in space, the material is called photonic crystal and may reflect light at a specific wavelength from ambient incident light. This particular wavelength of light corresponds to the forbidden photonic energy gap, which is called photonic band gap. Therefore, regularly stacked layers of colloidal particles in a medium with different refractive index may possess photonic band gaps. The red color in Figure 3a corresponds to the photonic band gap of the built-in silica colloidal crystal of 300 nm in diameter. As shown in Figure 3b, the colloidal crystal strip patterns reflected vivid iridescent red color after water was removed from the channels. As noted from the image, the crystallized patterns display uniform color without any noticeable cracks which usually occur during the conventional evaporation-induced crystallization process.

![Fig. 3 Silica colloidal crystals in centrifugal microfluidic chips. (a)~(b) show completely crystallized silica spheres of 300 nm in diameter which reflect red colour after centrifugation: (a) top view of a fluidic cell which has a set of parallel microchannels, (b) colloidal crystal strips of silica spheres in microchannels.](image)

When the PS particles were dispersed in water, the PS particles in aqueous medium did not settle down readily under the action of centrifugal force because of small density difference. In this case, as the spin velocity was increased over the threshold value, the PS particles were burst out together with water. Therefore, the PS particles were able to crystallize in the microfluidic channels only at slow rotation speeds far below the burst value. Under these conditions, evaporation-induced crystallization was dominant and consequently the time required for crystallization was quite long compared to the case of silica spheres. To solve this unfavorable problem for the case of aqueous suspension of PS particles, the surfaces of PS beads were modified by adsorbed PVP and the surface modified PS beads were redispersed in ethanol. The density of ethanol is 0.78 g/mL, which is much smaller than that of water. Therefore, the density contrast between PS particles and ethanol became much larger and the same procedure could be applied as used for the silica suspension. Figure 4a and 4b display the optical microscope images of the PS colloidal crystal patterns which were fabricated with 290 nm PS particles. As noted, the patterned PS colloidal crystal strips reflected uniform reddish iridescent color.
An SEM images of the silica colloidal crystals formed in the microchannels were shown in Figure 5a and 5b. As noted from the insets, the facing surface of silica colloidal crystal shows the hexagonal arrangement, which implies that the facing surface is the (111) plane of face-centered cubic (fcc) lattice. Therefore, the reflected colors displayed in Figure 3b corresponded to the stop band in the (111) direction (i.e., L-gap) of fcc lattice.

### 5. FLUIDIC MODULATION OF THE PHOTONIC CRYSTALS

Although the exact wavelength of reflected color can be predicted by solving Maxwell equations under periodic boundary conditions, Bragg’s equation also provides a simple and practical relation between refractive index, particle size and wavelength of reflected color, as represented in Eq. (4). \(^21\)

\[
\lambda = 2n_{\text{eff}} d_{111} \sin \theta
\]

Here, \(\lambda\) is the wavelength of reflected light, \(n_{\text{eff}}\) is effective refractive index, \(d_{111}\) is interlayer distance in the (111) direction and \(\theta\) is the incident angle to the (111) plane. Tunable photonic band gaps are of practical significance for adaptive photonic devices and can be achieved by integrating microfluidics with photonic crystals. Recently, microfluidic tuning of 2-dimensional silicon based photonic crystals was demonstrated for integrated optofluidic devices such as spectral filters and miniaturized nonlinear optics. \(^22\) In the present study, we fabricated hybrid colloidal crystals by the sequential injections of two different colloidal suspensions. The hybrid crystal is an ideal optofluidic platform for microfluidic sieves, optical filters and optical chromatography without any undetectable blind region of refractive index. By using this technique, colloidal crystals which have different lattice sizes, functionalities and dispersion media can be combined in a single microchannel. Figure 6 shows an SEM image of the interface between two different colloidal crystals. The background SEM image shows that the silica particles with two different sizes are crystallized at the two different regions with high quality ordering and the optical microscope image reproduced in the inset shows two different iridescent colors reflected from the hybrid colloidal crystals; red and yellowish green corresponding to their respective L-gaps.
If the refractive index of the medium is changed, the effective refractive index $n_{\text{eff}}$ is modulated according to the following relation (5).

$$n_{\text{eff}} = \sqrt{f_m n_m^2 + (1-f_m) n_p^2}$$

(5)

Here, $n_m$ and $n_p$ represent the refractive indices of the medium and the particle, respectively, and $f_m$ denotes the void fraction (0.26 for fcc crystals). Since the refractive index is the material property, the reflective color can be modulated by changing the medium. In Figure 7, the solid lines are the optical reflectance data of 255 nm silica colloidal crystal patterns in various solvent media. As the medium was changed from air to decaline, the wavelength of reflected light was shifted from 560 nm to 610 nm. However, the reflection peaks of the silica colloidal crystals in IPA and ethanol media were not strong enough to detect clearly because IPA and ethanol had the refractive indices similar to that of silica. Since the light recognizes the materials through the refractive index contrast, small refractive index difference between particles and medium allows transmission of light without causing noticeable reflection. Dotted lines indicate the reflection spectra of 250 nm PS colloidal crystal in various solvent media. The refractive index of PS is 1.595 which differs from 1.45 of silica, and therefore the PS colloidal crystal covers the blind region of the silica. Consequently, we could detect the peak of IPA and ethanol from the PS bead crystals which were not clearly noticeable in the silica colloidal crystals.
When the colloidal particles have surface moieties for specific binding of biomolecules, the photonic crystal structures can detect them by photonic band gap shifts or changes in reflected colors. Figure 8 shows the shifts of reflection spectra according to the specific binding of surface antigen of SARS corona virus and the antibody proteins. In addition, DNA and gold binding proteins were hybridized into the photonic crystals and their reflectance peak shifts were also detected. In this case, the colloidal crystals were made by conventional vertical coating method for the demonstrative purpose.

![Figure 8](image)

Fig. 8 Modulations of optical reflectance spectra of colloidal crystals by antigen-antibody interaction of the surface proteins of SARS corona virus.

6. CONCLUSIONS

We developed a high speed method for fabricating colloidal crystals inside of microchannels by using centrifugal microfluidics devices. Centrifugal force promoted the sedimentation process and the monodisperse silica and polystyrene particles were quickly packed into ordered state without forming noticeable cracks, which is practically advantageous over the conventional evaporation process. According to the geometrical feature of the microchannels, colloidal crystals were molded into designed patterns. The produced centrifugal microfluidic chip had multiplex microfluidic units. Centrifugal microfluidics approach enables us to perform a set of experiments over various microfluidic patterns at once. Moreover, the extremely small amounts of samples were used in this process since the volume of the microfluidic channel is nanoliter scale and 70 % of the channel volume is already occupied by the colloidal particles. Therefore, microscale multiplex centrifugal chip is useful for the multi-sample diagnostics and chemical and biological sensors with infinitesimal amount of samples. By using sequential injection technique, we could fabricate hybrid colloidal crystals in a single unit of microfluidic channels. Since the PS and silica colloidal crystals are complementary each other in photonic band gaps, they could span a wide range of the refractive indices of the solvent media. This result is demonstrative for the potential applications of our centrifugal microfluidic chips as optical chromatography devices without any blind region. Also, photonic crystals with fluidically modulated photonic band gaps can be applied for adaptive integrated photonic devices. Finally, the present centrifugal crystallization procedure is a versatile process and can be used for wide ranges of colloidal particles and solvent media with proper density mismatches.

ACKNOWLEDGEMENT

This work was supported by a grant from the Creative Research Initiative Program of MOST/KOSEF for “Complementary Hybridization of Optical and Fluidic Devices for Integrated Optofluidic Systems.” We also appreciate partial support from the Brain Korea 21 Program.
REFERENCES