Low temperature flow lithography

H. Lee, Y. H. Roh, H. U. Kim, and K. W. Bong

Chemical and Biological Engineering, Korea University, Seoul 02841, Republic of Korea

(Received 4 July 2018; accepted 29 August 2018; published online 24 September 2018)

Flow lithography (FL) is a microfluidic technique distinguished for its ability to produce hydrogel microparticles of various geometrical and chemical designs. While FL is typically performed in room temperature, this paper reports a new technique called low temperature flow lithography that uses low synthesis temperature to increase the degree of polymerization of microparticles without compromising other aspects of flow lithography. We suggest that decreased oxygen diffusivity in low temperature is responsible for the increase in polymerization. Microparticles that exhibit a higher degree of polymerization display a more developed polymer network, ultimately resulting in a more defined morphology, higher incorporation of materials of interest, and improved functional performance. This work demonstrates the increase in the degree of polymerization by examining the temperature effect on both the physical and chemical structures of particles. We show applications of this technique in synthesizing thin microparticles and enhancing microparticle-based detection of microRNA. Low temperature FL offers a simple and easy method of improving the degree of polymerization, which can be implemented in a wide range of FL applications. *Published by AIP Publishing.* <https://doi.org/10.1063/1.5047016>

I. INTRODUCTION

Flow lithography (FL), a microfluidic technique that synthesizes hydrogel particles via photolithography, has been gaining popularity because of its ability to assign complex geometrical and chemical designs to microparticles.¹ Such particles have demonstrated extended functionalities over homogenous spherical particles produced by conventional methods, such as emulsion polymerization^{2,3} and droplet-based microfluidics.^{4–6} For example, non-spherical particles of specific geometry^{7,8} have been utilized in photonics,⁹ self-assembly,¹⁰ microelectromechanical systems (MEMS),¹¹ and biomimicry systems, in which particles that structurally resemble biological elements, such as red blood cells, have been synthesized.^{12–15} Also, geometrical and chemical anisotropy has allowed for a precise control of particle retention and degradation in drug delivery.^{16–20} For multiplex bioassays, it has provided a new approach of encoding microparticles based on geometrical patterning and confined chemical labeling.^{21–23}

The capability of flow lithography to produce geometrically and chemically anisotropic microparticles stems from its particle synthesis process. With a photocurable prepolymer solution pressurized to flow inside a polydimethylsiloxane (PDMS) microfluidic channel, a pulse of photomask-defined UV light is flashed onto the solution. This results in the cross-linking of monomers into particles by free radical polymerization. Due to the oxygen permeability of PDMS, oxygen molecules can diffuse freely through the PDMS channel and react with radicals to inhibit polymerization.²⁴ This leads to the formation of polymerization inhibited layers on the oxygen-rich top and bottom of the channel, resulting in free-standing particles that can be flushed out with pressure.²⁵ The process is automated and repeated for mass production of particles. An improved adaptation to flow lithography called stop flow lithography involves stopping the flow before and during the UV exposure for higher particle resolution.²⁶ Unlike flow lithography in a continuous flow, a stopped flow allows for non-smeared UV exposures, resulting in a more defined particle shape with sharper edges. Geometrical anisotropy can be realized because the two-dimensional shape and size are determined by the photomask that the UV light passes through, while the particle height is determined by the channel height. Furthermore, chemical anisotropy is derived from

parallel laminar flows of different prepolymers, which are cross-linked adjacently, without mixing, to create chemically distinct regions within the particles.

For particles synthesized through flow lithography, their structural quality and functional performance are closely related to the degree of polymerization. Microparticles that exhibit a higher degree of polymerization display a more developed polymer network having a higher cross-linking density. This first results in a more defined particle morphology that more closely reflects the intended shape and size. Secondly, this results in a higher content of incorporated materials. For various applications, microparticles are often incorporated with functional materials, such as fluorophores,^{27,28} nanoparticles,^{29,30} antibodies,^{22,31} and DNA probes,²¹ etc. These materials can either be conjugated directly with the polymer network by free radical reaction or be encapsulated in between the network mesh. For both modes of incorporation, higher degree of polymerization leads to greater incorporation of materials, which results in higher functional performance.

Unfortunately, with the current flow lithography technique, there exist trade-offs between the degree of polymerization and other aspects of the technique. The most common methods of increasing the degree of polymerization include increasing the UV intensity and UV exposure duration. However, these may not be viable options for syntheses incorporating UV sensitive materials, such as DNAs and proteins, which can degrade upon exposure to high intensity 365 nm UV.^{32,33} Moreover, increasing the UV intensity often reaches a mechanical limit that is still insufficient for many synthesis conditions such as those incorporating UV absorbing materials (e.g., gold and magnetic nanoparticles)^{34,35} and in low height channels, where the inhibitory effect of oxygen is pronounced.²⁵ Also, increasing the duration of UV exposure decreases productivity since more time is assigned for each synthesis cycle, and above a certain UV dose, particles will adhere to the channel and disrupt further production.³⁶ Another method of increasing the degree of polymerization is by increasing the photoinitiator (PI) concentration in the prepolymer. However, photoinitiators, which are only slightly soluble in water, are poorly compatible with water soluble biomolecules, such as antibodies used for immunoassays. Increasing the PI concentration often causes the biomolecules to aggregate in the prepolymer.²² These problems illustrate that the common methods of increasing the degree of polymerization pose limitations on the prepolymer composition as well as the productivity of particle synthesis. There is a need for a method that can increase the degree of polymerization without compromising other aspects of flow lithography.

In this study, we present a simple and easy method that satisfies the need by lowering the synthesis temperature, which had been at room temperature, to below 15 °C. We first demonstrate that particles synthesized in low temperature exhibit a higher degree of polymerization by comparing the physical features of the microparticles synthesized at different temperatures. We highlight the synthesis of low height particles previously unattainable with FL in room temperature. We also examine the conjugation and encapsulation efficiencies of these particles to observe the changes in their chemical structure. In addition, we demonstrate the application of low temperature flow lithography and the enhanced particle performance in a microparticle based microRNA (miRNA) assay.

II. EXPERIMENTAL

A. Fabrication of the microfluidic devices

Identical microfluidic devices were used for all experiments except for the synthesis of low height particles. The first step of device fabrication is soft lithography of PDMS on a silicon master wafer that had been patterned using an SU-8,25 photoresist (MicroChem) to form positive relief microchannels of 50 μm height and 250 μm width. PDMS (SYLGARD 184, Dow Corning) mixed at a 10:1 base to curing agent ratio was poured over the master and cured for 4 h at 70 °C in an oven. Then, the cured PDMS was detached from the master and perforated with 1.0 mm and 10.0 mm punches (Miltex) to create inlet and outlet reservoirs, respectively. The inside center area of a glass Petri dish (D $\times$ H: 200 $\times$ 30 mm) was coated with PDMS and partially cured at 70 °C for 30 min. Then, the perforated PDMS block, with the negative relief pattern facing down, was attached onto the partially cured surface of the glass dish and fully cured overnight. An open ended cylinder (D $\times$ H: 40 $\times$ 50 mm) was glued to the center of the glass dish around the PDMS

channel. This created a donut-shaped reservoir inside the Petri dish outside the cylinder, which served as a constant temperature reservoir.

B. Stop flow lithography setup

The actuation of stop flow lithography involved using a UV-pressure synchronization system controlled by a customized circuit board and a LabView (National Instrument) script. With the microfluidic device mounted on an inverted microscope (Axiovert 200, Zeiss), the prepolymer solution was loaded into the device inlet in a pipette tip reservoir.³⁷ With a Tygon tube, the reservoir was connected to a compressed air tank, bridged by a pressure regulator (ITV0031-3BL, SMC Pneumatics). A photomask, designed using AutoCAD and printed using a high-resolution printer (50 000 dpi), was embedded into the field-stop of the microscope. A light-emitting diode (LED) lamp (M365LP1, Thorlabs) was used as the UV photo-polymerization source. For all experiments except for the synthesis of low height particles, the UV intensity was fixed at 1100 mW cm^{-2} as measured through a 20× objective by a UV power meter (GT-510, Giltron).

C. Control of the synthesis temperature

The synthesis temperature was controlled using the constant temperature reservoir around the device. The temperature of the glass underneath the microchannel was measured and intermittently monitored using an infrared thermometer (845, Testo). The glass temperature was taken to be the synthesis temperature since the microscale heat transfer to the fluid inside the microchannel is extremely efficient. To achieve the glass temperature of 5 °C, 10 °C, 23 °C, and 35 °C, a liquid pump (DWP-2760HW-B, Motorbank) was connected to the reservoir to circulate −9 °C 20% (v/v) ethanol, −4 °C 10% (v/v) ethanol, 23 °C water, and 65 °C water, respectively (Fig. S1 in the [supplementary material](#)). The coolants were cooled in an ultra-low temperature freezer (DFC-84CE, Operon) overnight at −70 °C. The temperatures of the coolants were maintained constant for the duration of melting inside a 30 × 22 × 15 cm insulated box. The water temperatures were controlled using a hot plate equipped with a temperature feedback probe. The glass temperature required a 5-min equilibrium time and remained constant as long as the coolant was in the process of melting. During particle synthesis, the temperature was monitored to deviate less than 0.5 °C from the target temperature.

D. Size experiment: Particle synthesis and analysis

The prepolymer solution consisted of 40% (v/v) poly(ethylene glycol) diacrylate (PEGDA700, Mn = 700 Da, Sigma-Aldrich), 20% (v/v) poly(ethylene glycol) (PEG200; Mn = 200 Da), 35% (v/v) deionized (DI) water, and 5% (v/v) 2-hydroxy-2-methylpropiophenone (photoinitiator, Sigma-Aldrich). One cycle of particle synthesis via stop flow lithography consisted of 300 ms of flow, 200 ms of stop, 75 ms of UV exposure, and 100 ms of hold time. A square (length = 40 μm) patterned photomask was used to yield one square prism (40 × 40 × 50 μm) particle per cycle. For each temperature condition, stop flow lithography was executed for 10 min, producing more than 800 particles for each temperature condition. After synthesis, the particles for each temperature were separately collected in a 0.5 ml microtube and rinsed five times in DI water containing 0.05% TWEEN® 20 (Sigma-Aldrich) before image analysis. Images for size analysis were taken using the inverted microscope equipped with a Canon EOS CD camera. The images were taken in the bright field through a 40× objective. The height and width of more than 20 particles for each temperature condition were recorded and analyzed using an image processing program (ImageJ).

E. Low height particles: Synthesis and analysis

Device fabrication followed the aforementioned process except the silicon wafer used for soft lithography was patterned with positive relief microchannels of 3.7 μm height and 100 μm width. The prepolymer solution consisted of 40% PEGDA700, 20% (v/v) PEG200, 25% (v/v) DI water,

5% (v/v) photoinitiator, and 10% (v/v) Rhodamine B methacrylate solution. The Rhodamine B methacrylate solution was prepared by dissolving Methacryloxyethyl Thiocarbonyl Rhodamine B (Polysciences, Inc.) in DI water at a concentration of 1 mg ml^{-1} . The prepolymer solution with an increased concentration of photoinitiator consisted of 40% PEGDA700, 20% (v/v) PEG200, 30% (v/v) photoinitiator, and 10% (v/v) Rhodamine B methacrylate solution. A photomask patterned with a circle of $30 \mu\text{m}$ in diameter was used. At the maximum UV intensity of 2200 mW cm^{-2} , one cycle of particle synthesis via stop flow lithography consisted of 700 ms of flow, 1000 ms of stop, 100 ms of UV exposure, and 400 ms of hold time. The particles synthesized in low temperature were collected in a 0.5 ml microtube and rinsed five times in DI water containing 0.05% TWEEN 20. The height of the particles in water was determined by atomic force microscopy (AFM) (NX-10, ParkSystems). Cantilever BL-AC40TS-C2 (Olympus) was used at a scan rate of 2.0 Hz and a set point of 3.0 nN. A line profile and a three-dimensional scan image were generated using the XEI software (ParkSystems).

F. Incorporation efficiency experiment: Particle synthesis

The prepolymer solution used for conjugation of Rhodamine B consisted of 40% PEGDA700, 20% (v/v) PEG200, 34% (v/v) DI water, 5% (v/v) photoinitiator, and 1% (v/v) Rhodamine B methacrylate solution. The Rhodamine B methacrylate solution was prepared by dissolving Methacryloxyethyl Thiocarbonyl Rhodamine B (Polysciences, Inc.) in DI water at a concentration of 1 mg ml^{-1} . One cycle of particle synthesis via stop flow lithography consisted of 300 ms of flow, 200 ms of stop, 75 ms of UV exposure, and 100 ms of hold time. Photomasks patterned with a circle (diameter = $150 \mu\text{m}$), square (length = $150 \mu\text{m}$), and hexagon (diameter = $200 \mu\text{m}$) were used to synthesize particles at 10°C , 23°C , and 35°C , respectively. For each temperature condition, stop flow lithography was executed for 10 min. After synthesis, the particles for each temperature were separately collected in a 0.5 ml microtube and rinsed five times in DI water containing 0.05% TWEEN 20 before image analysis. The particle synthesis for encapsulation of albumin-fluorescein isothiocyanate (FITC-albumin) is identical except the prepolymer solution consisted of 40% PEGDA700, 20% (v/v) PEG200, 30% (v/v) DI water, 5% (v/v) photoinitiator, and 5% (v/v) FITC-albumin solution. The FITC albumin solution was prepared by dissolving Albumin-fluorescein isothiocyanate conjugate (Sigma-Aldrich) in DI water at a concentration of 0.2 mg ml^{-1} .

G. Incorporation efficiency experiment: Image analysis

For direct comparison of the fluorescent intensity, the particles synthesized at different temperatures within the same mode of incorporation were mixed. Fluorescence images were taken on the inverted fluorescent microscope using the equipped Canon EOS CD camera in conjunction with a fluorescence light source (Illuminator HXP 120 V, Zeiss). The images were taken through the $10\times$ objective with the camera expose time of 250 ms. A red fluorescence filter set ($\lambda_{\text{ex}}/\lambda_{\text{em}} = 546/590 \text{ nm}$) was used for particles containing Rhodamine B methacrylate, and a yellow fluorescence filter set [$\lambda_{\text{ex}}/\lambda_{\text{em}} = (450\text{--}490)/(515\text{--}565) \text{ nm}$] was used for particles containing FITC albumin. The brightness of the fluorescent signal of more than 20 particles for each temperature condition were recorded and analyzed using an image processing program (ImageJ).

H. miRNA assay: Particle synthesis

We first prepared a base pre-polymer solution composed of 20% (v/v) PEGDA700, 40% (v/v) PEG200, 5% (v/v) photoinitiator, and 35% (v/v) $3\times$ Tris EDTA (TE) buffer (Sigma-Aldrich). Then, this base pre-polymer solution was mixed at a 9:1 ratio with a single-stranded DNA (ssDNA) probe solution, which was prepared by dissolving an acrydite-modified ssDNA oligomer (Integrated DNA Technologies) in $1\times$ TE buffer at a concentration of 1 mM. One cycle of particle synthesis via stop flow lithography consisted of 300 ms of flow, 200 ms of stop, 120 ms of UV exposure, and 100 ms of hold time. For all temperature conditions, a photomask patterned with a

circle (diameter = 150 μm) was used, and stop flow lithography was executed for 10 min. After synthesis, the particles for each temperature were separately collected and rinsed seven times in 1 $\times$ TET buffer (1 $\times$ TE buffer with 0.05% (v/v) TWEEN 20). The particles were stored in a total volume of 170 μl of 1 $\times$ TET buffer at 4 $^{\circ}\text{C}$ prior to use (~ 5 particles per 1 μl).

I. miRNA assay: miRNA detection and image analysis

miRNA detection assays were carried out in a total volume of 50 μl . First, 25 μl 1 $\times$ TET buffer was applied to each of three microtubes followed by adding 5 μl of the target miRNA (hsa-miR-1306-5p, Integrated DNA Technologies). The concentration of the target miRNA in the assay volume was 10 pM. The mixture was heated to 95 $^{\circ}\text{C}$ for 5 min in a thermal shaker (Hangzhou Allsheng Instruments Co., Ltd). After cooling to room temperature, 20 μl of particles from each temperature condition (a total of ~ 100 particles per tube) were added to separate microtubes. Each assay volume contained 350 mM NaCl. The assay microtubes were incubated at 55 $^{\circ}\text{C}$ for 90 min in the thermal shaker with agitation at 1500 rpm in order to allow for the hybridization of the target miRNAs with probes in the microparticles. The microparticles were rinsed three times with 1 $\times$ TET containing 50 mM NaCl. Then, 245 μl of ligation mixture was added, which was composed of 150 μl 10 $\times$ NEBuffer 2 (New England Biolabs), 1350 μl 1 $\times$ TET, 250 nM ATP (New England Biolabs), 40 nM universal adapter (Integrated DNA Technologies), and 800 U ml^{-1} T4 DNA ligase (Fermentas). The hybridization and ligation of the universal adapter (a total volume of 295 μl) were performed simultaneously at 21.5 $^{\circ}\text{C}$ for 45 min in the thermal shaker with agitation at 1500 rpm. After another rinsing process as described above, streptavidin–phycoerythrin (SA–PE; Life Technologies), diluted in 1 $\times$ TET by a factor of 50, was added. A final incubation step was allowed at 21.5 $^{\circ}\text{C}$ for 45 min. The particles were rinsed three times again before image analysis. Fluorescence images were taken on the inverted fluorescent microscope using the equipped Canon EOS CD camera in conjunction with a fluorescence light source (Illuminator HXP 120V, Zeiss). The images were taken through the 10 $\times$ objective with the camera expose time of 250 ms. A red fluorescence filter set ($\lambda_{\text{ex}}/\lambda_{\text{em}} = 546/590$ nm) was used throughout. The brightness of the fluorescent signal of more than 20 particles for each temperature condition were recorded and analyzed using an image processing program (ImageJ).

III. RESULTS AND DISCUSSION

A. Low temperature flow lithography

Flow lithography in low temperature (Fig. 1) can be actualized in many different forms. Examples include flow lithography in a refrigeration room, where the synthesis temperature is in equilibrium with the surrounding, and flow lithography using a commercialized cooling microscope stage, which is used in conjunction with the microscope and lowers the temperature of the microfluidic channel placed on top. Among many, we selected a simple and cost-effective cooling system that utilizes a constant temperature liquid reservoir around the microchannel to lower the temperature of the glass supporting the microfluidic channel (Fig. S1 in the [supplementary material](#)). Because heat transfer is highly efficient in the microscale, controlling the temperature of the glass provided an effective way to control the temperature of the prepolymer solution during particle synthesis. The range of operating temperature for low temperature flow lithography was established to be from 0 $^{\circ}\text{C}$ to 15 $^{\circ}\text{C}$ based on the experiment (Fig. S2 in the [supplementary material](#)).

Although seemingly counterintuitive, the increase in the degree of polymerization in low temperature can be explained by examining the change in a dimensionless Damköhler number [Eq. (1)],^{24,38} which quantifies the ratio of the rate of radical propagation to the diffusion of oxygen in the prepolymer solution. Since radical propagation is equivalent to polymerization, the rate of radical propagation governs the degree of polymerization.

$$\text{Da} = \frac{k_p k_O [O_2]_{\text{eq}} H^2}{2k_t D_O}. \quad (1)$$

FIG. 1. Particle synthesis using low temperature flow lithography. Unlike typical flow lithography, which is performed in room temperature, low temperature FL synthesizes particles at a lower temperature in the range of 0 °C–15 °C to increase the degree of particle polymerization. During particle synthesis, the temperature of the glass support is decreased using a constant temperature reservoir around the device. The increase in the degree of particle polymerization in low temperature is illustrated by the increase in fluorescent signal of particles encapsulating a fluorophore.

Here, the k values are rate constants of radical propagation (k_p), radical termination (k_t), and oxygen inhibition (k_o). $[O_2]_{eq}$ is the equilibrium concentration of oxygen in the prepolymer solution. H is the height of the microchannel, and D_O is the diffusion coefficient of oxygen in the prepolymer solution. The diffusion coefficients of monomer and initiator species have been excluded because of their relatively large molecular sizes.²⁴ As an illustration, the Damköhler numbers at a low temperature (4 °C) and room temperature (25 °C) were compared using the parameters shown in Table I. The details of D_O and k value acquisition for 4 °C are provided in the [supplementary material](#). Assuming the residence time of the prepolymer in the channel before UV exposure is too short to alter the equilibrium oxygen concentration, the Damköhler number at 4 °C was estimated to be 1.9 times greater than that at 25 °C. This indicates that the radical propagation with respect to diffusion of oxygen at 4 °C is almost as twice as fast compared to that at 25 °C. Such a dramatic change is observed primarily because the diffusion coefficient of oxygen D_O , which has the largest order of magnitude, is 2.88 times lower at 4 °C. Thus, the decrease in the diffusion coefficient of oxygen in low temperature can account for the majority of the increase in particle polymerization.

It is important to note that low temperature flow lithography is inherently different from oxygen purging, which is another measure that can be taken to increase the degree of particle polymerization. Oxygen purge chambers have been used with flow lithography to synthesize particles of more defined morphologies and previously unattainable sizes.^{12,25} This system utilizes an inert gas chamber around the microfluidic device to lower the effective concentration of oxygen in the air in contact with the device. Fewer oxygen molecules are available in the air to diffuse into the prepolymer solution and act as inhibitors, and this leads to the increase in the degree of polymerization. However, controlling the oxygen concentration is by no means an easy task as it requires the microchannel along with the precursor supply tubes to be placed inside a completely sealed chamber coordinated with an oxygen tank and an inert gas tank. Because the polymerization is sensitive to even slight perturbations of the oxygen concentration, the oxygen concentration in the chamber must be precisely monitored with a sensor. Moreover, the decrease in the oxygen concentration decreases the amount of effective oxygen in the PDMS-prepolymer interface, thereby increasing the likelihood of particle adhesion to the channel walls. On the other hand, temperature control in low temperature flow lithography is relatively easy and cost-effective because it takes place in open air without the use of specialized equipment. Also, because the oxygen concentration in the PDMS is unaltered, the adhesion of particles to the channel walls, often problematic for oxygen purging, is not observed.

TABLE I. Parameters used in Damköhler number calculation.

Parameter	Value	Units	Source
k_p (25 °C)	25	$\text{m}^3 \text{mol}^{-1} \text{s}^{-1}$	Ref. 39
k_p (4 °C)	14.7	$\text{m}^3 \text{mol}^{-1} \text{s}^{-1}$	Calculated ⁴⁰
k_t (25 °C)	2.52×10^3	$\text{m}^3 \text{mol}^{-1} \text{s}^{-1}$	Ref. 39
k_t (4 °C)	2.23×10^3	$\text{m}^3 \text{mol}^{-1} \text{s}^{-1}$	Calculated ⁴⁰
k_O (25 °C)	5×10^5	$\text{m}^3 \text{mol}^{-1} \text{s}^{-1}$	Ref. 41
k_O (4 °C)	5×10^5	$\text{m}^3 \text{mol}^{-1} \text{s}^{-1}$	Calculated ⁴²
D_O (25 °C)	2.84×10^{-11}	$\text{m}^2 \text{s}^{-1}$	Ref. 43
D_O (4 °C)	9.86×10^{-12}	$\text{m}^2 \text{s}^{-1}$	Calculated ⁴⁴
H	50	μm	Measured
$[O_2]_{\text{eq}}$	1.5	mol m^{-3}	Ref. 45

B. Temperature effect on particle size

To determine the effect of temperature on the degree of polymerization, the dimensions of particles synthesized at three different temperatures were compared (Fig. 2). The size of particles can be considered an appropriate indication of the degree of polymerization because the particle architecture is the result of polymerization of cross-linking monomers. Upon UV exposure, the oxygen concentration in the prepolymer, which had been at equilibrium with the oxygen concentration in the PDMS, begins to rapidly diminish. Because the oxygen concentrations are no longer in equilibrium, there is an influx of oxygen molecules from the PDMS to the prepolymer. Oxygen inhibition dominates until the oxygen concentration in the UV exposed region of the prepolymer is reduced by at least three orders of magnitude.²⁴ Polymerization starts and grows from the center of the region, where the rate of oxygen diffusion is much slower than the rate of oxygen inhibition. At and near the PDMS interfaces, however, the rates are balanced, resulting in unpolymerized layers of the prepolymer.¹² Since polymerization grows outwardly from the center of the particle, a larger particle is indicative of a higher degree of polymerization.²⁷

Square prism microparticles were synthesized at 10 °C, 23 °C, and 35 °C, while other conditions were kept constant. 23 °C was the representative temperature for the room temperature condition. 10 °C was chosen as the low temperature condition because it could be easily achieved with our system, and 35 °C was selected as the high temperature condition because of the comparable difference with room temperature. The particles synthesized at 10 °C displayed the largest dimensions that most closely reflected the intended size. It also exhibited sharper edges and corners. When the particle height and width were measured and averaged, we observed a clear correlation of the increase in size with the decrease in temperature. Compared to the particles synthesized at 23 °C, those synthesized at 10 °C showed an increase in height and width of approximately 5 μm and 4 μm , respectively, which are both an 11% increase. The analysis of the particles' physical structures clearly suggested that the degree of particle polymerization was increased in low temperature. It also demonstrated that low temperature flow lithography can be utilized to produce more structurally robust particles. It should be noted that the degree of polymerization can also be enhanced by increasing the UV intensity and exposure. To achieve the same degree of polymerization, the UV intensity, which is directly proportional to the diffusion coefficient of oxygen, must be 2.88 times higher at 25 °C than at 4 °C.²⁴ Similarly, the induction time, the minimum UV exposure time required for particle gelation, is 2.88 times longer at 25 °C than at 4 °C (supplementary material).

C. Low temperature fabrication of low height particles

The temperature effect on particles' physical structure was more clearly observed with the synthesis of low height particles. In this work, we synthesized particles of thinness previously unattainable with room temperature flow lithography using a 3.7 μm microchannel. In low temperature

FIG. 2. The effect of synthesis temperature on the size of particles synthesized at 10 °C, 23 °C, and 35 °C. The bright field microscopic images show the square prism particles on their sides. The bar graph plots the average heights and widths of particles synthesized in each temperature. The vertical error bars indicate the experimental standard deviation of >20 particles. The particles synthesized at 10 °C mostly closely reflected the intended particle size and shape. The ideal height is based on the channel height, and the ideal width is based on the photomask design.

(5 °C), we were able to synthesize circular disk-like particles of 30 μm in diameter [Fig. 3(a)] with a 100 ms exposure of the maximum UV intensity at 2200 mW cm⁻². However, the same setting did not produce any particles in room temperature (23 °C), even with an increase in the photoinitiator concentration to 30%. Video S1 in the [supplementary material](#) compared the particle synthesis between two temperatures. Atomic force microscopy (AFM) scan of the particles revealed the average particle height of 1.132 μm in water (Fig. 3). This result demonstrated that the vertical resolution of flow lithography can be improved in low temperature, which can be easily understood because the square root of a Damköhler number and the thickness of inhibition layers are inversely proportional to each other.²⁴ Because of the higher Damköhler number in low temperature, the thickness of the inhibition layer is decreased, increasing the degree of polymerization in the center region, resulting in particle formation. In our experiment, increasing the photoinitiator concentration and UV intensity, which was already at its maximum, proved ineffective at increasing the degree of polymerization in low height microchannels because of the pronounced inhibitory effect of oxygen. The synthesis of low height particles is a useful application of low temperature FL that cannot be reproduced in the typical room temperature flow lithography.

D. Temperature effect on particles' incorporation capacity

1. Conjugation

A chemical indication of the degree of polymerization is the incorporation capacity of particles. To perform a comprehensive analysis of the temperature effect on the incorporation capacity, we accounted for the two main modes of incorporation: conjugation and encapsulation. Conjugation describes the incorporation of materials that form covalent bonds with the polymer network by free radical reaction. If the degree of polymerization is increased, which results in more numerous cross-links of monomers, the conjugation of these materials is bound to increase as well. Therefore, we sought to examine the degree of polymerization by analyzing the amount conjugated.

Rhodamine B methacrylate, which is a red fluorescent compound, was used as the model material for conjugation. The fluorescence of Rhodamine B methacrylate was confirmed to be independent of temperature within the temperature range of this study. The particles' fluorescent

FIG. 3. Low height particles synthesized using low temperature flow lithography. Circular disk-like particles of 30 μm in diameter and 1.132 μm in height were produced in low temperature at 5 $^{\circ}\text{C}$. The same particle size is not achievable with room temperature flow lithography even at an increased concentration of photoinitiator. (a) Bright field image of the synthesized particles. (b) Surface scan of a particle using liquid atomic force microscopy (AFM). The green vertical line indicates the path line profile was taken. (c) Line profile of the particle height. The average height is determined to be 1.132 μm . (d) Three-dimensional image of the AFM scan. Note the Z scale and the XY scale are not in the same scale.

intensity served as a measure of their material content. Using a prepolymer solution containing Rhodamine B methacrylate, particles were synthesized at 10 $^{\circ}\text{C}$, 23 $^{\circ}\text{C}$, and 35 $^{\circ}\text{C}$. While other conditions were kept constant, photomasks of different shapes were used for each temperature to encode the particles based on their cross-sectional shape. This allowed us to directly compare the particles synthesized at different temperatures in the same microscopic frame. Particles synthesized at 10 $^{\circ}\text{C}$, 23 $^{\circ}\text{C}$, and 35 $^{\circ}\text{C}$ were identified by their circular, square, and hexagonal cross section, respectively. The shape of the cross section does not alter the conjugation of fluorophores because the differences in their cross-sectional areas are negligible. Also, it has no effect on the measurement of fluorescent intensity because only the fluorescent areas of the particles are measured.

We observed a clear effect of temperature on the conjugation efficiency of Rhodamine B methacrylate as shown in Fig. 4(a). The particles synthesized at 10 $^{\circ}\text{C}$ (circle) displayed the strongest signal followed by the particles synthesized at 23 $^{\circ}\text{C}$ (square). The increase in the fluorescent signal of particles synthesized in low temperature showed that the conjugation of materials, hence the degree of particle polymerization, increased in low temperature.

2. Encapsulation

The other mechanism through which materials of interest can be incorporated in microparticles is encapsulation. Encapsulation describes the incorporation of materials that are caught in between

FIG. 4. The effect of synthesis temperature on the incorporation capacity of particles. Both modes of incorporation are considered: (a) conjugation of Rhodamine B methacrylate and (b) encapsulation of FITC-albumin. The synthesis temperature of each particle is identified by its cross-sectional shape, which allows for the comparison of particles in the same microscopic frame. The vertical error bars indicate the experimental standard deviation of >20 particles. For both modes of incorporation, the fluorescent intensity of the particles, which correlates with the quantity of incorporated fluorophores, increases with the decrease in temperature.

the mesh-like polymer network. If the degree of polymerization is increased, more cross-links will form and the mesh size will become smaller. Smaller mesh size is more efficient in securely encapsulating the materials, preventing them from being released out of the microparticles. The fineness and the size of the mesh are especially critical for small materials. Identical to the conjugation analysis, we examined the degree of polymerization by analyzing the amount encapsulated in the microparticles. Albumin-fluorescein isothiocyanate conjugate (FITC-albumin), which is a green fluorescent nanosized protein, was used as the model material for encapsulation. The fluorescence of FITC-albumin was confirmed to be independent of temperature within the temperature range of this study. FITC-albumin is an ellipsoidal nanoparticle with diameters of 3.5 nm and 14 nm, which were considered a suitable size for this experiment considering the particle network density.⁴⁶ If larger nanoparticles, such as those whose diameter is greater than 100 nm, were to be used, the encapsulation would be less sensitive to the change in the mesh size and would not give a meaningful result concerning the degree of polymerization.

Microparticles were synthesized at 10 °C, 23 °C, and 35 °C using a prepolymer solution containing FITC-albumin. The particles were shape coded in the same manner as before. The effect of temperature on the encapsulation efficiency of FITC-albumin was clearly observed as shown in Fig. 4(b). The fluorescent intensity profile of FITC-albumin closely resembled that of Rhodamine B methacrylate. The circular particles synthesized at 10 °C displayed the strongest signal followed by the square particles synthesized at 23 °C. A stronger fluorescent signal is indicative of a higher content of FITC-albumin. Therefore, the high fluorescent signal of particles synthesized at 10 °C demonstrated the increase in encapsulation capacity and the degree of particle polymerization in low temperature. Furthermore, the result illustrated the capability of low temperature flow lithography to produce microparticles that incorporate an increased amount of materials of interest.

E. Temperature effect on detection of microRNA

Since the functionality of microparticles is assigned by the type of incorporated materials, a higher content of materials leads to an increase in performance. By using low temperature flow lithography to produce microparticles whose function is to detect a strand of microRNA (miRNA), we sought to confirm the increase in performance and also present another practical application of low temperature flow lithography. Microparticles used in microRNA assays are incorporated with complementary single stranded DNA (ssDNA) probes. Like Rhodamine B methacrylate, these DNA probes are incorporated by conjugation. Increasing the degree of polymerization of such particles by increasing the UV dose would be problematic because of the UV degradability of ssDNA probes. The synthesis and assay procedures closely followed the established procedures for microparticle based miRNA assays.^{21,47}

Using a prepolymer solution containing DNA probes, microparticles were synthesized at 10 °C, 23 °C, and 35 °C. The identical photomask pattern, a circle of 150 μm diameter, was used for all conditions to eliminate any shape dependency on the diffusion of additives during the assay. Because the assay involves the addition of detection compounds to the particles after synthesis, their diffusion into the particle is influenced by the particles' size and the shape. The assay began with the conjugation of target miRNAs with the DNA probes present in the microparticles by incubating the particles with miRNAs at a concentration of 10 pM. The conjugated DNA probes were then fluorescently labeled with streptavidin-phycoerythrin (SAPE) in a series of hybridization steps. So, a higher fluorescent intensity would indicate a higher detection of miRNA.

As seen in Fig. 5, the particles synthesized at 10 °C displayed the highest fluorescent signal intensity, especially around the edges of the particle. This is a common phenomenon caused by the high Damköhler number of the hybridization reaction.⁴⁸ The density of the DNA probes is too high for the SAPE molecules to diffuse deeper into the network before reacting, so most of the reactions take place around the edges. Regardless of the fluorescence pattern, the total intensity corresponds with the total detection of miRNA. Thus, the particles synthesized in low temperature resulted in the highest detection of miRNA, and this is due to the highest incorporation of the probes. Compared to the fluorescent intensity profile of Rhodamine B methacrylate conjugated microparticles, the difference between the miRNA detection microparticles synthesized at 23 °C

FIG. 5. The effect of synthesis temperature on microparticle based detection of miRNA. Single stranded DNA probes are incorporated in the particles via conjugation. The particles are hybridized with the target miRNA at a concentration of 10 pM and fluorescently labeled to identify the detection. The increase in fluorescent intensity of particles synthesized at 10 °C is due to the increase in the number of conjugated probes. The particles synthesized in low temperature resulted in the highest detection of the target miRNA. The vertical error bars indicate the experimental standard deviation of >20 particles.

and 35 °C is less apparent. This can be explained by the diffusion of SAPE in the polymer network. Unlike Rhodamine B methacrylate, which is a fluorescent marker already present in the prepolymer solution, the fluorescent marker SAPE is added after particle synthesis. Because the diffusion of SAPE into the microparticle is enhanced when the mesh size is large, the larger mesh size of particles synthesized at 35 °C can compensate for the smaller number of DNA probes. With the miRNA assay as a practical example, we demonstrated that the increase in particles' functional performance can be achieved using low temperature flow lithography.

IV. CONCLUSIONS

In this work, we demonstrated the increase in the degree of polymerization of particles synthesized in low temperature by examining the temperature effect on both their physical and chemical structures. The increase in the particle size and the conjugation and encapsulation capacities gave clear results for higher degree of particle polymerization in low temperature. We accentuated the capability of our technique to synthesize low height particles not producible with the typical room temperature flow lithography. We also presented an application of low temperature flow lithography in a microparticle based miRNA detection and confirmed the improvement in performance. All these results showed that low temperature flow lithography increases the degree of particle polymerization without decreasing productivity or placing a restriction on the prepolymer composition. Not only is the technique simple and easy, but it can also be applied to any flow lithography technique. The prepolymer is not limited to PEGDA species but can be expanded to other species as well. We believe this simple yet powerful technique will be easily implemented and possibly be utilized in a wide range of flow lithography applications.

SUPPLEMENTARY MATERIAL

See [supplementary material](#) for device setup (Fig. S1), calculation of parameters used for Damköhler number calculation, temperature range of low temperature flow lithography (Fig. S2), low height particle synthesis (Multimedia S1), and low temperature flow lithography using a different monomer (Fig. S3).

ACKNOWLEDGMENTS

This work was supported by the Engineering Research Center of Excellence Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (NRF-2016R1A5A1010148) and the Next-Generation Biogreen 21 Program funded by Rural Development Administration of Republic of Korea (No. PJ013158). This research was also supported by the Basic Science Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2018R1D1A1B07046577, NRF-2017R1D1A1B03029883, and NRF-2017R1D1A1B03029392).

- ¹D. Dendukuri, D. C. Pregibon, J. Collins, T. A. Hatton, and P. S. Doyle, *Nat. Mater.* **5**, 365 (2006).
- ²D. Dupin, S. Fujii, S. P. Armes, P. Reeve, and S. M. Baxter, *Langmuir* **22**, 3381 (2006).
- ³M. Zhang, M. Nowak, P. Malo de Molina, M. Abramovitch, K. Santizo, S. Mitragotri, and M. E. Helgeson, *Langmuir* **33**, 6116 (2017).
- ⁴D. Dendukuri and P. S. Doyle, *Adv. Mater.* **21**, 4071 (2009).
- ⁵S. Sugiura, M. Nakajima, H. Itou, and M. Seki, *Macromol. Rapid Commun.* **22**, 773 (2001).
- ⁶E. Y. Liu, S. Jung, and H. Yi, *Langmuir* **32**, 11043 (2016).
- ⁷K. S. Paulsen, D. Di Carlo, and A. J. Chung, *Nat. Commun.* **6**, 6976 (2015).
- ⁸S. H. Song, K. Kim, S. E. Choi, S. Han, H. S. Lee, S. Kwon, and W. Park, *Opt. Lett.* **39**, 5162 (2014).
- ⁹H. Kim, J. Ge, J. Kim, S. E. Choi, H. Lee, H. Lee, W. Park, Y. Yin, and S. Kwon, *Nat. Photonics* **3**, 534 (2009).
- ¹⁰S. E. Chung, W. Park, S. Shin, S. A. Lee, and S. Kwon, *Nat. Mater.* **7**, 581 (2008).
- ¹¹R. F. Shepherd, P. Panda, Z. Bao, K. H. Sandhage, T. A. Hatton, J. A. Lewis, and P. S. Doyle, *Adv. Mater.* **20**, 4734 (2008).
- ¹²H. Z. An, E. R. Safai, H. B. Eral, and P. S. Doyle, *Lab Chip* **13**, 4765 (2013).
- ¹³N. Doshi, A. S. Zahr, S. Bhaskar, J. Lahann, and S. Mitragotri, *Proc. Natl. Acad. Sci.* **106**, 21495 (2009).
- ¹⁴T. J. Merkel, S. W. Jones, K. P. Herlihy, F. R. Kersey, A. R. Shields, M. Napier, J. C. Luft, H. Wu, W. C. Zamboni, and A. Z. Wang, *Proc. Natl. Acad. Sci.* **108**, 586 (2011).

- ¹⁵K. Chen, T. J. Merkel, A. Pandya, M. E. Napier, J. C. Luft, W. Daniel, S. Sheiko, and J. M. DeSimone, *Biomacromolecules* **13**, 2748 (2012).
- ¹⁶Y. Hu, Q. Wang, J. Wang, J. Zhu, H. Wang, and Y. Yang, *Biomicrofluidics* **6**, 26502 (2012).
- ¹⁷H. U. Kim, D. G. Choi, Y. H. Roh, M. S. Shim, and K. W. Bong, *Small* **12**, 3463 (2016).
- ¹⁸N. A. Peppas, J. Z. Hilt, A. Khademhosseini, and R. Langer, *Adv. Mater.* **18**, 1345 (2006).
- ¹⁹S. Rahmani, T.-H. Park, A. F. Dishman, and J. Lahann, *J. Control. Release* **172**, 239 (2013).
- ²⁰H. U. Kim, D. G. Choi, H. Lee, M. S. Shim, and K. W. Bong, *Lab Chip* **18**, 754 (2018).
- ²¹S. C. Chapin, D. C. Appleyard, D. C. Pregibon, and P. S. Doyle, *Angew. Chem.* **50**, 2289 (2011).
- ²²D. C. Appleyard, S. C. Chapin, and P. S. Doyle, *Anal. Chem.* **83**, 193 (2010).
- ²³H. Lee, J. Kim, H. Kim, J. Kim, and S. Kwon, *Nat. Mater.* **9**, 745 (2010).
- ²⁴D. Dendukuri, P. Panda, R. Haghgoie, J. M. Kim, T. A. Hatton, and P. S. Doyle, *Macromolecules* **41**, 8547 (2008).
- ²⁵H. Z. An, H. B. Eral, L. Chen, M. B. Chen, and P. S. Doyle, *Soft Matter* **10**, 7595 (2014).
- ²⁶D. Dendukuri, S. S. Gu, D. C. Pregibon, T. A. Hatton, and P. S. Doyle, *Lab Chip* **7**, 818 (2007).
- ²⁷R. Haghgoie, M. Toner, and P. S. Doyle, *Macromol. Rapid Commun.* **31**, 128 (2010).
- ²⁸J. J. Kim, K. W. Bong, E. Reátegui, D. Irimia, and P. S. Doyle, *Nat. Mater.* **16**, 139 (2016).
- ²⁹K. W. Bong, S. C. Chapin, and P. S. Doyle, *Langmuir* **26**, 8008 (2010).
- ³⁰K. P. Yuet, D. K. Hwang, R. Haghgoie, and P. S. Doyle, *Langmuir* **26**, 4281 (2009).
- ³¹L. Chen, H. Z. An, R. Haghgoie, A. T. Shank, J. M. Martel, M. Toner, and P. S. Doyle, *Small* **12**, 2001 (2016).
- ³²J. G. Peak, B. Pilas, E. J. Dudek, and M. J. Peak, *Photochem. Photobiol.* **54**, 197 (1991).
- ³³C. Kiehlbassa, L. Roza, and B. Epe, *Carcinogenesis* **18**, 811 (1997).
- ³⁴W. Haiss, N. T. Thanh, J. Aveyard, and D. G. Fernig, *Anal. Chem.* **79**, 4215 (2007).
- ³⁵M. Răcuciu, D. Creangă, A. Airinei, D. Chicea, and V. Bădescu, *Mater. Sci. Pol.* **28**, 609 (2010).
- ³⁶J. Kim, H. An, Y. Seo, Y. Jung, J. S. Lee, N. Choi, and K. W. Bong, *Biomicrofluidics* **11**, 24120 (2017).
- ³⁷J. H. Sim, H. J. Moon, Y. H. Roh, H. W. Jung, and K. W. Bong, *Korean J. Chem. Eng.* **34**, 1495 (2017).
- ³⁸S. K. Suh, K. W. Bong, T. A. Hatton, and P. S. Doyle, *Langmuir* **27**, 13813 (2011).
- ³⁹S. Kızılel, V. H. Pérez-Luna, and F. Teymour, *Macromol. Theory Simul.* **15**, 686 (2006).
- ⁴⁰S. Beuermann and M. Buback, *Prog. Polym. Sci.* **27**, 191 (2002).
- ⁴¹C. Decker and A. D. Jenkins, *Macromolecules* **18**, 1241 (1985).
- ⁴²M. Lázár, *Free Radicals in Chemistry and Biology* (CRC Press, FL, 1989).
- ⁴³H. Lin and B. D. Freeman, *Macromolecules* **39**, 3568 (2006).
- ⁴⁴P. Gonzalez-Tello, F. Camacho, and G. Blazquez, *J. Chem. Eng. Data* **39**, 611 (1994).
- ⁴⁵M. D. Goodner and C. N. Bowman, *Chem. Eng. Sci.* **57**, 887 (2002).
- ⁴⁶M. Gekle, *Annu. Rev. Physiol.* **67**, 573 (2005).
- ⁴⁷Y. H. Roh, S. J. Sim, I.-J. Cho, N. Choi, and K. W. Bong, *Analyst* **141**, 4578 (2016).
- ⁴⁸H. Lee, S. J. Shapiro, S. C. Chapin, and P. S. Doyle, *Anal. Chem.* **88**, 3075 (2016).