Supporting Information

Three-dimensional Flash Flow Microreactor for Scale-up Production of Monodisperse PEG-PLGA Nanoparticles

Kyoung-Ik Min, a Do Jin Im, b Hyune-Jea Lee, a Dong-Pyo Kim a,*

a Center of Applied Microfluidic Chemistry, Department of Chemical Engineering, Pohang University of Science and Technology (POSTECH), Pohang, Republic of Korea

b Department of Chemical Engineering, Pukyong National University, Busan, Republic of Korea
Fabrication of parallel 3D flash flow microreactor (3D-FFM)

As shown in Figure 1a, the parallel 8 sets of microchannels with 3D flow focusing geometry were designed exactly symmetrical in its structure which ultimately allowed us to attain homogeneous condition at each microchannel for high-throughput production. To fabricate the proposed polyimide (PI) film based 3D flash flow microreactor (3D-FFM) system, we used UV laser ablation technique (355 nm, ESI, USA) as previous report.[10] The laser spot size was fixed to become a 25 μm diameter circle, and the stage was controlled to fully ablate the 125 μm thick PI films (Kapton HN film, Dupont, USA) directly without mask. Each layer of PI film (95 mm x 70 mm) were ablated by laser to form desired shape along the edge of microchannel (300 μm width and 125 μm depth) and holes (1 mm diameter circle for in/outlet, 300 μm square shape for junction between microchannels) as shown in Figure S1, and the end of microchannel overlapped with other microchannel or square hole (300 μm x 300 μm) when assembling all films with simple alignment. The dimensions of main microchannel for flow focusing were 300 μm of width, 125 μm of height and 1.5 cm of length, respectively.

After laser ablation, the films were cleaned by washing with acetone and dried under nitrogen. Next, all ablated films were coated with fluoropolymer (ND-110, Neoflon, DAIKIN, Japan, 60 % solids fluorothermoplastic aqueous dispersion) which is 150 nm of FEP (fluoroethylene propylene) nanopowder dispersed in aqueous medium with a softening point at 260~280 °C as thermal adhesive layer. The coating thickness was 3 μm of packed nanopowders layer after spin coating at 1500 rpm of speed for 30 sec and evaporation of solvent at 100 °C for 10 min as shown in Figure S2. And 4 corners of each film holed with 1 mm diameter were passed through metal pins to align the film patterns. Additionally, the plain PI films were placed as top and bottom cushions to homogeneously distribute the applied pressure over the film so as to enhance the bonding reliability. All patterned and aligned PI films were placed between flat metal plate, and then finally the PI film based microchannels were sealed by simple one-
step bonding of seven layered films that pressing together at 300 °C under a pressure of 10 kPa for at least 2 hr (Figure S3). Finally the fabricated 3D-FFM was assembled with metal holders to connect tube as shown in Figure 1e.

**Figure S1.** Photo image of laser-ablated polyimide film with microchannel design on each layer. The end of microchannel exactly overlapped with other microchannel or square hole when assembling all films after one-step multilayered bonding process with simple alignment by inserting metal pin. The inset shows order of level in aligned films.

**Figure S2.** SEM images of a) cross-sectional and b) top-view of FEP nanoparticles right after spin coating on polyimide film.
Figure S3. Scheme for fabrication of 3D-FFM device by one-step multilayer bonding process at 300 °C for 2 h after alignment using metal pin.
**Pressure test of 3D-FFM system**

The pressure tolerance test of the sealed 3D-FFM device was measured by HPLC pump (SP-930D, Younglin, Korea). The HPLC pump was connected with 3D-FFM device to infuse water. Then, water was introduced into the 3D-FFM device by controlling HPLC pumps in the range of 1~30 mL/min. Durability was tested by pumping water at 30 mL/min for 3 h. In addition, evolved burst pressure inside the 3D-FFM device was recorded while rapidly pumping water at 10 mL/min through the device with closed outlet.

**Numerical modeling and simulations**

The incompressible Navier-Stokes equation and the continuity equation were numerically solved to simulate the flows inside the microchannels. An Advection-diffusion equation was also solved to predict the species transport by the fluid flows using the user defined scalar. All the governing equations were discretized using the finite volume method and a commercial numerical software FLUENT is used for the simulation. The semi-implicit method for pressure-linked equations (SIMPLE) algorithm is used for the coupling of velocity and pressure fields. The physical properties of the fluid and the diffusivity for the scalar species ($10^{-10} \text{ m}^2/\text{s}$) was assumed to be that of water at room temperature.[7] No-slip boundary conditions on all the channel walls were used and flow rates were specified at five (for 2D model three) inlets. The grid sensitivity and convergence of the model have been tested for different numbers of volume elements. The convergence limits were set so that velocities and the mass fraction converged within 0.001%.
Table S1. Summarized experimental conditions and results of simulation for 3D hydrodynamic flow focusing pattern.

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Reynolds number</th>
<th>Total flow rate (ml/min)</th>
<th>3D Flow Condition (ml/min)</th>
<th>Expected Flow pattern</th>
<th>Mass fraction</th>
<th>Pathline profile</th>
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<tr>
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<td>F₁: F₂ = 7 : 3</td>
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<td>F₃ = 8 / F₄ = 50 / F₅ = 216</td>
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<td>1.9</td>
<td>13.3</td>
<td>0.8</td>
<td>F₀: F₇ = 7 : 3</td>
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<td>F₈ = 80 / F₉ = 560 / F₁₀ = 216</td>
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<td>Unit microchannel: F₁₁ = 40 / F₁₂ = 90</td>
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<td>6.6</td>
<td>13.3</td>
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<td>F₀: F₇ = 7 : 3</td>
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<td>F₈ = 400 / F₉ = 2520 / F₁₀ = 1680</td>
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<td>Unit microchannel: F₁₁ = 100 / F₁₂ = 450</td>
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<td>3.7</td>
<td>13.3</td>
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<td>F₈ = 240 / F₉ = 302 / F₁₀ = 168</td>
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<td>6.6</td>
<td>13.3</td>
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<td>F₀: F₇ = 7 : 3</td>
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<td>F₀: F₇ = 7 : 3</td>
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<td>F₀: F₇ = 7 : 3</td>
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Preparation of PEG-PLGA nanoparticles

The PEG$_{5k}$-PLGA$_{20k}$ and PEG$_{5k}$-PLGA$_{55k}$ (PolySciTech Division, Akina Inc.) were dissolved in acetonitrile (ACN) at varying concentrations from 10 to 50 mg/mL, respectively. For PEG-PLGA nanoparticles, a 20 mL syringe for polymer injection was mounted on a syringe pump (Harvard Apparatus, Holliston, MA, USA), two 20 mL syringes for water injection were mounted on a different syringe pumps. Polymer solution and water flow rates were controlled by syringe pump at the same conditions as shown in Table S1 for each experiment. For example, flow rate of polymer solution was maintained at 2.4 mL/min and two water flow rates were maintained at 2.8 mL/min for both horizontal and vertical focusing flows where each unit microchannel had a 300 $\mu$L/min of polymer solution flow and 700 $\mu$L/min of water flow.

Reynolds number (Re) was calculated using the following equation.

\[
Re = \frac{\rho U_c L_c}{\mu} = \frac{U_c L_c}{\nu}
\]

where $\rho$ represents the fluid’s density; $U_c$ represents the fluid’s average velocity; $L_c$ represents the channel width $\mu$ is the fluid’s viscosity; $\nu$ is the kinematic viscosity.

To prepare nanoparticles by 2D flow focusing, we applied exactly identical dimensions of 2D microreactor for only horizontal focusing, and the total flow rates of polymer and water were identical with unit microreactor in 3D-FFM system. The 30 mg/mL of PEG$_{5k}$-PLGA$_{20k}$ and water were infused into the 2D flow focusing device at 0.9 mL/min and 2.1 mL/min, respectively. For bulk synthesis, 0.9 mL of PEG$_{5k}$-PLGA$_{20k}$ (30 mg/mL) was added to 2.1 mL of water phase by dropwise addition during 5 min.
**Transmission electron microscopy (TEM) analysis**

The TEM sample was prepared by depositing 5 μL of the nanoparticle suspension (1.0 mg/mL) onto a 200-mesh carbon-coated copper grid (FCF-200-Cu, Electron Microscopy Sciences, UK). Samples were blotted away after 1 min incubation and grids were negatively stained for 1 min at the room temperature with 3% (w/v) uranyl acetate aqueous solution (Sigma-Aldrich). The grids were then washed with double-distilled water and air-dried prior to imaging. Nanoparticles were imaged by transmission electron microscopy (JEOL JEM-1011, JEOL Ltd., Japan) at an acceleration voltage of 100 kV.

**Particle sizing**

Particle sizing was performed using dynamic light scattering (Zetasizer Nano, Malvern Instruments Ltd., U.K.). For each measurement, 500 μL or more volume of the sample was loaded in a disposable cuvette. More than five measurements were performed on each sample. All measurements were performed at concentrations of less than 10% ACN to ensure that any observed variation in particle size was not due to the solvent.
Figure S4. The size distributions of PEG-PLGA nanoparticles prepared by 3D-FFM system as a function of flow rate, flow ratio, molecular weight and concentration of polymer. (*Flow ratio of polymer solution to water).
Opacity of nanoparticle solutions

The opacity image of nanoparticle solutions using 10 mg/mL of PEG$_{5k}$-PLGA$_{55k}$ shows a wide difference of opaque with various sizes synthesized from different flow rates at low and high polymer ratio as shown in Figure S5. Larger particles can reflect more light, so the solution containing larger particles looks milky with low transparency, besides high polymer ratio increase reflection due to concentration of polymer nanoparticle.

![Flow ratio images](image)

**Figure S5.** The opacity images of nanoparticle solutions prepared from 10 mg/mL of PEG$_{5k}$-PLGA$_{55k}$ with different ratio of polymer solution to water under various total flow rates. The opacity indicates that the size and concentration of nanoparticles in the each solution depends on flow rate and flow ratio.

**Supporting Movie - Mass production of nanoparticles at high flow rate**

The movie shows actual production of nanoparticles in the 3D-FFM system from 30 mg/mL of PEG$_{5k}$-PLGA$_{20k}$. Flow rates were maintained at 7.2 mL/min of polymer solution and 16.8 mL/min of water where each unit microchannel had 0.9 mL/min of polymer solution and 2.1 mL/min of water. The average size 50 nm of PEG-PLGA nanoparticles were produced 12.9 g h$^{-1}$ of production rate under flash flow that spurt from outlet as shown in Supporting Movie.
Individual size distribution of PEG-PLGA nanoparticles prepared by 3D-FFM system as a function of flow rate, flow ratio, molecular weight and concentration of polymer

PEG_{5k}-PLGA_{20k}; 10 mg/mL; 0.008 mL/min of polymer; 0.072 mL/min of water
(F_{polymer} = 0.001 mL/min; F_{water} = 0.009 mL/min at unit microchannel)

PEG_{5k}-PLGA_{20k}; 10 mg/mL; 0.08 mL/min of polymer; 0.72 mL/min of water
(F_{polymer} = 0.01 mL/min; F_{water} = 0.09 mL/min at unit microchannel)

PEG_{5k}-PLGA_{20k}; 10 mg/mL; 0.4 mL/min of polymer; 3.6 mL/min of water
(F_{polymer} = 0.05 mL/min; F_{water} = 0.45 mL/min at unit microchannel)

PEG_{5k}-PLGA_{20k}; 10 mg/mL; 0.8 mL/min of polymer; 7.2 mL/min of water
(F_{polymer} = 0.1 mL/min; F_{water} = 0.9 mL/min at unit microchannel)
PEG<sub>5k</sub>-PLGA<sub>20k</sub>; 10 mg/mL; 0.024 mL/min of polymer; 0.056 mL/min of water

(F<sub>polymer</sub> = 0.003 mL/min; F<sub>water</sub> = 0.007 mL/min at unit microchannel)

PEG<sub>5k</sub>-PLGA<sub>20k</sub>; 10 mg/mL; 0.24 mL/min of polymer; 0.56 mL/min of water

(F<sub>polymer</sub> = 0.03 mL/min; F<sub>water</sub> = 0.07 mL/min at unit microchannel)

PEG<sub>5k</sub>-PLGA<sub>20k</sub>; 10 mg/mL; 1.2 mL/min of polymer; 2.8 mL/min of water

(F<sub>polymer</sub> = 0.15 mL/min; F<sub>water</sub> = 0.35 mL/min at unit microchannel)

PEG<sub>5k</sub>-PLGA<sub>20k</sub>; 10 mg/mL; 2.4 mL/min of polymer; 5.6 mL/min of water

(F<sub>polymer</sub> = 0.3 mL/min; F<sub>water</sub> = 0.7 mL/min at unit microchannel)
PEG5k-PLGA20k; 30 mg/mL; 0.008 mL/min of polymer; 0.072 mL/min of water
(F_{polymer} = 0.001 mL/min; F_{water} = 0.009 mL/min at unit microchannel)

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PEG<sub>5k</sub>-PLGA<sub>20k</sub>; 50 mg/mL; 0.008 mL/min of polymer; 0.072 mL/min of water
(F<sub>polymer</sub> = 0.001 mL/min; F<sub>water</sub> = 0.009 mL/min at unit microchannel)

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(F<sub>polymer</sub> = 0.05 mL/min; F<sub>water</sub> = 0.45 mL/min at unit microchannel)

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