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Continuous Fabrication of Biocatalyst Immobilized Microparticles Using Photopolymerization and Immiscible Liquids in Microfluidic Systems

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We report a novel technique for manufacturing polymeric microparticles containing biocatalysts by the behavior of immiscible liquids in microfluidic systems and in situ photopolymerization. The approach utilizes a UV-polymerizable hydrogel/enzyme solution and an immiscible oil solution. The oil and hydrogel solutions form emulsions in pressure-driven flow in microchannels at high values of the dimensionless capillary number (Ca). The resultant hydrogel droplets are then polymerized in situ via exposure to 365 nm UV light. This technique allows for the generation of monodisperse particles whose size can be controlled by the regulation of flow rates. In addition, both manufacturing microparticles and immobilizing biocatalysts can be performed simultaneously and continuously.

In open microchannels having dimensions on the order of 100 μm, miscible liquid streams exhibit laminar flow characteristic of low Reynolds numbers (*Re*).[1] Therefore, chemical reactions occur either between the streams and the interior surface of the microchannels or at the interface between the streams.[2,3] Such a liquid behavior has been successfully applied for microfluidic diffusion-based separation and analytical purposes, fabrication of various microstructures, and patterning inside microchannels.[4−7] However, the flow behavior of immiscible liquids is observed to be different from that of liquids of the same or similar nature and such properties can be used in various fields.[8,9] Several applications using the flow behavior of immiscible liquids at the microscale have recently been reported. For example, Song et al.[10] presented a microfluidic system that formed aqueous droplets in a continuous flow of a water-immiscible fluid. The droplets acted as microreactors that mix the reagents rapidly and transported them with no dispersion. Zhao et al.[11] reported a strategy to control the flow of immiscible liquids in microchannels by patterning surface free energies, and this method was applied to the fabrication of a semipermeable membrane. Hisamoto et al.[12] used an immiscible system to develop a new methodology to perform multiion sensing. The proposed immiscible system maintained stable multilayer interfaces for a long distance, completing the ion pair extraction reaction inside the microchannel.

In this paper, we report a novel technique for manufacturing polymeric microparticles containing biocatalysts by the immiscible property of hydrogel and oil solutions in microfluidic systems combined with "on the fly" photopolymerization. The UV-polymerizable hydrogel solution mixed with enzyme and the immiscible oil solution enter into the two inlet channels as a sample and a sheath flow, respectively. The hydrogel droplets surrounded with oil solution are formed in pressure-driven flow in microchannels at high values of the dimensionless capillary number (Ca), and then, the hydrogel droplets are polymerized by exposure to 365 nm UV light. This technique allows the generation of monodisperse particles whose size can be controlled simply by the regulation of sheath flow rate. In addition, both manufacturing microbeads and immobilizing biocatalysts can be performed simultaneously and continuously. Contrary to the chemical methods, there is no chemical or optical damage to the entrapped biological materials, as the UV exposure time to the polymerized beads is within 1 s.

The apparatus for fabricating the polymeric microparticles is constructed by a simple and cost-effective process, as illustrated in Figure 1. This device was prepared by hybridizing poly(dimethylsiloxane) (PDMS) substrate with a preformed main channel and a pulled micropipet. The pulled micropipet was fabricated from aluminosilicate glass pipet using a micropipet puller (P-97, Sutter Instrument Co.). By employing the microforge (MF-900, Naris-

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(1) Kovacs, G. T. A. *Micromachined Transducer Sourcebook*; McGraw-Hill: Boston, MA, 1998.

(2) Bard, A. J. *Integrated Chemical Systems: A Chemical Approach to Nanotechnology*; Wiley: New York, 1994.

(3) Vilkner, T.; Janasek, D.; Manz, A. *Anal. Chem*. **2004**, *76*, 3373−3386.

(4) Weigl, B. H.; Yager, P. *Science* **1999**, *283*, 346−347.

(5) Gao, J.; Xu, J.; Locascio, L. E.; Lee, C. S. *Anal. Chem*. **2001**, *73*, 2648−2655.

(6) Kenis, P. J. A.; Ismagilov, R. F.; Whitesides, G. M. *Science* **1999**, *285*, 83−85.

(7) Zhao, B.; Moore, J. S.; Beebe, D. J. *Science* **2001**, *291*, 1023−1026.

(8) Link, D. R.; Anna, S. L.; Weitz, D. A.; Stone, H. A. *Phys. Rev. Lett.* **2004**, *92*, 054503.

(9) Sugiura, S.; Nakajima, M.; Seki, M. *Langmuir* **2002**, *18*, 5708−5712.

(10) Song, H.; Tice, J. D.; Ismagilov, R. F. *Angew. Chem., Int. Ed.* **2003**, *42*, 768−772.

(11) Zhao, B.; Viernes, N. O. L.; Moore, J. S.; Beebe, D. J. *J. Am. Chem. Soc*. **2002**, *124*, 5284−5285.

(12) Hisamoto, H.; Horiuchi, T.; Uchiyama, K.; Tokeshi, M.; Hibara, A.; Kitamori, T. *Anal. Chem.* **2001**, *73*, 5551−5556.

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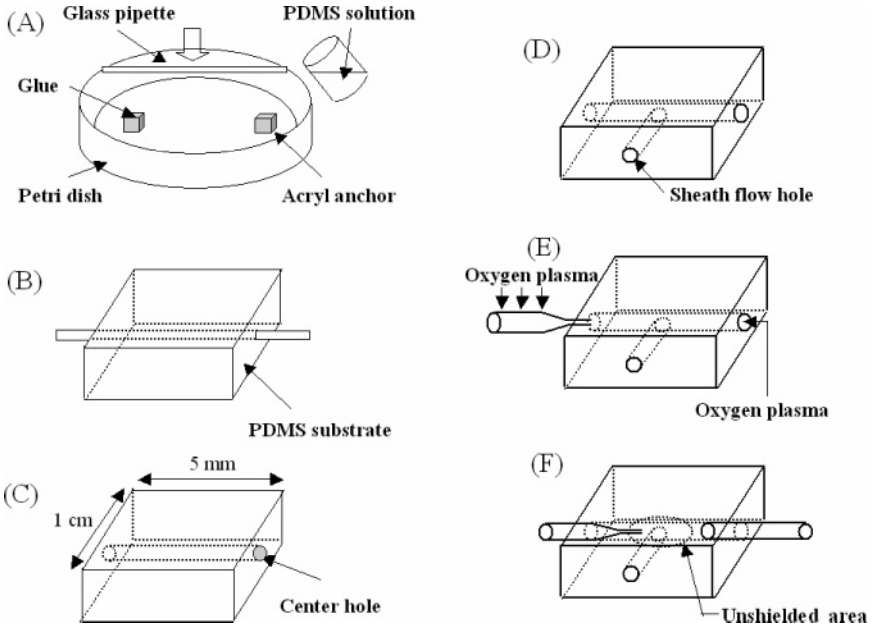

Figure 1. Schematic illustration of the process for fabricating the apparatus used to manufacture microparticles: (A) fixation of the acryl anchor, bonding of the glass pipet on the anchor, pouring PDMS prepolymer, and curing on the hot plate; (B) taking out the cured PDMS and cutting; (C) pulling out the micropipet; (D) puncturing the hole for sheath flow; (E) oxygen plasma exposure and bonding of the pulled micropipet; (F) inserting and bonding of the outlet pipet and shielding.

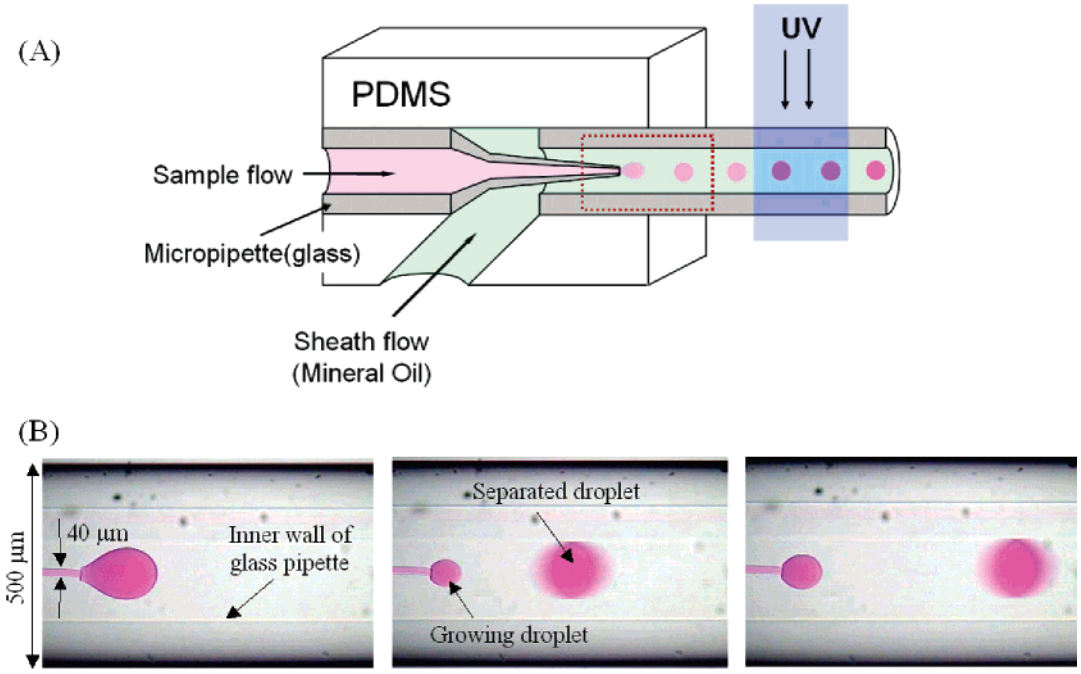

Figure 2. (A) Schematic illustration of the apparatus used to fabricate microparticles. The produced microdroplets are polymerized by the continuous radiation of 365 nm UV light at the unshielded area. (B) Snap photographs of the separated microdroplets. Rhodamine B was mixed with the sample fluid for better visualization. The hydrogel droplets in the main channel were monitored by the optical microscope (sample flow, 1 μL/min; sheath flow, 150 μL/min).

hige), the end of the tip was cut for its diameter to be 20, 30, and 40 μm, respectively. On the surface of the Petri dish, the acryl anchor was bonded using double-sided tape. The pulled glass pipet was placed onto the anchor using the glue. A mixture of PDMS prepolymer and curing agent (Sylgard 184 silicone elastomer kit, Dow Corning, Midland, MI) was prepared in a 10:1 ratio, poured over the Petri dish, and cured for 2 h at 80 °C on a hot plate. The Petri dish was removed, and the imbedded pipet was carefully extracted. The cured PDMS substrate with center hole was cut to a suitable size by a razor, and the inlet for the sheath flow was cored out by a 12-gauge needle at a 45° angle. After both the prepared PDMS substrate and the pulled micropipet were exposed to the oxygen plasma, they were combined by inserting the pulled pipet into the main channel with a 1 mm diameter and bonded by curing for 4 h at 80 °C. The normal micropipet for the extrusion of photopolymerized microparticles was inserted at the opposite site of the main channel and bonded in the same way. All the apparatus were shielded using the cooking foil except the unshielded area, as illustrated in Figure 1F.

To make microparticles, the polymerizable sample fluid (85 wt % 4-hydroxybuthyl acrylate, 11 wt % acrylic acid, 1 wt % ethyleneglycol dimethacrylate, and 3 wt % 2,2-dimethoxy-2-phenyl-acetonphenone) and the immiscible nonpolymerizable sheath fluid (mineral oil) were intro-

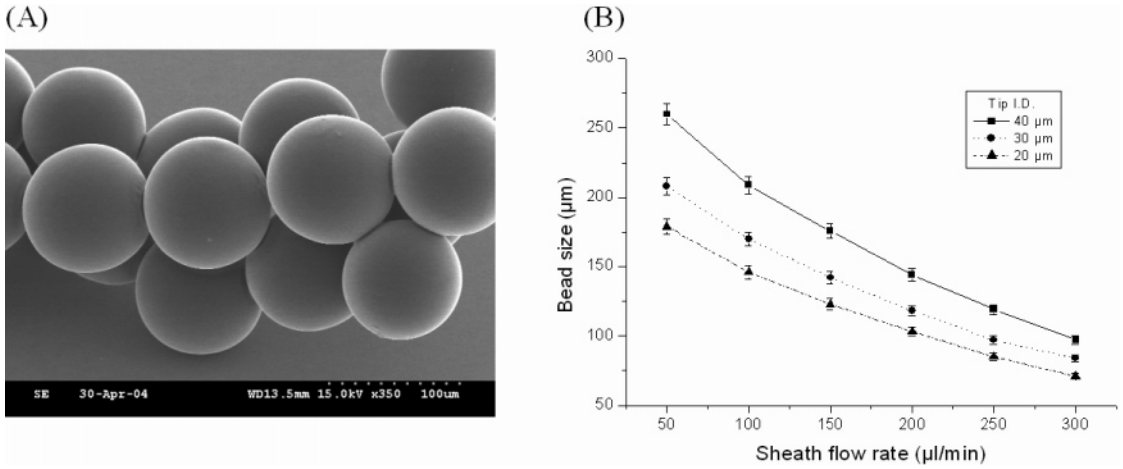

Figure 3. (A) Scanning electron micrograph of microparticles that were polymerized at a 1 μL/min sample flow rate and a 225 μL/min sheath flow rate. The produced microparticles have a 90 μm diameter. (B) Influence of sheath flow rate on the diameter of microparticles. All the sample flow rates were 1 μL/min. The mean diameter decreases with flow rate; error bars represent the standard deviation of the diameter.

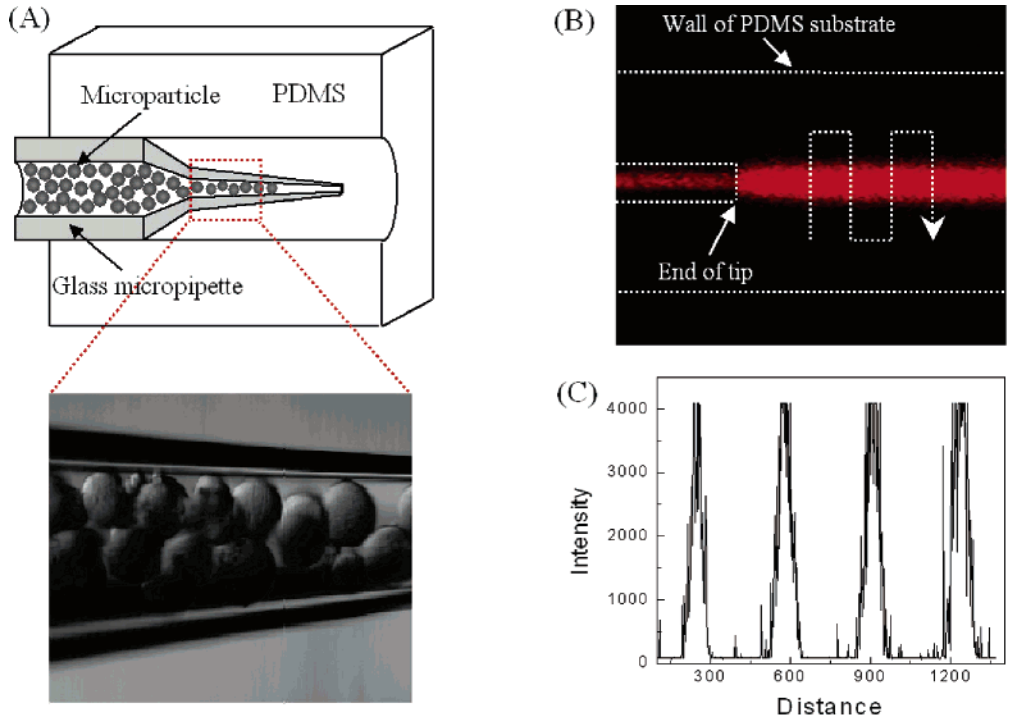

Figure 4. (A) Schematic illustration and optical image of microparticles packed in the micropipet. (B) Fluorescence micrograph of the outlet stream just after the glucose oxidase- and HRP-catalyzed reactions. (C) Fluorescence intensity line scans at the locations indicated by the dashed line in part B. Excitation wavelength, 563 nm; maximum emission wavelength, 587 nm.

duced into inlet channels of the sample and sheath flow, respectively. Both fluids were combined at the tip of the pulled micropipet (dotted area in Figure 2A), producing hydrogel droplets floating among the sheath stream (Figure 2B). The separated droplets traveled through the main channel without touching the inner wall of the channel, and then, the hydrogel droplets were polymerized by the continuous UV exposure (365 nm, 1.2 mW/cm^2, Novacure, Photonic Solutions Inc.) at the unshielded area.

Figure 3A shows a scanning electron micrograph of polymerized microparticles with diameters below 100 μm. Droplet formation is achieved by a competition between surface tension and shear force generated at the junction of two microfluidic channels, generating picoliter-scale droplets. The simplest model for droplet formation is based on the shear force generated between the hydrogel solution and the oil solution at the flow junction. The predicted size of a droplet under external shear force is approximated by equating the Laplace pressure with the shear force: $r \sim \sigma/\eta\epsilon$, where r is the final droplet radius, σ is the interfacial tension between the hydrogel and oil, η is the viscosity of the continuous phase, and ϵ is the shear rate.[13] As shown in Figure 3B, the size of the polymerized microparticles could be easily and precisely controlled by changing the sample and sheath flow rates. The generated microparticles had a size range from 70 to 260 μm and a coefficient of variation below 2%. Furthermore, the formation rate of microparticles with a 90 μm diameter was 2.6 $\times$ 10^3/min at a 1 μL/min sample flow rate and a 225 μL/min sheath flow rate. The production of monodisperse, stable, and controllable microparticles has been

(13) Thorsen, T.; Roberts, R. W.; Arnold, F. H.; Quake, S. R. *Phys. Rev. Lett.* **2001**, *86*, 4163−4166.

of interest for many applications in science and technology, including targeted drug delivery, immobilization of catalysts in chemical production, food additives, and so forth. Several new approaches for the formation and control of microparticles have recently been reported. Electrohydrodynamic (EHD) forces were used to generate coaxial jets of immiscible liquids.[14] The spray generated from the varicose breakup of the jet consists of monodisperse compound droplets. Also, the process of selective withdrawal of one fluid through a second immiscible fluid was applied to coat small particles with polymer films.[15] Our approach has several advantages; the fabrication process can be carried out under ambient conditions without an electrical field, the biological materials can be immobilized without any damage, and the change of the particle size is easy and predictable.

On the basis of these technologies, a wide variety of functional microparticles can be produced by mixing other chemical or biological materials with sample fluid. The photopolymerizable hydrogel solution uniformly mixed with 2 mg/mL glucose oxidase and 1 mg/mL horseradish peroxidase (HRP) was introduced and polymerized by the continuous UV exposure. The microparticles immobilizing glucose oxidase and HRP were introduced into a pulled glass micropipet inserted into the main channel of PDMS substrate using pump-driven pressure, as shown in Figure 4A. The diameter of the pulled glass micropipet ranged from 20 to 40 μm. Therefore, microparicles having a diameter of 90 μm were packed easily within the glass micropipet. To demonstrate the enzyme immobilization without damage and the multiple enzyme reactions, a mixture of 50 mM glucose and 10 μM nonfluorescent amplex red in 100 mM PBS buffer (pH 7.0) was introduced into the channel at a flow rate of 2 μL/min. The glucose is converted to gluconic acid and H_2O_2 by the glucose oxidase-catalyzed reaction, and then, fluorescent resorufin is formed via the HRP-catalyzed reaction between H_2O_2 and amplex red.[16] A fluorescence image of the main channel at the end of tip is shown in Figure 4B, and the corresponding line scan is shown in Figure 4C.

In conclusion, we developed a simple and cost-effective method for the fabrication of polymeric microparticles by the immiscible property of hydrogel and oil solutions in microfluidic systems and in situ photopolymerization. This method allows for the continuous manufacture of microparticles with a wide variety of physical and chemical properties by controlling the flow parameters, material parameters, and polymerization parameters. The flexibility of the method across different materials, geometries, and scales is a key advantage over many existing methods that require some form of retooling to realize different outcomes. In addition, the apparatus for fabricating the microparticles can be constructed even in a laboratory in a simple and cost-effective way without using complicated machining tools. This technique can also be extended to a continuous process for the creation of microscale cylindrical structures (e.g., microfibers and microtubes)[17] and scaled up by using an array of microchannels in parallel.

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(14) Loscertales, I. G.; Barrero, A.; Guerrero, I.; Cortijo, R.; Marquez, M.; Ganan-Calvo, A. M. *Science* **2002**, *295*, 1695−1698.

(15) Cohen, I.; Li, H.; Hougland, J. L.; Mrksich, M.; Nagel, S. R. *Science* **2001**, *292*, 265−267.

(16) Seong, G. H.; Crooks, R. M. *J. Am. Chem. Soc.* **2002**, *124*, 13360−13361.

(17) Jeong, W. J.; Kim, J. Y.; Kim, S. J.; Lee, S. H.; Mensing, G.; Beebe, D. J. *Lab Chip* **2004**, *4*, 576−580.