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Notes

Micropillars Fabricated on Poly(methyl methacrylate) Substrates for Separation of Microscale Objects

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Development of polymeric microfluidic devices has played an important role in the recent, rapid progress of biomedical research. Here we report a fabrication method for micropillars on poly(methyl methacrylate) (PMMA) substrates for separation of microscale objects. The fabricated micropillars enable continuous separation of microparticles only by introducing fluids. The present method offers a new strategy to fabricate polymeric prototype devices for R&D work.

Keywords Micropillars, poly(methyl methacrylate) (PMMA), microparticles separation

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Development of polymeric microfluidic devices has played an important role in the recent, rapid progress of biomedical research. Polymeric microfluidic devices have been fabricated using molding techniques, such as imprinting and injection molding.[1,2] These methods allow researchers to mass-produce the polymeric microfluidic devices at low cost. Since the molding techniques involve time- and labor-intensive processes, methods based on them are not appropriate to make prototype devices, especially for a few devices needed early-stage R&D work. In this paper, we report a method for rapid and easy fabrication of polymeric microfluidic devices to separate microscale objects. Our fabricated devices have micropillars embedded in a microchannel, and sufficiently smooth surfaces to allow easy flow of microscale objects.

Here we report a fabrication method for micropillars on poly(methyl methacrylate) (PMMA) substrates for separation of microscale objects. This method uses reactive ion etching (RIE) for the micropillar fabrication.[3] RIE allows us to make fine microstructures with high aspect ratio. The fabrication process, consisting of six steps, is easy and simple as shown in Fig. 1a, and it is suitable for quick and precise fabrication of polymeric microfluidic devices. In our method, the PMMA substrate was etched under only O_2 gas ambient, which is more environmentally-friendly and cost effective than the reported method (O_2–CF_4 gas mixture ambient).[3]

The fabrication starts with deposition of a 100 nm thick titanium layer, which functions as a mask for the following RIE, on a 1 mm thick PMMA substrate (Tateyama Machine Co., Ltd.) (Fig. 1a). Photoresist (OFPR-8600, Tokyo Ohka Kogyo Co., Ltd.) was coated on the substrate by a spinner, and then, the substrate was heated at 90°C for 10 min. After UV irradiation, the substrate was heated at 90°C for 15 min, and then developed for 90 s, after that, the developed substrate was rinsed in water for 1 min, and then heated at 90°C for 15 min. The titanium layer and the PMMA substrate were etched using an RIE apparatus (Tateyama Machine Co., Ltd.) under CF_4 and O_2 gas ambients, respectively. The residue of the titanium layer was removed using 1% hydrofluoric acid for 1 min, and the substrate was rinsed in distilled water for 5 min. The inlet and outlet *via* holes (3 mm diameter) for the microfluidic system were drilled with a driver. For the bonding step, the patterned substrate and a 0.5 mm thick non-patterned PMMA substrate were rinsed in ethanol quickly, and then rinsed once in distilled water. After drying out both substrates, the substrates were treated using a UV ozone cleaner (Filgen, Inc.) for 60 min, and finally, the substrates were bonded to each other under 5.0 Nm at 75°C for 10 min.

The fabricated device showed good light permeability of the PMMA substrates (Fig. 1b). SEM images revealed that the micropillars were embedded in a microchannel and the bottom surface of the microchannel was sufficiently smooth to allow easy flow of microscale objects (Figs. 1c and 1d). We estimated

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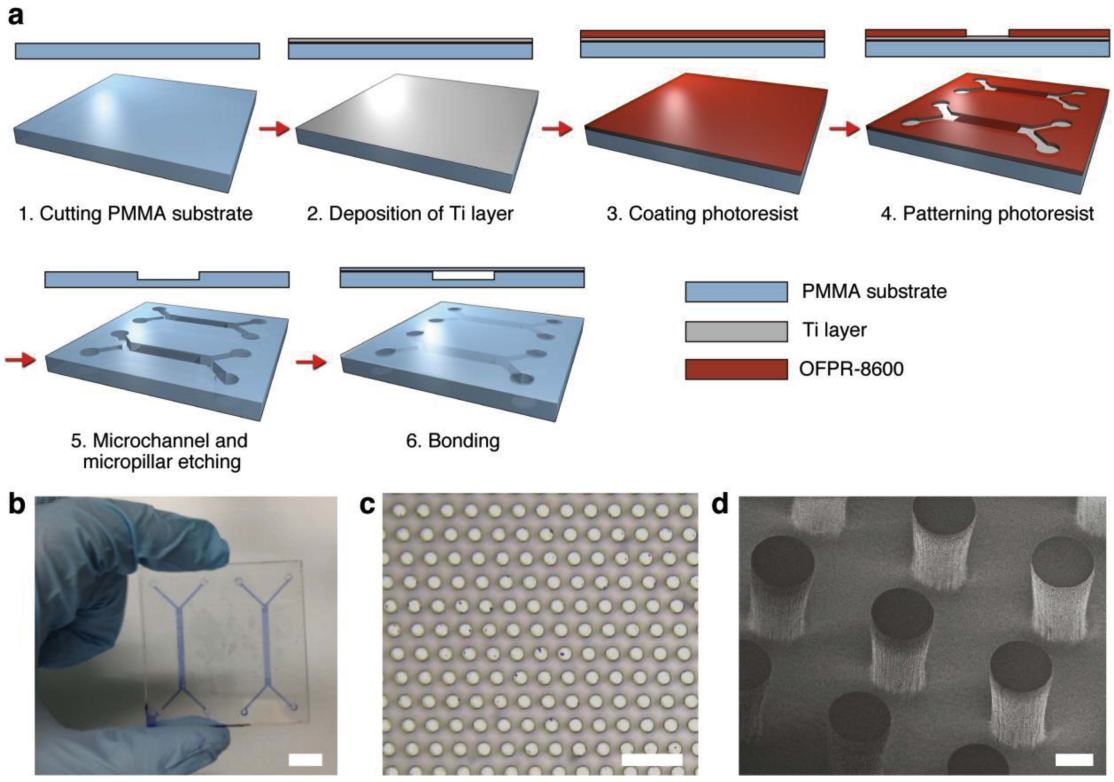

Fig. 1 Micropillars on poly(methyl methacrylate) (PMMA) substrates. (a) Schematic illustrations showing fabrication of micropillars on PMMA substrates. (b) A photo of the PMMA substrates with fabricated micropillars; scale bar, 1 cm. Trypan blue made it easy to confirm the microchannel pattern. (c) SEM image of micropillars embedded in a microchannel; scale bar, 100 µm. (d) Enlarged SEM image of the PMMA micropillars; scale bar, 10 µm. Each micropillar was 30 µm in diameter and 60 µm high.

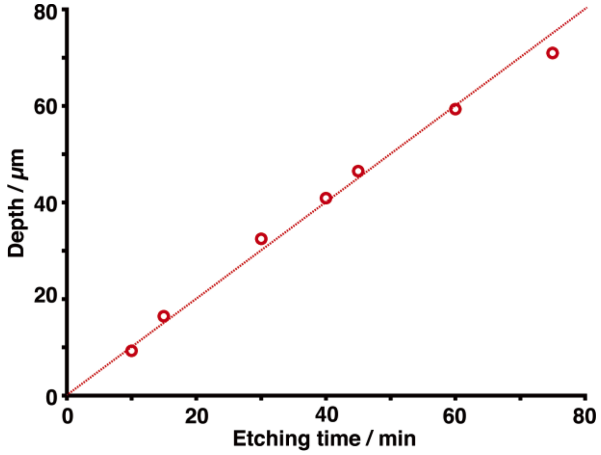

Fig. 2 Relationship between etching time and etched depth of PMMA substrates. There was a linear relationship between the etching time and etched depth (red line). Estimated etching rate was 1 µm/min.

the etching rate was 1 µm/min by measuring the etched depth at each etching time: 10, 15, 30, 40, 45, 60, and 75 min (Fig. 2). To fabricate micropillars of the desired 60 µm height, we etched PMMA substrates under O_2 gas ambient for 60 min. This produced the PMMA microfluidic devices, which consisted of micropillars 30 µm in diameter, 60 µm in height, and with a separation distance of 15 µm between micropillars (Fig. 3a).

For the separation of microscale objects in the fabricated device, we used deterministic lateral displacement (DLD), which is one of the continuous separation methods. Since DLD was firstly reported by Huang *et al.*[4] in 2004, a number of papers have applied DLD to separate microscale objects, such as small particles,[5,6] blood cells,[7,8] cells,[9] trypanosomes,[10] circulating tumor cells,[11] and platelets.[12] In this paper, we designed the micropillar array to separate microscale objects in the polymeric microfluidic devices. We arrayed micropillars with an angle, tan $\theta = 0.15$, in a microchannel of 2 mm width and 20 mm length (Fig. 3a).

We prepared samples to confirm the function of the devices and used them in the following evaluations. The samples were a suspension of beads consisting of a mixture of 6 µm microbeads (Fluoresbrite® YO carboxylate microspheres 6.0 µm, Polysciences, Inc.) and 10 µm microbeads (Fluoresbrite® YG carboxylate microspheres 10.0 µm, Polysciences, Inc.). The bead suspension and water included 0.1 v/v% tween-20 (Sigma-Aldrich, Inc.). The fabricated micropillars enable continuous separation of microbeads only by introducing fluids. For confirmation of microbead displacement, we focused on the bead suspension fluids on a microchannel sidewall (Fig. 3a). The beads were directed toward the outlets marked A and B. Based on DLD, we could confirm that the 6 µm diameter microbeads snaked around the micropillars, while the 10 µm diameter microbeads mainly flowed along them. Displacement, representing the lateral travel distance from the microchannel sidewall, of the 10 µm diameter microbeads increased gradually

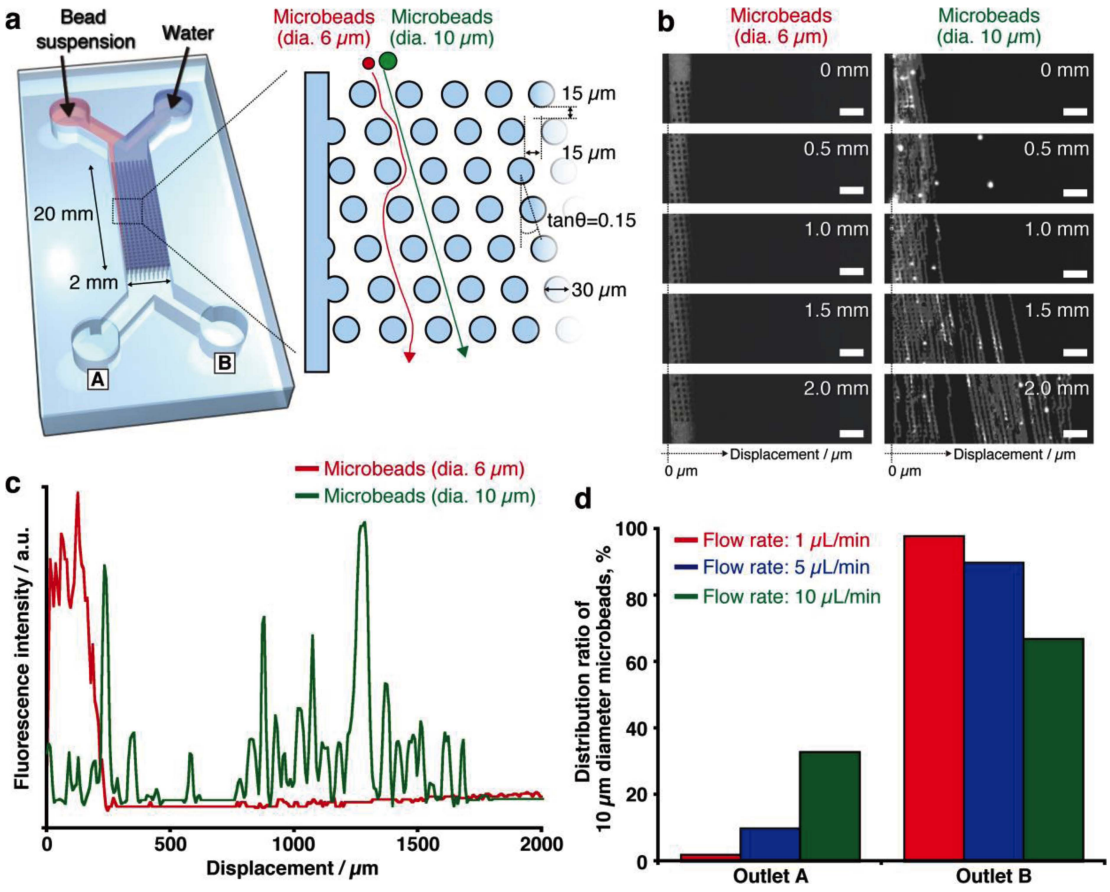

Fig. 3 Separation of microscale objects using PMMA micropillars embedded in a microchannel. (a) A schematic illustration showing sample introduction into a polymeric microfluidic device. Microbeads with a 10 µm diameter (green circle) flow along the micropillars (green arrow), while microbeads with a 6 µm diameter (red circle) snake around the micropillars (red arrow). (b) Fluorescence trajectories of both diameters of microbeads at 0, 0.5, 1.0, 1.5, and 2.0 mm points from the junction of the inlets for the suspension and water; scale bars, 200 µm. Flow rate was 1 µL/min. (c) Fluorescence intensities of microbeads with 6 µm (red line) and 10 µm (green line) diameters at the 2.0 mm point from the junction. Displacement represents the lateral travel distance from the microchannel sidewall, as defined in (b). (d) Dependence of flow rate on separation efficiency.

as the microbeads flowed downstream in the microchannel. And, we could achieve continuous separation of 6 and 10 µm diameter microbeads using the PMMA microfluidic devices (Fig. 3c). The detection of continuous separation was demonstrated by measuring fluorescence intensity at a point 2.0 mm from the junction of the inlets for the suspension and water. As seen in Fig. 3c, the averaged displacement of 6 µm diameter microbeads was limited to 200 µm, while the averaged displacement of 10 µm diameter microbeads was around 1000 µm.

Separation efficiency using PMMA micropillars depended on flow rates (Fig. 3d). For evaluation of separation efficiency, we used a high-speed camera to monitor the number of 10 µm diameter microbeads and calculate the distribution ratio of 10 µm diameter microbeads at each outlet. Using a flow rate of 1 µL/min, we could achieve over 95% displacement of 10 µm diameter microbeads to outlet B, leading to highly efficient separation. As we increased flow rate from 1 to 10 µL/min, the displacement ratio of 10 µm diameter microbeads to outlet B decreased. From these results, we can say that our separation seems to rely on DLD and diffusion. Larger flow rates, such as 10 µL/min, have a lower diffusion effect on microbeads, resulting in less displacement compared to lower flow rates. Meanwhile, smaller flow rates, such as 1 µL/min, have a bigger diffusion effect on microbeads, resulting in greater displacement.

In summary, we have demonstrated the fabrication method for micropillars on poly(methyl methacrylate) (PMMA) substrates for separation of microscale objects. Using RIE etching under O_2 gas ambient, we could control the height of the micropillars and get a smooth surface at the bottom of the microchannel. The device with fabricated micropillars on PMMA substrates could achieve continuous separation of 6 and 10 µm diameter microbeads. This method offers a new strategy to fabricate polymeric prototype devices for early-stage R&D work.

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