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3D micro fractal pipettes for capillary based robotic liquid handling

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Miniaturized and mobile liquid handling devices are essential elements to biological or clinical applications. This will innovate the conventional liquid handling methods like manual or automated pipetting systems. Here we propose the micro fractal pipette as the candidate device for this objective. It is made of Epoxy polymer and printed by innovative 3D nanoprining technology based on two-photon absorption polymerization with sub-microns resolution. We demonstrated the efficient liquid handling performance by the micro fractal pipette between the source droplet and the target hydrogel substrate. Thanks to the high porosity up to 78% of total volume and 8.5 times higher cavity surface area compared to the full pyramid with same dimensions. The biomimetic inner cavity microchannel networks contributes to the low pressure drop. The proposed micro fractal pipette could also innovate the versatile and miniaturized liquid handling system promising to various biological or clinical applications.

Miniaturization of intelligent systems such as lab-on-a-chip which integrates several microelectromechanical components allows highly resolved, rapid, safe and low-cost diagnostics with small volume of specimens¹. However the diagnostics of clinical specimens require additional steps of liquid handling such as collection, preparations, and injection of specimens to biosensors. In biological laboratories, robotic pipetting systems are widely used by automating tedious liquid handling steps but they are limited to be used inside laboratory. To extend the applications out of laboratory, a portable microfluidic sample handling system is inevitable. Conventional syringe pump and needle require to be tethered by external syringe pump and tubing for precise liquid volume handling. This makes the entire system becomes bulky with several disadvantages such as high priming/dead/residual volumes which are limiting factors towards remote and microfluidic sample handling. Another alternative technology could be the porous micro needles². Although this shows some advanced features such as low dead volume and high portability, the liquid handling performance strongly depends on the geometrical parameters such as the wettable surface area, the porosity and the in/outlet aperture size. Moreover it is essential to control the cavity geometries for the repeatable liquid handling. Most of the porous micro needles have limited porosity and their cavity dimensions are less controlled². Our aim is to propose a microfluidic specimen handling system with enhanced porosity towards biomedical applications requiring specimen/drug collection and delivery. We propose the liquid handling system consisting of highly porous and bioinspired design

FIG. 1. (Top) Proposed concept of the MFP for liquid handling. The MFP approaches to fill the liquid by capillary force and it moves to the target (2% agarose gel) to release the liquid by capillary force of hydrogel. (bottom) Design and the fabrication of MFP. The fractal pyramid 5 levels with 1mm^2 base was fabricated by two-photon 3D lithography.

with fractal pyramid microstructure. The fabrication is realized by two-photon absorption 3D nanolithography having sub-micron fabrication resolution. The proposed micro fractal pipette (MFP) allows enhanced porosity, light weight and less invasive liquid handling. Moreover the fractal structures allow facile development of photore-sist for inner cavity channels after photopolymerization because the developer medium has better access through large in/outlet openings and also biomimetic microchan-nel architecture. The MFP does not require external syringe pump and the dead volume of the proposed MFP is close to zero in theory. The liquid filling in and releasing out of the MFP are made by capillary force of the MFP and the target to deliver. This capillary force driven liquid handling is facilitated by low pressure drop (less than 20% from input pressure) thanks to the biomimet-ically distributed inner cavity channel network. This elim-

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inates the needs for external pump and additional tube connections for it which are the major source of dead volume for fluidic system. Considering that the MFP is a stand-alone liquid handling system containing exclusively the inner fluidic storage, it can realize near zero dead volume. Similar fractal microstructures were fabricated for surface wettability study^{3,4} or for gas permeation membrane⁵. However to the best of our knowledge, our approach of applying the fractal microstructure for the micro pipette is unique. Here we present the MFP's design, fabrication and the liquid handling performances.

Figure 1 shows the schematic concept of the proposed system. First, the MFP is fixed at the end of the capillary probe and the entire system is mounted on the microstage. It should be noted here that the used capillary tube was only fixed to the closed bottom part of the MFP thus the absorbed liquid remains exclusively inside the MFP. Then the petri-dish containing the 2% hydrogel substrate and the red colored ink droplet was mounted onto another microstage. The MFP approaches to the ink droplet to fill itself by capillary force. Our assumption of using MFP is to increase absorption efficiency thanks to the increased in/outlet openings, increased wettable surface area through the cavity and the increased cavity volume. Once the ink is filled, the MFP is moved onto the target hydrogel substrate by stage motion. The ink-filled MFP is approached to get in contact with hydrogel substrate. The filled ink is released onto the hydrogel substrate due to the higher capillary force due to the nanometric scale porosity of hydrogel. Several more attempts can be made onto other position of the hydrogel substrate to completely release the ink out of the MFP. The capillary filling and releasing of the MFP can be understood by the simplified Lucas-Washburn model⁶: $x^2 = \frac{\gamma R \cos \theta}{2\eta} \cdot t$ by ignoring inertial forces, where x is the liquid filled up distance, η is the viscosity of the liquid, R is the mean capillary radius, t is the time and θ is the contact angle. The contact angle θ is assumed to be static, the capillary liquid motion is proportional to the square root of the mean capillary radius R . Considering the estimated R_{MFP} as around $10.7\mu\text{m}$ and the sub-microns scale pore sizes of hydrogel, the capillary releasing is made to the hydrogel substrate. Further analyses were made by finite element method simulation to understand the pressure drop of the MFP during liquid handling with the assumption of the laminar flow condition and a static solution. The pressure drop of the MFP was remained to be around 20% which is reasonable value at an input pressure of 100kPa. This can be explained by the fact that the MFP has the inner cavities fluidic channels are close to the biological vascular networks⁷.

To prove the liquid handling by experiments, we designed and fabricated the MFP prototype by two-photon 3D nanolithography system (Nanoscribe, GmbH). This multiscale fabrication capability is essential to fabricate the sub-millimetre scale MFP prototype. After photopolymerization process, the fractal structure with large openings with the connected cavities allowed facile access

FIG. 2. (Left) Liquid handling experiments by MFP to hydrogel. (Right) Performance comparison between full pyramid and the MFP. The scale bar is 3mm.

of the developer medium to the inner cavities for the development of photoresist. The developed structures were rinsed several times by isopropyl alcohol and dried carefully. Scanning electron microscope imaging was made to observe the quality of the process. As shown in the figure 1, the MFP was fabricated with good quality of inner cavity development. For the target substrate, 2% agarose hydrogel samples was prepared. For the scaled-up MFP, polylactic acid (PLA) was 3D printed to have young's modulus around 3.5GPa similar to the epoxy resist (around 4GPa). The MFP was scaled up by 55 times thus the width of the base part is 55mm.

The fabricated MFP was applied to demonstrate the liquid handling performance. The performance of liquid handling can be understood by the efficient absorption from source, handling and release to target with minimal loss. Since the liquid handling typically comprises several inefficiencies at each steps, liquid handling performance tests were made by following the schematic concept shown in the figure 1. As shown in the figure 2, the MFP mounted at the end of capillary probe was operated by micro stages. The MFP entered the red dye droplet to fill it up. Once it is filled, it moves out of the droplet. The MFP's gradually decreasing inner cavities allow the filled liquid to remain inside the cavities. The surface area of whole cavities inside single MFP is 8.5 times larger than full pyramid. The filled liquid through connected cavity channels could avoid rapid evaporation during handling. Then the filled liquid was released to target hydrogel substrate by stage motion. The MFP demonstrated to be able to release several spots by repeating the process without getting dried. After this process, we can confirm that the red dye filled MFP was completely drained out and released to the hydrogel. We estimate the filled liquid volume is around 185 nl which can obtain the porosity around 78%. This contributed to the 28 times higher absorbability of the MFP compared to the full pyramid. Furthermore the penetration of the MFP through the hydrogel was guaranteed thanks to the solid mechanics from the photopolymerized epoxy based resist having young's modulus around 4GPa superior to the target hydrogel substrate (2% agarose) having the young's modulus in the range of 14 kPa⁸. If the struc-

ture has lower young's modulus compared to the hydrogel target substrate, the absorbed liquid in the pipette could be lost at the interface of the hydrogel substrate due to the deflection by compression during the insertion. Therefore as in the proposed case, forming the MFP with hard epoxy avoids the loss of the liquid during insertion process. This can also be compatible to the human's skins having large range of young's modulus⁹. The very performing liquid handling of the MFP is also partly supported by the large surface opening area (1.32mm^2 thus 76% of 4 sides of pyramid) of the MFP allowed facile liquid absorption and release. This can be explained by the fact that there are large openings at the contact surface of MFP which reduces the fluidic resistance at the interface to the target hydrogel substrate. In comparison with the conventional micro porous structures, there are much less chance to get also clogged in the openings. Mechanical resistance of insertion to hydrogel is also important for the successful liquid delivery. Highly resistive pipettes could lead to the application of excessive insertion force thus the insertion could increase the pain or the incision. The proposed MFP has only 0.42mm^2 (24%) of the outer surface in contact with hydrogel thus the friction is reduced compared to the full pyramidal shape. We suppose that this reduced contact surface thus the insertion friction can contribute to reduce the insertion pain or incision. The reduced contact surface area also reduces the liquid loss at the outer surface of the pipette during insertion. Together with small gravity force, the MFP can be simply fixed to capillary tube by double side adhesive film. Large capacity of liquid absorption by single MFP reaching up to 185nl which is 78% of the volume total of pyramid could cause eventual loss of liquid during displacement step as well due to the gravity. During the displacement step, the liquid filled MFP contained the liquid without any loss. This is thanks to the 8.5 times larger surface area in contact with the filled liquid. This increases drastically the surface capillary force which contributes to hold the liquid stably during displacement even with mechanical vibrations. The minimum spot size achievable with the current dimension of the MFP could be as small as below $10\text{ }\mu\text{m}$ in radius which can be estimated from the minimum deliverable volume of liquid is 3.8 pl. Considering the smallest the cavity dimension of the tetrahedron is with the side of $20\text{ }\mu\text{m}$ and the sub-micron resolution 3D nanolithography, further miniaturization could be attempted. To test the scalability of the concept, we used the 3D printed scaled-up MFP. After making the same steps shown in the figure 1, the absorbed volume of liquid (10 ml) was transported without any loss during handling. Considering the fact that we did not make Reynolds number matching notably the viscosity of the liquid in the scaled-up model tests, stable liquid handling is highly confirmed.

The proposed MFPs demonstrated several enhanced features compared to the conventional approaches. First, they can fill up large volume percentage of liquid up to 185nl which is 78% of full pyramid thanks to enhanced

	Classical needle ¹⁰	Porous μ -needle ²	Micro fractal pipette (this work)
Applications	Drug delivery, specimen collection for biosensing		
Priming volume	~ few ml	~ few nl	few nl ~ few ml
Dead volume	High	Low (close to zero)	Low (close to zero)
Mechanics/material	Stable/metal	Depends/various	Stable/epoxy
Pump requirement	Required	Less required	Not required
Liquid loss	Back pressure	High	Almost zero
Insertion resistance	High	Mid	Low
Fluidic resistance	Mid	High (e.g. clogging)	Low

FIG. 3. Comparisons with classical needle, porous microneedle and the proposed MFP.

porosity. Second, thanks to the increased contact surface area and stable mechanics, the filled-up liquid was able to be delivered stably to target hydrogel substrate without showing any loss during the displacement and also insertion steps. As summarized in the figure 3, the proposed concept also demonstrated to be effective in the liquid handling scalable volume from picolitre to millilitre without any support from external microfluidic pumps or syringe. Compared to porous microneedle with equivalent porosity, the MFP shows advantages in the lower mechanical resistance of insertion and fluidic resistance thanks to biomimetic inner cavity channel network promising to drug delivery through the skin or other tissues. The MFP is easily washable for more than hundreds of times of repeatable use and did not show ageing effect at least for few months so it is theoretically semi-permanent in its lifetime. Considering also the miniaturization and the ignorable dead volume feature, the MFP could be promising to portable liquid handling tool thus the applications could range from the field specimen collection to the liquid handling inside laboratory.

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