ADJUSTMENT OF SURFACE CONDITION FOR SELF-GENERATION OF DROPLET ARRAY USING OIL-WATER REPLACEMENT

Hiroki Yasuga and Norihisa Miki
Keio University, Japan

ABSTRACT

This paper demonstrates a method to self-generate a two-dimensional (2D) array of nanoliter-scale droplets. 2D droplet arrays are powerful tools for high throughput and low-cost processes in the field of biology or medicine. We recently developed a self-generation method of droplet array without precise liquid control such as precise pipetting or pumping. The next challenge of the self-generation is the further miniaturization of generated droplets. Here, we addressed it by applying a photoresist off-stoichiometry thiol-ene (OSTE), of which surface energy can be tuned by polymer chain grafting. We experimentally found the surface conditions to self-generate an array of nanoliter-scale droplets.

KEYWORDS

Droplet generation, water-in-oil droplets, capillary, off-stoichiometry thiol-ene (OSTE), Surface modification

INTRODUCTION

The small sample volume of microdroplets enables high-throughput biological assays or medical diagnosis due to reduction of sample/analytes and acceleration of reactions [1]. Arrays of microdroplets further enhances the efficiency of such experiments because they facilitate microscopic observation and enable access to individual droplets owing to the arrayed registration [2]. Through previous works, several applications of droplet arrays have been reported, including digital PCR [3] and cell/microbial assays [4]. In conventional studies, the droplet arrays were prepared with pipetting [5], in microfluidic channels [6] or in the slip-chip [7]. However, such systems generally require precise pipetting or pumping operations, which needs bulky system and degrades the virtue of microsystems.

Recently, we have developed the method to self-generate droplets in a well array without precise pipetting or pumping [8]. In the array, wells are connected with each other through channels (Fig. 1a). First, aqueous solution is impregnated into the well-channel systems (Fig. 1b). Then, immiscible solvent (ex., oil) is added on the well array including the aqueous solution (Fig. 1c). If the solvent is more highly wet on the array material, it spontaneously propagates into the array. In the channel connecting wells, the solvent is totally filled and splits aqueous solution into droplets at every single well (Fig. 1d). Using this method, droplet array including 2D concentration gradient of samples was demonstrated, suggesting the applicability of the system to multiplexed analysis in the field of biology or medicine. As for such droplet generation system, the next challenge is further miniaturization of droplets. Smaller-scale droplets are more preferable to certain application, such as digital PCR.

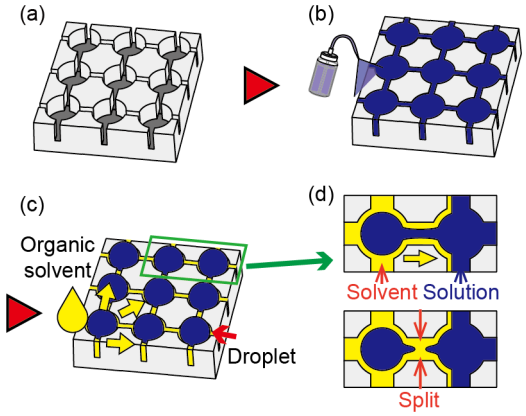

Figure 1: (a) A connected well array. (b) Impregnated aqueous solution. (c) Droplet generation. (d) Mechanism of splitting.

To generate small droplets using this method, it is necessary to finely adjust the wettability of the surface to water and solvent. Currently, successful surface modification relies on fluoropolymer coating, limiting the fabrication scalability; in small dimension, channels are clogged. It precludes the fabrication of the array in small dimension and, hence, droplet volume that can be produced is limited to hundreds of nanoliter or more.

Here, we studied a surface modification method for self-generation of the droplet array in nanoliter-scale or even smaller. Applied polymer material of OSTE is known to be capable of tuning surface energy based on polymer chain grafting [9]. First, we investigated it via the contact angle measurement whether the OSTE surface could be modified with several different monomers. Then, conditions of the self-generation of droplet array were explored among the monomers and solvent species.

FABRICATION

OSTEmer^{TM}322 (OSTE, Mercene Lab, Sweden) was selected as material of the connected circular well arrays. This OSTE is known as dual-cure polymer by UV and thermally [10]. It remains thiol groups on the surface after the UV curing. Monomers with methacrylate functional groups can be reacted and bonded with the thiol groups on the surface as previously reported [9].

The fabrication of the connected well array was conducted in two steps; mold and well array fabrications. The mold was fabricated as follows and as shown in Fig. 2a-c. First, uncured OSTE was exposed to UV for 100 s through shadow-mask (Tokyo process service, Japan) on a prepared flat cured OSTE (Fig. 2a). Then, the well array pattern was developed in a bath of isobutyl acetate for 3 min with ultrasonication. After the development, the mold was coated with fluoropolymer (CYTOP®, Asahi glass, Japan). It was kept on hot plate at

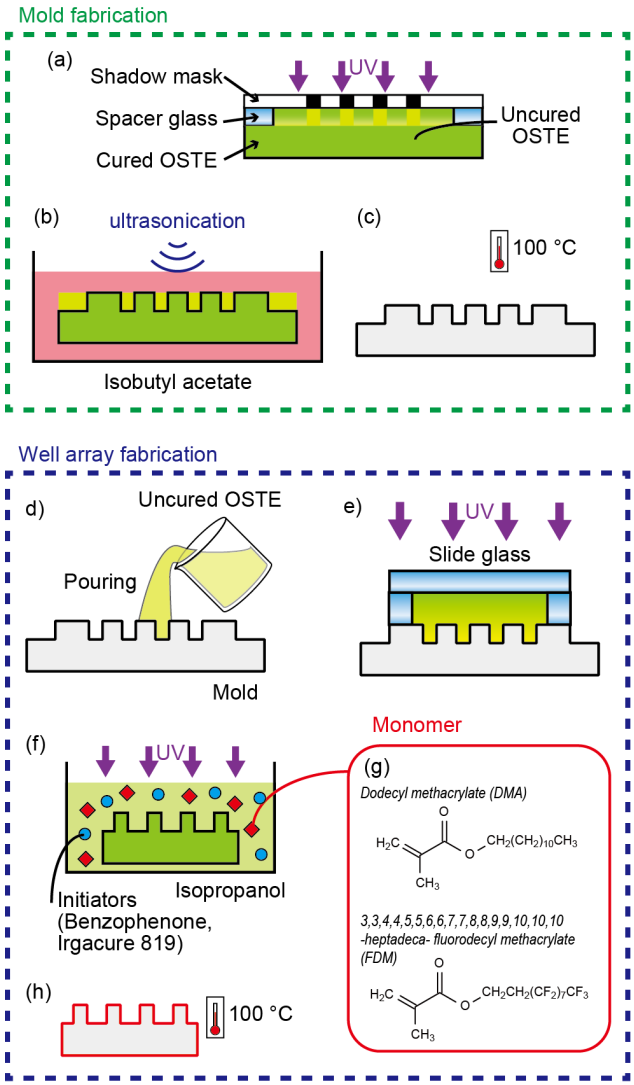

Figure 2: Fabrication procedures. (a) Photolithography of of a well array mold. (b) Development of the well array mold. (c) Thermal curing of the mold. (d) Poring of OSTE onto the mold. (e) UV exposure. (f) Surface modification based on grafting. (g) Structural formula of applied monomers. (h) Thermal curing of a well array.

100°C for 1 hour, for the complete polymerization of the OSTE.

The well array device was prepared as shown in (Fig. 2d-h). Into the mold, uncured OSTE was poured (Fig. 2d) and exposed to UV for 300 s (Fig. 2e) after being degased in a vacuum chamber. Then, the array was peeled off from the mold and the surface of the array is modified by polymer chain grafting with a UV exposure (Fig. 2f). The solution that the array was soaked in was isopropanol including two photo initiators (0.5% w/w Irgacure 819 from BASF, Germany, and Benzophenone, from Wako pure chemical, Japan), and monomers with a methacrylate functional group. For the hydrophobic surface modification, we used either 5% w/w *dodecyl methacrylate* (DMA, Tokyo chemical industry, Japan) or 20% w/w *3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10 heptadecafluorodecyl methacrylate* (FDM, Wako pure chemical, Japan) or both of them (Fig. 2g). Next, it was left in pure isopropanol for300 s to stabilize the surface modification. Finally, the well array was completely cured by leaving it at 100°C for 1 h (Fig. 2h).

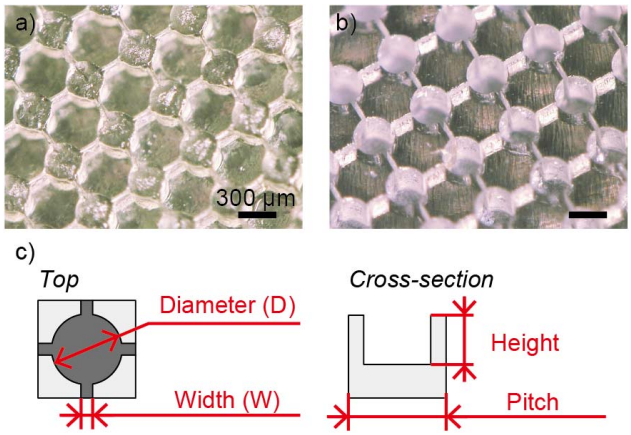

Figure 3: (a) An image of fabricated mold with D 300 µm and W 30µm. (b) An image of fabricated connected well array with D 300 µm and W 30µm. (c) Parameter of a single well unit of the array.

Table 1: Dimension of fabricated well array.

Pattern num.	*D [µm]*	*W [µm]*	*Capacity [nl]*
1	300	30	21
2	300	60	21

Images of the fabricated mold and the connected well array are shown in Fig. 3a and b. The well array has four fabrication parameters; diameter of the circular well (D), width of the channels (W), height of the well (which is set as same value as the D) and pitch of wells (which is set 1.5 times the D value), as shown in Fig. 3c. Table 1 summarizes the dimensions of well array and the expected capacity in a single well. The number of wells in a single array is 32×32.

EXPERIMENTAL

Contact angle test

To evaluate the reliability of surface modification based on grafting, we conducted contact angle measurement on surface modified with DMA, FDM and the mixture of DMA and FDM (the ratio is 5:1, 1:1 and 1:5). The contact angle was measured through a half-angle method: 2 µl of purified water (with Milli-Q system, Millipore, MA, USA), *n*-decane (Wako pure chemical, Japan) or fluorocarbon solvent (FC, CT-SOLV180, Asahi Glass, Japan) was dispensed onto the target surface. The images were acquired by a microscopic observation system (VHX-600, Keyence Corporation, Japan).

Droplet generation test

We investigated the yields of droplets generated in a well array in terms of well geometries, immiscible solvent species, and surface conditions.

The procedure of droplet generation was as follows: Firstly, ethanol was filled into the connected well array. Then, it was washed out with aqueous solution including 5% w/w indigo carmine (Millipore, MA, USA) to help visualization of the droplet generation. Finally, 2 µl of immiscible solvent was dispensed at the edge of the well array. The droplet generation events were observed with the microscopic observation system. The residual solution

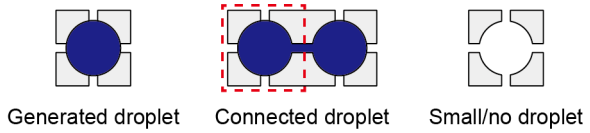

Figure 4: Classification of residual solvent in a single well unit.

states produced by the dispensed solvent were categorized into three groups; a generated droplet, a connected droplet and a small/no droplet in a circular well as shown in Fig. 4. The droplet generation rate was investigated, which is defined as the ratio of the number of generated droplets to that of the available wells. Because droplet generation occurring around the edge of the array is affected by pipetting of the solvent, 10×10 wells in the center of the array were used for the evaluation. The generated droplets were investigated 20 min after the solvent injection.

First, we measured the generation rate using the number 1 and 2 arrays in Table 1 to investigate the effect of well geometry (with FC as solvent and FDM for the surface modification). Then, using the number 1 of the well array, *n*-decane and FC was tested for the droplet generation on the surfaces modified with FDM, DMA and the mixture of FDM and DMA (the ratio is 5:1, 1:1 and 1:5).

RESULT AND DISCUSSION

Contact angle test

Fig. 5a-c shows image of water, *n*-decane and FC droplets, respectively, on the surface modified with the 1:5 mixture of DMA and FDM. Figure 5d shows the three liquid contact angles on all tested surface. As shown in this figure, water contact angles on all modified surface increased from that on the unmodified surface. It indicates the hydrophobic surface modification based on polymer chain grafting was successfully conducted. The contact angle of water increased with the ratio of FDM. We considered that this is because grafted FDM groups

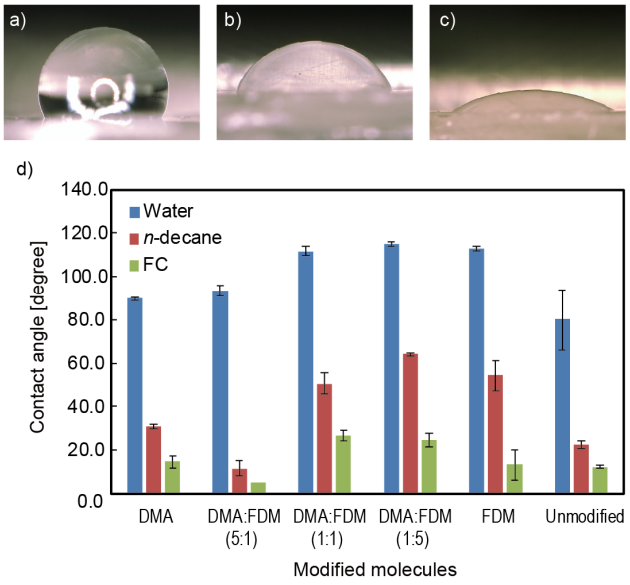

Figure 5: Images of droplets of water (a), n-decane (b) and FC (c) on the surface modified with1:5 of DMA and FDM mixture. (d) Measured contact angles of three liquids on the surfaces modified with 5 different conditions in addition with unmodified surface.

increased on the surface with the ratio of FDM, which is more hydrophobic than the DMA. Similar tendency to water was observed on the contact angle of *n*-decane. FC showed the contact angle less than 30 degree on all tested surfaces.

Droplet generation test

In the experiment using number 1 and 2 well arrays in Table 1, the droplet generation successfully occurred with only the number 1 array. This result indicates that it is necessary for the D of the well to be 10 times or more of the W to generate droplets.

Figure 6a shows droplet generation rate as for FC and *n*-decane using well array surface modified with DMA, FDM and the mixture of them. For the FC, the highest generation rate was obtained with 1:5 mixture of DMA and FDM. Although FC successfully propagated into well arrays of all the surface condition, the droplet generation rate increased with the ratio of the FDM. Only FDM modification did not show the highest, indicating that a certain ratio of the DMA existence effectively worked.

On the other hand, for *n*-decane, the highest rate was obtained with the 1:1 mixture. On the well array modified with the DMA and the 5:1 mixture, *n*-decane successfully propagated but droplet generation rate was low. The well

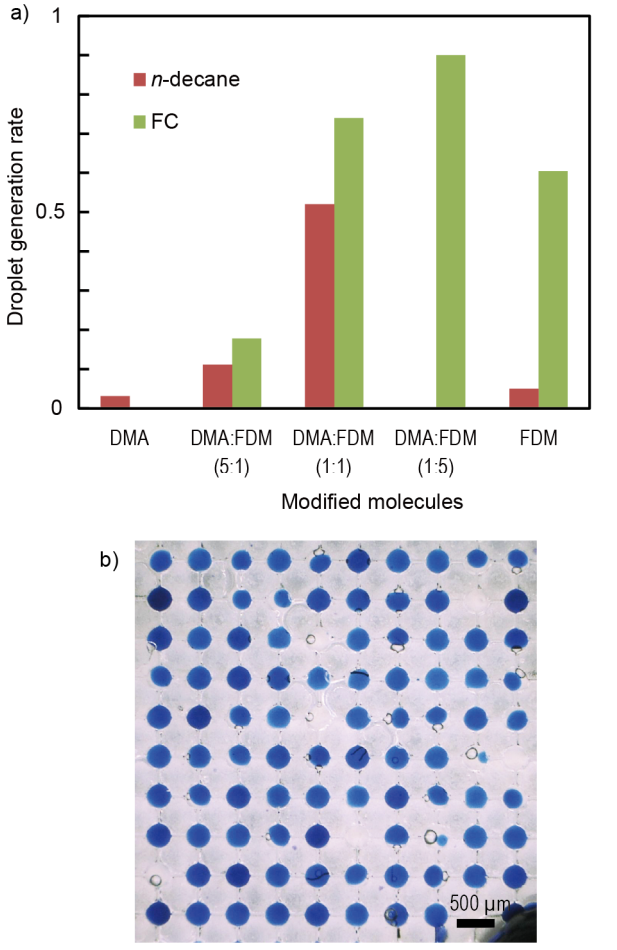

Figure 6: (a) Droplet generation rate using FC and n*-decane with the well array modified with 5 different conditions; DMA, FDM, 5:1, 1:1, 1:5 mixtures. (b) Generated droplets on the well array modified with 1:5 of DMA and FDM using FC.*

arrays modified with 1:5 mixture and FDM showed quite low value of the generation rate. During the experiments, it was often observed that air in spite of the *n*-decane pushed the aqueous solution out of the well. We consider that it is because the contact angle of *n*-decane is relatively high on the surface including FDM as shown in Fig. 5. FC and *n*-decane showed the highest generation rate on different surfaces. This result indicates that the combination of the surface condition and used solvent is important to obtain high droplet generation rate.

We found the surface modification scheme based on grafting to self-generate droplet array. Although volume of generated droplets in this work was estimated to be tens of nanoliters, the result indicates the dimension of the well array is possible to further miniaturize and hence it is possible to generate smaller-scale droplets, which would be beneficial for some applications, such as digital PCR.

CONCLUSIONS

In this work, we experimentally studied a surface modification scheme of OSTE for self-generation of nanoliter-scale droplet arrays by oil-water immiscibility and replacement. The OSTE surface was successfully modified based on polymer chain grafting. The modified surface showed higher contact angle with the increase of the ration of FDM. Droplet generation was observed on both cases using FC and *n*-decane but the highest generation rate with each solvent was obtained on differently modified surface.

The proposed manner is possible to miniaturize volume-scale of generated droplets to nanoliter or even smaller. Note that all the liquid manipulation process for the droplet generation did not need any precise process unlike previous methods. It illustrates that the proposed array drastically facilitates the preparation on the 2D droplet array. We believe that the proposed system can be a universal platform in the field of biological assay or medical diagnosis based on microdroplets.

ACKNOWLEDGEMENTS

One of the authors is supported by a Grant in Aid for JSPS Research Fellows (Grant Number JP16J06211) in Japan. We would like to thank Dr. Tommy Haraldson at KTH Royal Institute of Technology for fruitful discussions about polymer chain grafting.

REFERENCES

[1] T.S. Kaminski, O. Scheler, and P. Garstecki, "Droplet microfluidics for microbiology: techniques, applications and challenges", Lab Chip, 16, 2168-2187 (2016).

[2] R.R. Pompano, W. Liu, W. Du, and R.F. Ismagilov, "Microfluidics using spatially defined arrays of droplets in one, two, and three dimensions", Annu. Rev. Anal. Chem., 4, 59-81 (2011).

[3] F. Shen, W. Du, J.E. Kreutz, A. Fok, and R.F. Ismagilov, "Digital PCR on a SlipChip", Lab Chip, 10, 20, 2666-2672 (2010).

[4] H.H. Jeong, S.H. Jin, B.J. Lee, T. Kim, and C. S. Lee, "Microfluidic static droplet array for analyzing microbial communication on a population gradient", Lab Chip, 15, 3, 889-999 (2015).

[5] Y. Zhu, Y.X. Zhang, W.W. Liu, Y. Ma, Q. Fang, and B. Yao, "Printing 2-dimentional droplet array for single-cell reverse transcription quantitative PCR assay with a microfluidic robot", Sci. Rep., 9551 (2015).

[6] M. Sun, S.S. Bithi, and S.A. Vanapalli, "Microfluidic static droplet arrays with tuneable gradients in material composition," Lab Chip, 11, 23, 3949-3952 (2011).

[7] X.H. Yan, L. Hu, Y. Li, X.J. Feng, and B.F. Liu, "Microfluidic Slipchip-based Reaction Microarray with Dual Concentration Gradient," Chinese J. Anal. Chem., 43, 10, 1520-1525 (2015).

[8] H. Yasuga and N. Miki, "Imbibed immiscible solvent splits aqueous solution into a two-dimensional droplet array without precise pipetting or pumping", in proceedings of MicroTAS2016 conference, Dublin, Ireland, October 9-13, (2016), pp. 83 - 84.

[9] X. Zhou, C.F. Calborg, N. Sandström, A. Haleem, A. Vastesson, F. Saharil, W. van der Wijngaart and T. Haraldsson, "Rapid fabrication of OSTE+ microfluidic devices with lithographically defined hydrophobic/hydrophilic patterns and biocompatible chip sealing," in proceeding of Microtas2013, Freiburg, Germany, October 27-31, (2013), pp. 134-136.

[10] N. Sandström, R.Z. Shafagh, A.Vastesson, C.F. Carlborg, W. van der Wijngaart, and T. Haraldsson, "Reaction injection molding and direct covalent bonding of OSTE+ polymer microfluidic devices," J. Micromechanics Microengineering, 25, 7, 75002 (2015).

CONTACT

*H.Yasuga, tel: +81-45-563-1141; hy-3@keio.jp