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研究ノート

Production of monodisperse oil-in-water emulsions using asymmetric micro through-holes compactly arranged on a metallic chip

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Abstract

The aim of this study was to produce monodisperse oil-in-water (O/W) emulsions using a metallic microchannel emulsification (MCE) chip containing 171 compactly arranged asymmetric micro through-holes. Each asymmetric micro-though-hole that was fabricated on an aluminum chip consisted of a circular microhole with a 100-µm diameter (1650-µm depth on the inlet side) and a microslot on the outlet side with a 100 × 700-µm cross-section and 350-µm depth. Uniformly sized silicone oil droplets with an average diameter (d_{av}) of 291.5 µm and the coefficient of variation (CV) of 3.9% were smoothly formed by means of the asymmetric micro through-holes at the flow rate of the dispersed phase (Q_d) of 1.0 mL/h without a cross-flowing continuous phase. Metallic MCE chips have advantages over silicon MCE chips in terms of durability vis-à-vis alkaline cleaning and shocks as well as modifications of through-hole dimensions.

Key words: Microchannel emulsification; oil-in-water emulsion; metallic chip

Introduction

Emulsions are thermodynamically metastable systems, meaning that emulsion stability can be analyzed within a finite period. Droplet coalescence, which eventually causes phase separation, is strongly influenced by droplet size and by its distribution of emulsion products. Emulsions with narrow distributions of droplet sizes are usually called monodisperse emulsions. Such emulsions are advantageous owing to improved stability versus droplet coalescence and easier control and interpretation of major emulsion properties (McClements, 2004). These characteristics have attracted researchers from various fields including the food industry. Monodisperse emulsions are also available as templates useful for producing monodisperse microparticles and microcapsules.

Microchannel emulsification (MCE) is a promising method for producing monodisperse emulsions (Kobayashi and Ichikawa, 2015) because this approach involves a robust and very mild process of droplet formation. Microchannel (MC) arrays with unique geometric features

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enable stable droplet formation that is insensitive to the flow rate of each phase below a critical value (Kobayashi et al., 2010). Previous MCE studies showed production of monodisperse emulsions (containing bioactive compounds) with droplet sizes of >1 µm and the minimal coefficient of variation (CV) of <5% (Kobayashi and Ichikawa, 2015). Monodisperse emulsions obtained by MCE have been used for fabricaing food-grade monodisperse micromaterials, e.g., lipid microparticles, gel microbeads, and coacervate microcapsules (Kobayashi and Ichikawa, 2015).

To date, silicon-based MC array chips have been mainly developed for MCE, which can be ascribed to precise microfabrication of MC arrays, extremely flat chip surfaces, and controlled modification of chip surfaces (Vladisavljević et al., 2012). Studies on MCE have also been performed on nonsilicon MC array chips made of a polymer (poly[methyl methacrylate]) (Liu et al., 2005; Kobayashi et al., 2008) or metal (stainless steel) (Kobayashi et al., 2012). MC arrays can be categorized into parallel microgrooves and micro through-holes (Kobayashi and Ichikawa, 2015). Asymmetric micro through-holes that are compactly arranged on a chip have higher droplet productivity in comparison with the other types of MC arrays. In MCE, asymmetric micro through-holes that are currently available are formed on a silicon-based chip; their drawbacks include intolerance toward alkaline cleaning, shock fragility, and difficulties with modification of through-hole dimensions.

We therefore explored the fundamental production characteristics of oil-in-water (O/W) emulsions by MCE using asymmetric micro through-holes compactly arranged on an aluminum chip.

Materials and methods

Reagents and preparation of the solution

Silicone oil with dynamic viscosity of 48.5 mPa·s at 25°C (KF96-50) was purchased from Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan). Polyoxyethylene (20) sorbitan monolaurate (Tween 20; hydrophilic-lipophilic balance [HLB] 16.7) was purchased from Wako Pure Chemical Industries Co., Ltd. (Osaka, Japan). A continuous-phase solution was prepared by dissolving Tween 20 in Mill-Q water at the emulsifier concentration of 1.0 wt%. All the reagents were used as received.

Experimental setup and the procedure

The emulsification experiments were performed on a custom-made MCE system (Kobayashi et al., 2014). Figure 1(a) schematically depicts an aluminum MCE chip containing asymmetric micro through-holes. Each asymmetric micro-through-hole consists of a circular microhole with a 100-µm diameter and 1650-µm depth on the inlet side and a microslot

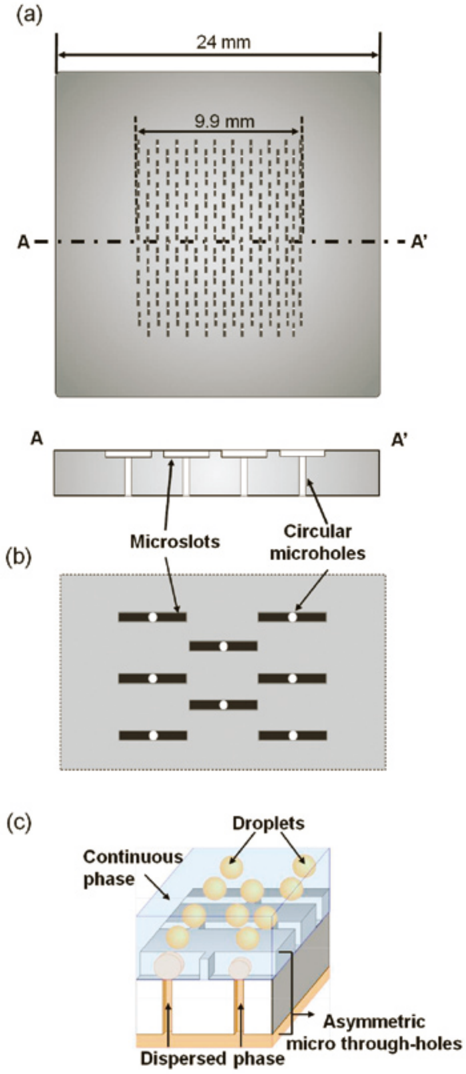

Fig. 1 **(a) Schematic top and cross-sectional views of aluminum MCE chip containing asymmetric micro through-holes.**
(b) Magnified schematic top view of asymmetric micro through-holes.
(c) Three-dimensional schematic drawing of droplet generation via asymmetric micro through-holes.

on the outlet side with a 100 × 700-μm cross-section and 350-μm depth. As shown in Fig. 1(b), the asymmetric micro through-holes are compactly arranged at the center of the chip. One hundred seventy-one asymmetric micro through-holes were fabricated by microdrilling to obtain circular microholes and by means of subsequent electric discharge machining for forming microslots.

Prior to each experiment, the aluminum MCE chip was surface-oxidized using a plasma reactor (PR-500, Yamato Scientific Co., Ltd., Tokyo, Japan) to make its surface hydrophilic. This MCE chip was fixed in a module prefilled with a continuous phase. A dispersed phase from a 10-mL glass syringe was fed into the module by means of a syringe pump (Model 11 Plus; Harvard Apparatus, Inc., Holliston, USA). The continuous phase from a 1-L plastic tank was fed into the module by means of hydrostatic pressure. To form oil droplets, we injected the dispersed phase introduced into the module via the asymmetric micro through-holes in the presence or absence of the cross-flowing continuous phase at ambient temperature (~25°C; Fig. 1(c)). A microscopic video system (Kobayashi et al., 2014) was used for *in situ* and real-time monitoring of the droplet formation from the slot outlets.

Measurement of droplet size

The diameters of the oil droplets formed were manually measured using image analysis software (WinRoof, Mitani Co., Fukui, Japan). The number- average droplet diameter (d_{av}) was determined by dividing the number of the measured droplets (n = 200) by the sum of the droplet diameters. CV was used as an indicator of the droplet size distribution and was calculated as follows:

$$CV = (\sigma/d_{av}) \times 100 \qquad (1)$$

where σ is the standard deviation of the droplet diameter.

Results and discussion

Figure 2(a) shows typical formation behavior of oil droplets on an aluminum MCE chip containing asymmetric micro through-holes at the flow rate of the dispersed phase (Q_d) of 1.0 mL/h and the flow rate of the continuous phase (Q_c) of 200 mL/h. This Q_d corresponds to the dispersed-phase flux (J_d) of 10 L/(m^2·h). Oil droplets smoothly and periodically formed and detached from the slot outlets. Such successful droplet formation was observed in ~85% of slot outlets, and the droplet formation rate for each active micro-through-hole was between 0.11 and 0.13 s^{-1}. As shown in Fig. 2(b), the formed oil droplets were of uniform size and were closely packed on top of the compartment above the chip surface in the absence of the cross-flowing continuous phase. No droplet coalescence occurred during microscopic observation for 1 h.

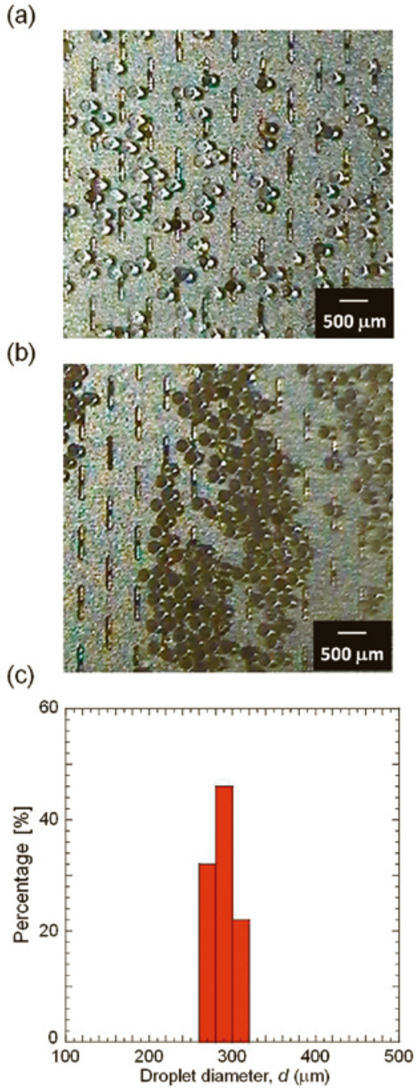

Fig. 2 **(a), (b) Typical generation and detachment of oil droplets from the outlets of asymmetric micro through-holes fabricated on an aluminum chip. (c) Size distribution of the oil droplets obtained using the aluminum MCE chip.**

The size distribution of the oil droplets that were formed by means of the aluminum MCE chip is presented in Fig. 2(c). The resultant oil droplets had d_{av} of 291.5 μm and CV of 3.9%; these data are suggestive of their monodispersity. Their size distribution also contained a sharp monomodal peak; this pattern is similar to that of the oil droplets formed by means of a silicon MCE chip (Kobayashi et al., 2010). This d_{av} was 2.9-fold greater than the microhole diameter. In MCE, d_{av} is mainly dependent on the microhole diameter and slot depth and ranges from 20 to 180 μm (Kobayashi et al., 2010; Khalid et al., 2015), indicating that d_{av} of the oil droplets obtained here is acceptable. The droplet formation rate for the whole MCE chip was found to be 7.7×10^4 h^{-1}. The aluminum MCE chip that we used here contained compactly arranged micro through-holes, just as silicon MCE chips do (Kobayashi et al., 2010), suggesting that it is possible to produce monodisperse emulsions at high productivity in terms of droplets. The cross-flowing continuous phase that we used here did not affect the resultant oil droplet size (data not shown). The Reynolds number of this cross-flowing continuous phase (Re_c) can be calculated by means of the formula

$$Re_c = \rho_c U_c d_{eq,ch} / \eta_c = \rho_c U_c \, (4A_{ch} / L_{ch}) / \eta_c \qquad (2)$$

where ρ_c is the continuous-phase density, U_c is the average continuous-phase velocity in the compartment above the chip surface, $d_{eq,ch}$ is the equivalent diameter of the compartment, L_{ch} is the wetted perimeter of the compartment, and η_c is the continuous-phase viscosity. The Re_c value of 0.7 that we obtained here means laminar flow of the cross-flowing continuous phase. We believe that shear stress resulting from the cross-flowing continuous phase is too low to enhance droplet detachment from the slot outlets.

The results of this study are discussed below. Although it was possible to microscopically examine formation of droplets from the slot outlets, the contours of the expanding and detached droplets were less clear-cut than those for a silicon chip. This difference is attributable to the difference in surface roughness of silicon and aluminum MCE chips. The surface roughness of silicon MCE chips is less than 0.01 μm, whereas that of aluminum MCE chips is thought to be greater than 0.1 μm. Diffuse light reflection, in principle, increases with the increasing surface roughness. The greater surface roughness of the aluminum MCE chips causes greater diffused reflection of light from the chip surface; this situation may make the contours of expanding and detached droplets blurry. The asymmetric micro through-holes that we fabricated were highly uniform: the size distribution of < 4%. This result fulfills the necessary condition of producing monodisperse emulsions. In contrast, the size of the oil droplets that were formed in this study is quite large for emulsion droplets; this situation is due to the large size of the asymmetric micro through-holes fabricated on the aluminum MCE chip. Further downsizing of such asymmetric micro through-holes is required to attain production of monodisperse emulsions with droplet sizes smaller than 100 μm for food-related and biotechnological applications.

In conclusion, a monodisperse O/W emulsion with uniformly sized oil droplets ~300 μm in diameter was reliably produced here by means of a metallic MCE chip containing compactly arranged asymmetric micro through-holes. The surface roughness of MCE chips is the main factor affecting sharpness of the contours of expanding and detached droplets during microscopic examination.

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金属チップ上に密に配置された非対称マイクロ貫通孔を用いた単分散水中油滴型エマルションの作製

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要　旨

本研究では，金属製のマイクロチャネル乳化チップを利用した単分散水中油滴（O/W）型エマルションの作製特性について検討した．前記チップの中央部には，171個の非対称マイクロ貫通孔が密に配置されている．個々の非対称マイクロ貫通孔は，入口側のマイクロホール（直径100 µm，深さ1650 µm）および出口側のマイクロスロット（断面サイズ100×700 µm，深さ350 µm）が連結した状態で構成されている．分散相流量速度が1.0 mL/hの場合において，サイズが均一な微小油滴（平均液滴径291.5 µm，変動係数3.9%）が，連続相のせん断流れを必要とせずにマイクロスロットの出口から安定的に作製された．金属製マイクロチャネル乳化チップは，耐アルカリ性，耐衝撃性，および貫通孔サイズの変更容易性の面においてシリコン製マイクロチャネル乳化チップよりも有利であるといえる．