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Dissolution-Guided Wetting for Microarray and Microfluidic Devices

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Abstract

The trapping of air bubbles presents a substantial impediment for the user in the increasingly widespread use of lab-on-a-chip products having microcavities in the forms of microwells, traps, dead ends and corners. Here we demonstrate a simple, effective, and passive method to eliminate air bubbles by coating hydrophilized microarray and microfluidic devices with a monosaccharide such as D-glucose or D-sorbitol, where the microcavities are filled with a conformal, elliptical, cone-shaped monosaccharide solid. These devices were stored in air for up to 6 months with a complete rewetting of the microcavities by dissolution of the monosaccharide with an aqueous solution.

Introduction

Lab-on-a-chip technology has made rapid progress for applications in cell biology and biochemical assay.[1] Lab-on-a-chip systems that enable the efficient performance of assays with low reagent consumption typically contain microfluidic channels and other microstructures such as microwells,[2] microposts,[3, 4] traps,[5] and corners or dead ends,[6] where air bubbles can be easily trapped upon the addition of aqueous solution when the surface is not sufficiently hydrophilic. The trapped air bubbles result in Cassie-state wetting on the surface. This wetting phenomenon has been exploited for specific applications, such as selective deposition of proteins and cells to the areas that are in contact with the aqueous solution, for example, on the surface between microwells,[7] or on the top surface of micropallets.[8] Nevertheless, for a majority of applications, the trapped air bubbles in microfabricated devices pose a challenge for the end user and bubbles need to be removed to generate a Wenzel-state wetting to allow the entire surface to be in full contact with aqueous solutions such as analytes or cell-culture medium.[9, 10]

Microwell arrays are examples of microfabricated devices possessing microcavities, and have emerged as useful platforms for cell culture and assays at single-cell resolution.[2, 11] For example, our lab has developed a modified microwell array platform in which each well possesses a bottom formed by a loosely attached microelement, termed a microraft, with proven utility in isolation and cloning of live adherent cells.[12, 13] Since microwell arrays are generally made from polymers, such as polydimethylsiloxane (PDMS), which are either hydrophobic or only slightly hydrophilic in their native form, trapping of air bubbles inside the microwells are encountered whenever the array is covered with an aqueous solution. To solve this problem, plasma treatment is generally used to produce a hydrophilic surface; however, in many of the common polymers this hydrophilization is only temporary, and

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either a partial or complete hydrophobic recovery is usually observed.[14, 15] In addition to surface oxidation to prevent air bubble entrapment, several methods are currently used for removing trapped air bubbles in cavities, including vacuum application, pressurization, centrifugation, vibration and sonication.[10, 16–18] Alternatively, low surface tension liquids (*e.g.* ethanol, $\gamma = 22.4\ mN{\cdot}m^{-1}$) can be used to initially wet the surface prior to exchange with water ($\gamma = 72.9\ mN{\cdot}m^{-1}$) or an aqueous buffer.[19, 20] Although the above methods are effective in removing air bubbles in specific cases, there remains a need for a simpler, passive method in preventing bubble formation, especially for the use of microdevices having microcavities in field and clinical applications. Bubble formation also poses challenges for any polymer-based, lab-on-a-chip product with cavities for commercial applications where ease of use is paramount. This study provides a simple, effective, and passive approach to avoid air bubble formation in lab-on-a-chip systems by priming the cavities with a monosaccharide such as D-glucose or D-sorbitol. Upon addition of aqueous solution, dissolution of monosaccharide guides a rapid, complete rewetting of the cavities.

Results

Trapping air bubbles in microcavities

The trapping of air bubbles in cavities on microfabricated devices upon addition of aqueous solution can be explained by the scheme shown in Fig. 1a where a microwell is used as an example. ϕ is defined as the angle that a diagonal through the well makes with the well side wall and θ is the angle of the aqueous solution on the side wall of the cavity. For a hydrophilic surface, such as a freshly oxidized polymer surface, $\theta < \phi$, the advancing liquid can wet the vertical wall and bottom before reaching the other edge of the well. As a result, air can be pushed out from the well resulting in a homogeneous wetting (Wenzel state). For a hydrophobic surface, $\theta > \phi$, advancing liquid reaches the other edge of the well before it can wet the side and bottom walls. As a result, air can be trapped inside the well resulting in heterogeneous wetting (Cassie state).

The native surface for common polymers used in lab-on-a-chip devices is generally not hydrophilic. Among ten different polymers of interest for microfabrication, PDMS has the most rapid hydrophobic recovery,[15] so it was selected as the model material to create the structured surfaces in this study. We found the water contact angle on PDMS films immediately after plasma treatment was 10° ± 5°, but recovered to 42° ± 8° (n = 3) after 3 days, and 65° ± 11° (n = 3) at day 6. Air bubbles were trapped inside microwells (diameter D = 100 μm, height H = 55 μm, Fig. 1b) upon addition of water. Similarly, air bubble entrapment was present in corners (Fig. 1c) and dead ends (Fig. 1d) of PDMS microfluidic channels on day 7 after channel fabrication. A similar trend was observed for devices made from other polymers, such polystyrene, poly(D,L-lactide) and SU-8 epoxy photoresist (data not shown), although these materials exhibited a slower hydrophobic recovery than PDMS consistent with a recent report regarding hydrophobic recovery of commonly used polymers.[15] The trapped air bubbles must be eliminated prior to use of the devices for many biological applications, for example, culture of cells within PDMS microwells.

Priming the hydrophilic microcavities with monosaccharide

We report a simple method to eliminate air bubbles on structured surfaces possessing microcavities, corners and dead ends. The process is composed of two steps: the surface is first made hydrophilic such as by plasma treatment, then primed with an aqueous monosaccharide solution, whereupon it can be placed in dry storage. Prior to use of the microdevice, the end user simply adds water to the surface to dissolve the monosaccharide followed by rinsing to remove any trace of the sugar on the surface. The dissolution of the sugar guides a complete wetting, leaving the surfaces bubble free. Microwells and

microfluidic channels made from PDMS were used as the model to demonstrate this dissolution-guided wetting.

PDMS microwells were first oxidized with air plasma to generate a hydrophilic surface. An aqueous solution of glucose was added to the microwells forming Wenzel-state wetting on the surface. Excess volume above the wells was then removed by aspiration. Upon drying, a conformal coating of solid glucose lined the microwell walls and floor (Fig. 2a). Plasma treatment of the PDMS was required to generate a conformal coating of solid glucose; otherwise, a discontinuous coating of solid glucose was formed in native PDMS microcavities (supplemental data #1). For the microwells primed with glucose at a concentration of 30%, the glucose layer appeared to coat only a portion of the microwell walls and floor (Fig. 2b). Lower glucose concentrations (22%) resulted in even lower coverage of the microwell surface (Fig. 2b). The angle of the glucose layer on the side wall was also steeper at the lower glucose concentrations. This observation is consistent with the assumption that the contour of the dried glucose layer is elliptical in shape (supplemental data #2). With this assumption, the concentration of the glucose solution must be >33% in order to generate full coverage of the interior surface of the microwells, *e.g.* 37% glucose in Fig. 2b. The relative coverage of dry glucose in the microwell is dependent only on the concentration of glucose c, not on the dimension of microcavities (diameter and height). Therefore, the microcavities can be fully primed by using a glucose solution with $c > 33\%$, even for a surface possessing microcavities with a wide range of sizes (10 μm – 3 mm) and depths.

In the case of microfluidic devices, PDMS channels were molded from a master and then bonded with glass slides through plasma oxidation. To prime the corners and dead ends, 5 μL of the sugar solution was added to one entrance of the microfluidic channel immediately after plasma treatment and bonding. The microfluidic channel was then purged with nitrogen expelling the sugar solution from the main channel (supplemental data #7). After purging, residual sugar solution remained trapped in the corners and dead ends. The devices were then stored at room temperature. The residual sugar solution within the device was allowed to gradually dry over 1–2 days by evaporation. A 30% glucose solution was used to prime the corners, and a 50% sorbitol solution was used to prime dead ends. The higher solubility of sorbitol (59.6 vol%) over glucose (37.1 vol%) was found to be required for the dead ends used in these studies due to the small volumes trapped in these structures. Upon drying, a residue of solid sugar remained in the corners and dead ends (Fig. 4c).

In the study, solid sugar polyols, such as D-glucose and D-sorbitol, were selected as they rapidly dissolve in water and are biocompatible and non-toxic. The solid sugars are also non-volatile, and stable at a wide range of temperatures due to their high melting point (146 °C for glucose and 95 °C for sorbitol). Phosphate buffered saline (PBS), a common salt solution used in biology, was also assessed, but large salt crystals formed in the wells instead of a conformal coating on the well walls (supplemental data #1). Water soluble polymers were excluded due to their relatively low dissolution speed in water. In addition to sugars, other components of biofluids, such as albumin, amino acids and lipids, might be used to guide rewetting as long as a conformal coating can be generated.

Prevention of bubble formation by dissolution-guided rewetting

In the case of microwell arrays, the glucose coating alters both θ for the side wall and ϕ for the well, which facilities the rewetting of the microwells by water. Since water can rapidly dissolve glucose, it was reasoned that the dissolution could guide the rewetting of the microwells (Fig. 3a). Rewetting of PDMS microwell arrays (D = 50 μm, H = 55 μm) was assessed one month after oxidation and priming with a 37% or 0% glucose solution. Fig 3b shows the wetting on PDMS microwells with glucose priming (left panel) and without

glucose priming (right panel). For microwells without glucose priming, air bubbles formed in the microwells. For microwells primed with a 37% glucose solution, no air bubbles formed. This anti-bubble effect was present for 6 months, the longest time tested, suggesting that the changes in θ and ϕ were successful in maintaining wettability over time. To visualize the rate of glucose dissolution, a fluorescent glucose solution (37% glucose with 200 μg/mL TRITC-dextran, MW= 500,000) was used to prime a PDMS microwell array (microwells with D = 200 μm, H = 55 μm) one month prior to the experiment. The loss of fluorescence in the well was then tracked over time to follow glucose dissolution. After addition of water, the microwells were observed to completely wet followed by a loss of the priming layer over a time period of 25 s (Fig. 3c). Two video clips (supplemental data) show the difference in the wetting characteristics of microwells with and without glucose coating.

In the case of microfluidic chips, after storing the devices at room temperature for 7 days, water was introduced into the channels (Fig. 3d and e). For the device with corners, water passing through the channel dissolved the glucose in the corners over a period of 30 s (Fig. 3d, video clip in supplemental data). The dissolution of glucose was guided the wetting of the corners in a manner similar to that seen in the microwells. A similar observation was made for the sorbitol-primed dead ends (Fig. 3e, video clip in supplemental data). Due to the relatively smaller dimension (50 × 50 μm for the dead end) compared to corners (500 μm square), the dissolution was faster (~10 s). Air bubble entrapment was not observed in the primed devices, but was present in identical unprimed devices (video clips in supplemental data).

Rewetting of the devices was guided by sugar dissolution and not by a delay in the hydrophobic recovery of PDMS created by the glucose coating. Glucose priming did not delay hydrophobic recovery of the underlying PDMS surface (supplemental data #3). Following glucose dissolution, no glucose residue was detectable on the PDMS surfaces as confirmed by an attenuated total reflectance (ATR)-FTIR spectrometer (supplemental data #4). Thus the glucose-guided wetting did not occur due to the presence of a permanent glucose-surface coating. In another test, water was used to remove the glucose from the primed PDMS microwells (day 7 after priming), and the microwells were immediately dried with nitrogen, then tested for rewetting. Air bubbles formed in the microwells in the absence of glucose. These results suggest that the presence of solid glucose, not the surface properties of the underlying PDMS, was the critical determinant in guiding microcavity rewetting. This anti-bubble strategy functioned equally well for microwells made from other materials, such as polystyrene (supplemental data #5). Sorbitol was also tested as a priming layer for PDMS microwells with results similar to those shown above (supplemental data# 6).

Conclusions

A simple method has been developed to prevent air-bubble entrapment in cavities, corners and dead ends of microfabricated devices, thus solving a common problem encountered when using microwell arrays and microfluidic channels with corners and dead ends in a variety of lab-on-a-chip applications. The devices were hydrophilized by plasma treatment and primed with glucose or sorbitol. The primed microwells could be kept in dry storage for a prolonged period without loss of efficacy. Air bubble formation was prevented by simply adding water or aqueous buffer to the device to dissolve the sugar. Simple, robust, and easily implemented methods such as this are needed to increase the rate of adoption of microfluidic technologies in everyday laboratory practice as well as in the field and in clinical applications. The current technique represents an example of combining robust simplicity with functionality. Since glucose is an energy source for microbial metabolism, end users may be concerned about bacterial or fungal contamination during storage. This issue can be

addressed by sterilization after the coating is applied using gamma-ray irradiation or ethylene oxide. Sugars that are poor energy sources such as sorbitol, xylitol or mannitol can also be used to replace glucose.[23] The method is applicable to a variety of polymer-based, lab-on-a-chip products with cavities, corners and dead ends including microwell arrays and enclosed microfluidic systems where wetting complicated geometries is particularly challenging.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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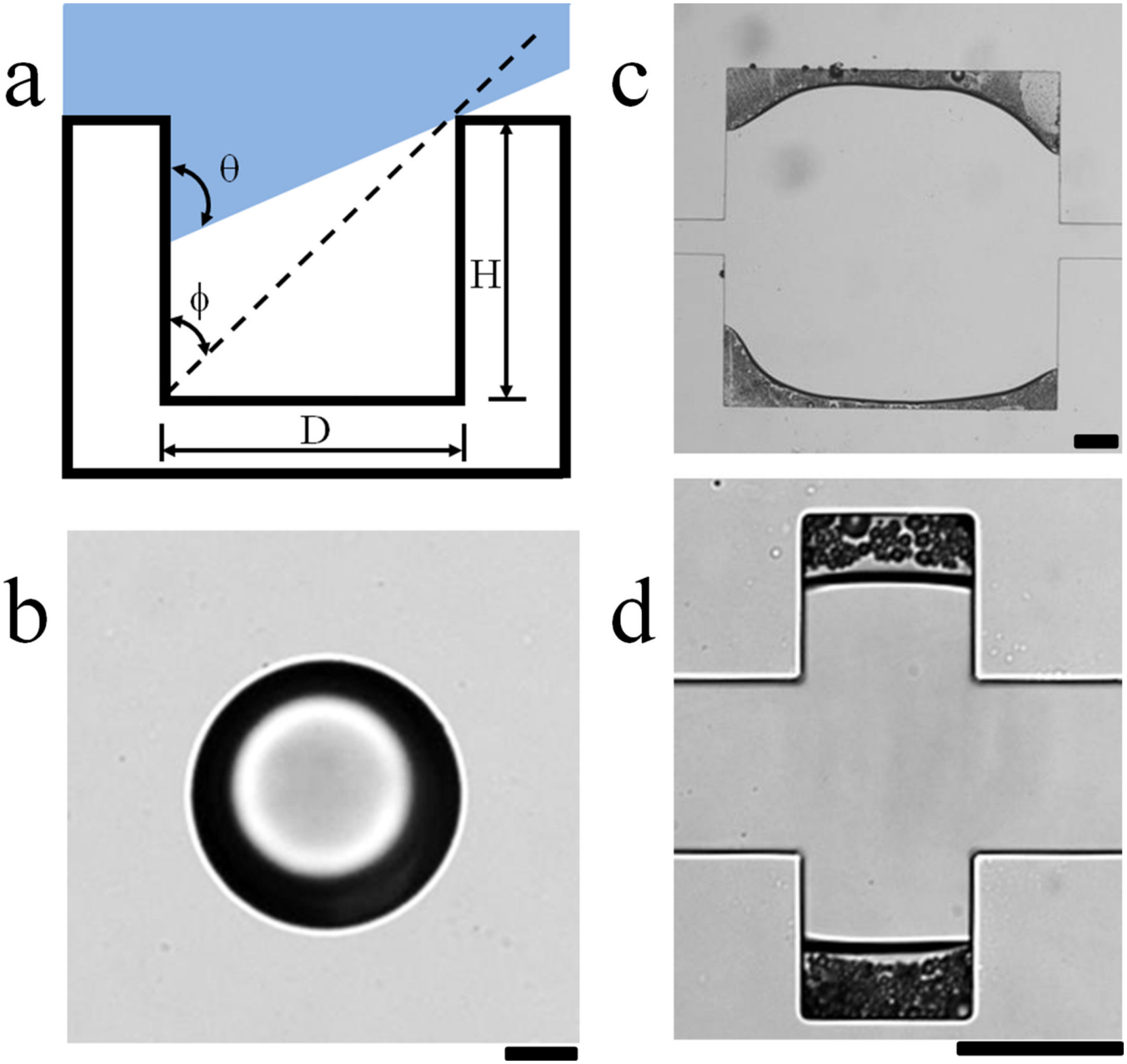

Figure 1.
Wetting of the microwell, corner and dead end in devices made from PDMS. (a) Schematic showing the wetting of a microwell is dependent on both θ and ϕ. (b–d) Brightfield images showing air bubble entrapment was present in a microwell (a), corners (c) and dead ends (d) on day 7 after plasma treatment. Scale bar = 50 μm.

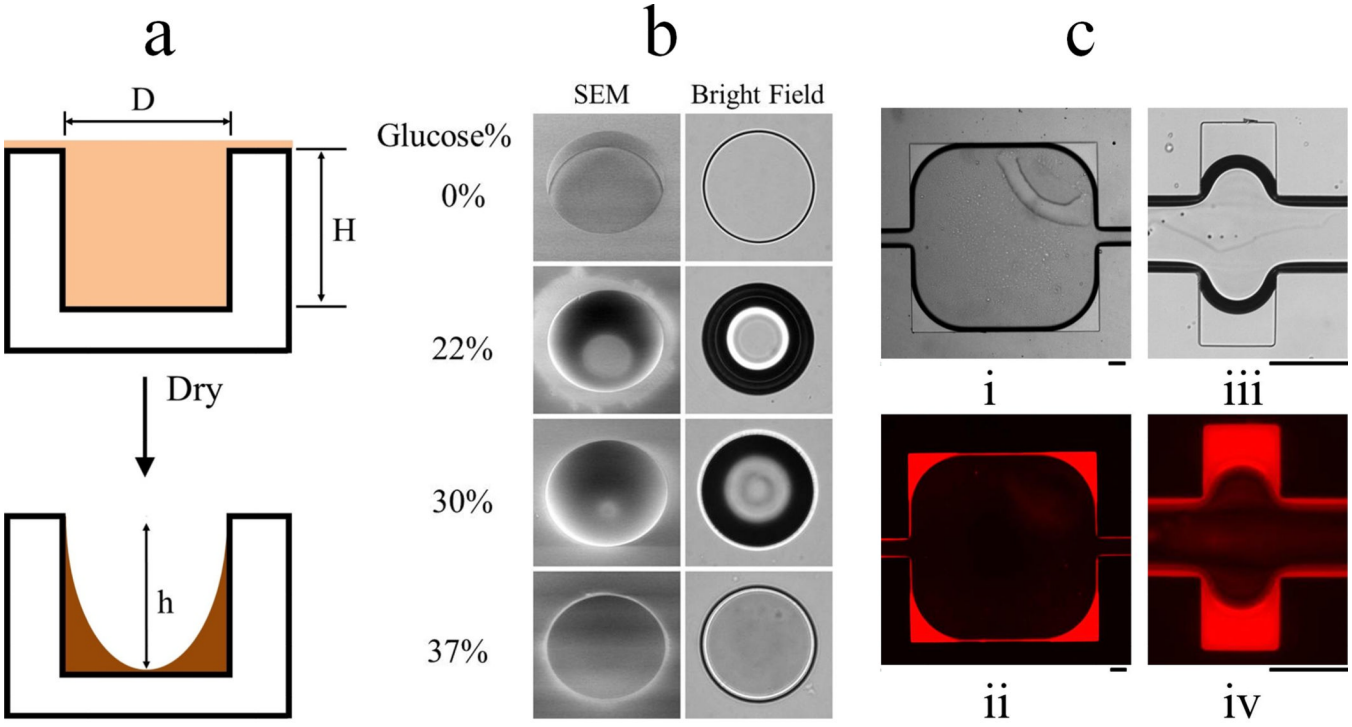

Figure 2.

Priming the hydrophilic microcavities with monosaccharide. (a) Schematic showing that the drying of glucose solution in a microwell results in a conformal, elliptical, cone-shaped coating of solid glucose. The degree of coverage (h/H) depends on the volumetric concentration of glucose. (b) SEM and brightfield images showing a microwell (D = 200 μm, H = 55 μm) filled with a glucose solution and then dried. The concentration of glucose was varied as shown in the figure. SEM images were obtained at a tilt angle of 30°. (c) Priming the corners (i, ii) with glucose, and dead ends (iii, iv) with sorbitol. Transmitted light (i and iii) and fluorescence (ii and vi) images clearly show the corners and dead ends were occupied with sugar. The sugar was mixed with 200 μg/mL TRITC dextran for fluorescence imaging. Scale bar = 50 μm.

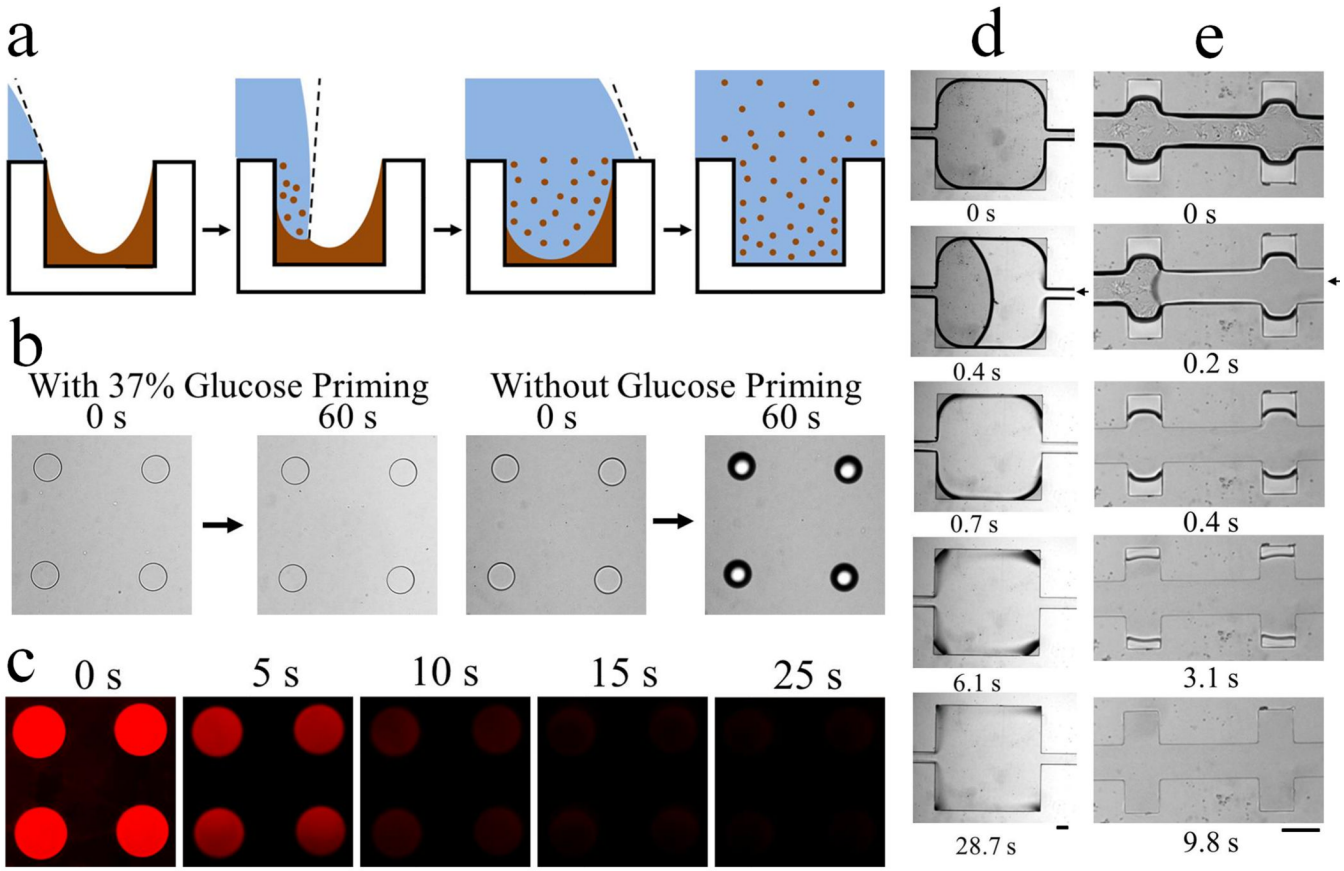

Figure 3.

Dissolution guided wetting in microcavities. (a) Schematic showing the wetting process on a microwell guided by dissolution of glucose. (b) Wetting in PDMS microwells (D = 50 μm, H = 55 μm) with 37% glucose priming (left panel) and without glucose priming (right panel). Prior to wetting, PDMS samples were treated with air plasma for 2 min, primed with 37% glucose (or not primed), and stored at room temperature in air for one month. (c) Time-lapse fluorescence images showing the dissolution of glucose (mixed with 200 μg/mL TRITC dextran) in a microwell array (D = 200 μm, H = 55 μm). (d–e) Time-lapse transmitted light images showing the dissolution of glucose in corners (d) and sorbitol in dead ends (e). Arrows indicate the direction of water flow. Prior to test, the channels were stored at room temperature in air for one week. Scale bar = 50 μm.