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Short Communication

Biomolecular interactions control the shape of stains from drying droplets of complex fluids

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HIGHLIGHTS

- Streptavidin-biotin model chosen for its wide variety of bio-applications.
- Direct influence of a biomolecular interaction on drying submicroliter droplets.
- Two different experimental manifestations are exposed.

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ABSTRACT

When a sessile droplet of a complex fluid dries, a stain forms on the solid surface. The structure and pattern of the stain can be used to detect the presence of a specific chemical compound in the sessile droplet. In the present work, we investigate what parameters of the stain or its formation can be used to characterize the specific interaction between an aqueous dispersion of beads and its receptor immobilized on the surface. We use the biotin-streptavidin system as an experimental model. Clear dissimilarities were observed in the drying sequences on streptavidin-coated substrates of droplets of aqueous solutions containing biotin-coated or streptavidin-coated beads. Fluorescent beads are used in order to visualize the fluid flow field. We show differences in the distribution of the particles on the surface depending on biomolecular interactions between beads and the solid surface. A mechanistic model is proposed to explain the different patterns obtained during drying. The model describes that the beads are left behind the receding wetting line rather than pulled towards the drop center if the biological binding force is comparable to the surface tension of the receding wetting line. Other forces such as the viscous drag, van der Waals forces, and solid–solid friction forces are found negligible. Simple microfluidics experiments are performed to further illustrate the difference in behavior where is adhesion or friction are present between the bead and substrate due to the biological force. The results of the model are in agreement with the experimental observations which provide insight and design capabilities. A better understanding of the effects of the droplet–surface interaction on the drying mechanism is a crucial first step before the identification of drying patterns can be promisingly applied to areas such as immunology and biomarker detection.

1. Introduction

Understanding how a drop of complex fluid dries on a solid surface and leaves a signature stain has various applications, ranging from manufacturing to medical diagnostic and forensic identification (Maltoni, 2003; Dugas et al., 2008; Maillard et al., 2001). Recent studies have focused on transport processes such as the pinning of a wetting line (Sangani et al., 2009; Thiele, 2014), the effect of the solute particle size on the drying process (Sangani et al., 2009), local velocity profiles (Hu and Larson, 2005), and evaporation fluxes at the droplet surface (Hu and Larson, 2002). A few studies have been centered on the influence of surface properties on drying process (Sefiane and Bennacer, 2009; Baughman et al., 2010; Xu et al., 2011; Accardo et al., 2011; Lee et al., 2012; Ristenpart et al., 2007; Larson et al., 2010), but never

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concentrated on the role of biomolecular interaction between the solute and the substrate. A molecular recognition event is different from nonspecific van-der-Waals and electrostatic interactions studied so far. Such selectively sticky behavior is a powerful tool to differentiate between beads of different surface chemistry, as shown in the adhesion-based cell-separation microfluidic (Miwa et al., 2008).

In the current study, we investigate how the drying process of a nanoliter droplet is affected by molecular interactions between the dispersed particles and a chemically modified surface with specific selective receptor. The biotin-streptavidin system was selected for its known strong non-covalent protein-cofactor interaction (Holmberg et al., 2005). Aqueous dispersions of biotin- or streptavidin-coated fluorescent particles are used to track the flow lines and visualize the effect of the streptavidin-coated surface on drying sequence. Our approach is analog to a recent study (Trantum et al., 2012) but does not use external perturbations to force the drying process, which facilitates the development of future applications.

2. Experimental methods

Streptavidin- or biotin-coated fluorescent polystyrene (PS) particles (diam. 0.4–0.5 μm) were ordered from Spherotec (Lake Forrest, IL) and diluted 500 or 1000 times in volume with ultrapure biotechnology performance certified (BPC) water. Streptavidin-coated glass slides (Product Code SMS, Arrayit Corporation, Sunnyvale, CA) were ultrasonicated in ultrapure BPC water for 2 min, then rinsed and blown dry using filtered nitrogen. The 0.1 μL droplets were deposited at room temperature of 22–24 °C with a relative humidity (RH) of 20–24% using a micropipette. Fluorescence images were recorded on a Nikon Eclipse Ti-U inverted microscope with a 10× objective (Modulation Optics, Inc. Plan Fluor ELWD, NA=0.30), a mercury light source, and an amplified EM-CCD (Andor Technology iXon, South Windsor, CT) cooled to −20 °C using a 500 ms exposure. Both of biotin- and streptavidin-coated beads have different dyes embedded in their core; therefore the streptavidin surface was simultaneously imaged using fluorescein isothiocyanate (FITC) and Cy3 filters.

3. Results and discussion

The evolution of the fluorescent particles coated with either biotin or streptavidin in the microdroplet is visualized on a glass surface coated with streptavidin. Fig. 1 demonstrates that, in both cases, the particles first gather into forming a large peripheral ring. This indicates strong contribution from radial flow and that the drying process is driven by evaporation at the wetting line (Bhardwaj et al., 2009; Bhardwaj et al., 2010). As the water evaporates, particles experience Brownian motion and the ring diameter stays constant. The two systems behave differently in the final stage. Whereas most streptavidin-coated beads are deposited in the central region of the droplet residue with fewer particles in the peripheral ring, the biotin-coated particles are distributed more uniformly (Fig. 1(a) and (b)). The same observation can be drawn by comparing the radial particle distribution profile in a 90° quadrant area centered at the geometrical midpoint of the droplet and with the radius of the external peripheral ring (Fig. 1(c) and (d)). About 30 s after the deposition, the distribution profiles show uniformly distributed beads around the center for both biotin- and streptavidin-coated solutions. However, the biotin-coated beads will retain a uniform angular distribution whereas the streptavidin-coated beads are concentrated within the first 35 μm. Here, we make the case that the different outcome is due to the existence of biomolecular force that resists the wetting force of the receding wetting line.

In order to understand how biomolecular interactions can prevent beads, which are attached to a solid–liquid interface, from sliding along with a moving fluid, we first need to consider the balance of the forces acting on a bead. We formulate therefore a mechanistic analytical model for one bead near the wetting line of the evaporating droplet. As shown in the force diagram (Fig. 2(b)), the forces acting on the beads are the hydrodynamic drag force, a biological bonding force, a friction force, a Van der Waals force, and a surface tension force.

To evaluate the drag force caused by the moving fluid with the binding forces, we assembled a Hele-Shaw flow cell out of polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning, Midland, MI). By flowing water at increasing flow rates after biotin and streptavidin beads have been deposited on the bottom surface (streptavidin-coated glass slide), we can increase the drag force on the bead and qualitatively compare it to the resisting forces (van der Waals and friction forces for both types of beads together with a biological bonding force, only for biotin-coated beads). The flow channel is rectangular ($0.5 \times 2 \times 10$ mm). Using syringe pumps, the pressure drop Δp along the channel is varied and the presence of beads on the glass slide is monitored by fluorescence. The PDMS flow cell was first flushed with a 1:500 dilution of biotin-coated beads at a low flow rate (0.2–0.5 mL/min). The beads were then allowed to settle in the dark for 30 min, before a 1:500 dilution of streptavidin-coated beads was flowed through the cell at an equally slow flow rate. The cell was left to rest in the dark for another 30 min. The flow cell was then flushed with water at increasing flow rates of 0.5, 1, 2, 5, 10, and 14 mL/min. The number of beads of each type is counted using image processing software (ImageJ, NIH, Bethesda, MD) by thresholding the grayscale image and performing particle analysis. The selection criteria were particle sizes and a minimum circularity (or roundness) of 0.8. The number of streptavidin beads present on the surface for the lowest rinsing flow rate tested is much smaller than the number of biotin beads due to higher affinity of the biotin beads for the surface. When the rinsing flow rate is increased from 0.5 to 1.0 mL/min, most of the streptavidin beads are flushed away from the surface whereas the high number of biotin beads on the surface remains unchanged (Supplementary Fig. S1). Table 1 gives the number of particles after the deposition step and for each flow rates of rinsing water, as well as the drag force. The results in Table 1 confirm that a hydrodynamic drag force of 0.2–0.8 pN is sufficient to wash out the streptavidin beads which are not attached by biomolecular forces, while the biotin beads remain attached to the surface even for a drag force one order of magnitude larger due to the presence of biomolecular interactions.

In the Stokes flow conditions, which are typical for drop drying, the *drag force* on a bead attached to the solid–liquid interface is estimated at a distance z with Faxen's law (Leach et al., 2009) to:

$$F_{drag} = 6\pi\mu R v_{rad} \Big/ \left(1 - \frac{9}{16}\left(\frac{R}{z}\right) + \frac{1}{8}\left(\frac{R}{z}\right)^3\right), \quad (1)$$

where μ is the dynamic viscosity of the droplet liquid, R is the radius of the bead, and v_{rad} is the radial velocity inside the droplet due to the evaporation. The radial velocity v_{rad} scales as j/ρ, where j is evaporative flux [kg m^{-2} s^{-1}] and ρ is density of the liquid (Bhardwaj et al., 2010).

The *bonding force* involves the biological and van der Waals interaction between the biotin-coated bead and the streptavidin-coated substrate. The biological force is modeled by assuming that each bond between the ligand covalently bound to the bead and the receptor on the substrate exerts either a radial outward (from the center of the bead) tensile force or no force at all, i.e. bonds cannot exert a compression force (Bell, 1978). To quantify the force,