Supplementary information

Contact Angle Changes Induced by Immunocomplex Formation

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1. Contact angle measurement on bare gold surfaces

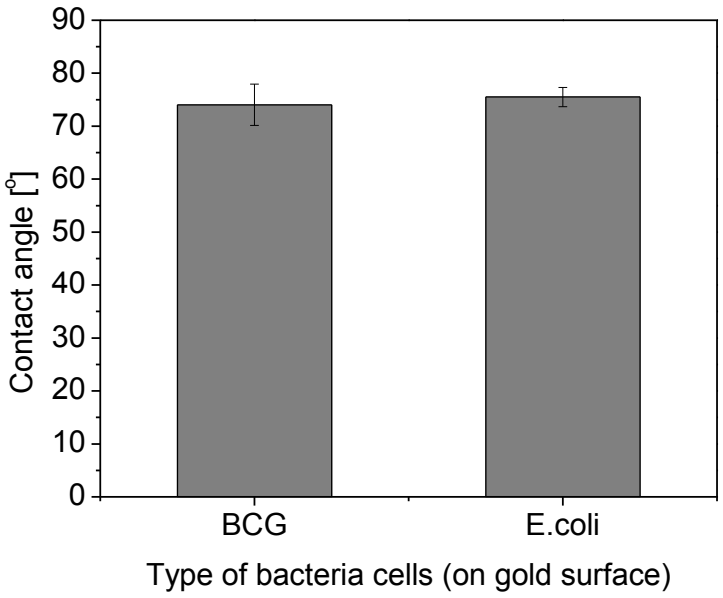


Fig. S1. Contact angle measurements for BCG and *E.coli* cells on bare gold surfaces (n=3).

To evaluate if the type of bacteria cells in the suspension affects the contact angle of a liquid drop, rectangular gold-coated Si substrates (2.5x5 mm^2) were utilized. On a Si wafer, a 1µm-thick oxide layer was thermally grown. A 20 nm-thick gold layer was then evaporated onto the oxide layer by electron-beam evaporation. To visualize binding of bacterial cells on the bare gold surface, BCG and *E. coli* cells at 10^7 cfu/mL in 1x phosphate buffered saline (PBS) buffer were stained with an intercalating dye (SYTO 9® green fluorescent nucleic acid stain; molecular probes L7007, Invitrogen, Carlsbad, CA). To eliminate unbound staining dyes, the solution was centrifuged to collect the pellet of stained cells and the supernatent was discarded. The collected pellets were then resuspended in PBS. Stained BCG and *E. coli* cells were used for the contact angle measurement on gold surface.

A 0.5µL-droplet of stained BCG or *E. coli* cells was deposited on the gold coated substrate. When the droplet was stabilized, the contact angle at the initial state was measured by using a goniometer (Rame-Hart, model 500 Adv G/T). Each case was repeated three times. As shown in Fig. S1, the type of bacteria cells did not affect contact angle measurement on gold surface without antibodies.

2. ELISA measurement results

The specificity of anti-BCG and anti-E. coli antibodies was evaluated by using an ELISA kit. The binding activities of anti-BCG IgY and anti-*E. coli* IgG were evaluated by ELISA. Equal concentrations of two bacterial strains (BCG and E. coli, approximately 10^7 cells in 100 µL PBS) were assayed for binding to anti-BCG IgY and anti-*E. coli* IgG antibody using a 0.45 micron filter plate (Millipore Billerica, MA, #MAHVN4510). Aliquots of the bacterial suspensions were added to the 96-well filter bottom plate and washed with PBS. Subsequently, a 100 µl aliquot of 5 µg/ml IgY-BCG or IgG-E. coli antibody in PBS was added to the washed cells and incubated for 1 hour at 37°C. After the PBS wash, a secondary antibody was added (rabbit anti-IgY-HRP conjugate for IgY-BCG, rabbit anti-IgG-HRP conjugate for IgG-*E. coli*, Thermo Scientific) and incubated for 1 hour at 37°C. The sample was then washed with PBS, followed by addition of 100 µL ABTS substrate (Thermo Scientific #37615). The mixture was then incubated for 10 minutes, and filtered into a 96-well receiver plate. Absorbance was recorded at 405 nm using a UV spectrophotometer (Nanodrop ND-1000, Thermo Scientific).

In the experimental results, both antibodies bound more avidly to the corresponding target bacteria cells as shown in Fig. S2.

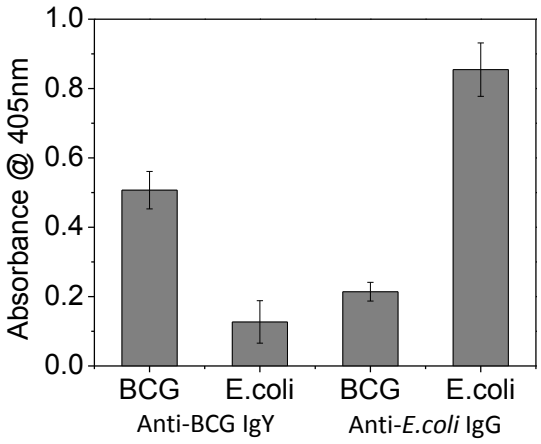
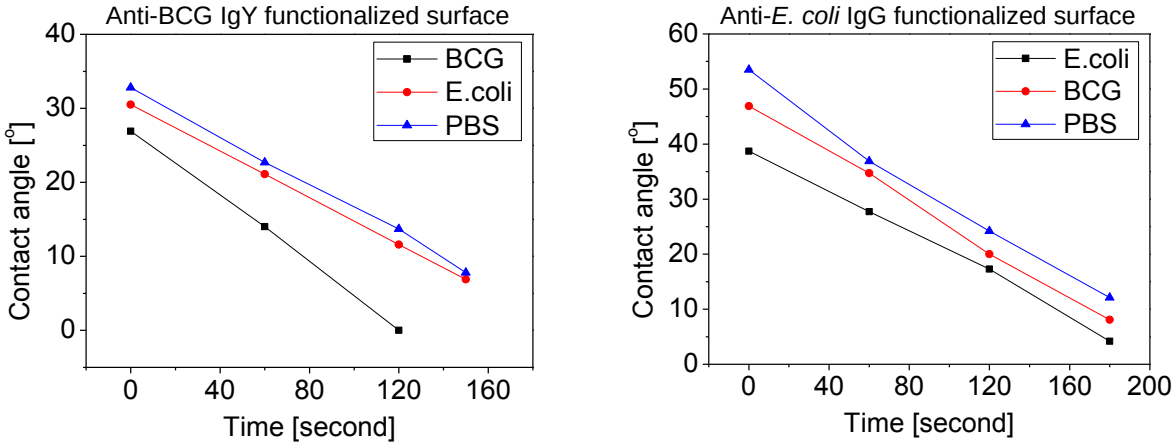


Fig. S2. ELISA measurement results. The background absorbance was substracted from each value (n=3).

3. Contact angle measurement during the evaporation

Three kinds of liquid samples of BCG in PBS, *E. coli* in PBS, and PBS buffer without cells were used for the contact angle measurement. The substrate was coated with anti-BCG IgY and anti-*E. coli* IgG. A 0.5μL-droplet of BCG, *E. coli* cells, or PBS was deposited on the antibody functionalized substrate. The contact angle at the initial state and during evaporation were measured by using a goniometer (Rame-Hart, model 500 Adv G/T). The ambient temperature and humidity during the measurement were 24.2 ± 0.49 °C and 23.2 ± 1.09%, respectively.

Fig. S3 shows the contact angle measurement during the evaporation. The contact angle was reduced as the liquid drops evaporated. The contact angle during the evaporation was the highest for the specific binding case for the contact angle measured at the initial state (time=0 second)



(a) (b)

Fig. S3 Contact angle measurement during the evaporation for both BCG and *E. coli* cells on a surface functionalized with (a) anti-BCG IgY and (b) anti-*E. coli* IgG.

4. Analytical model

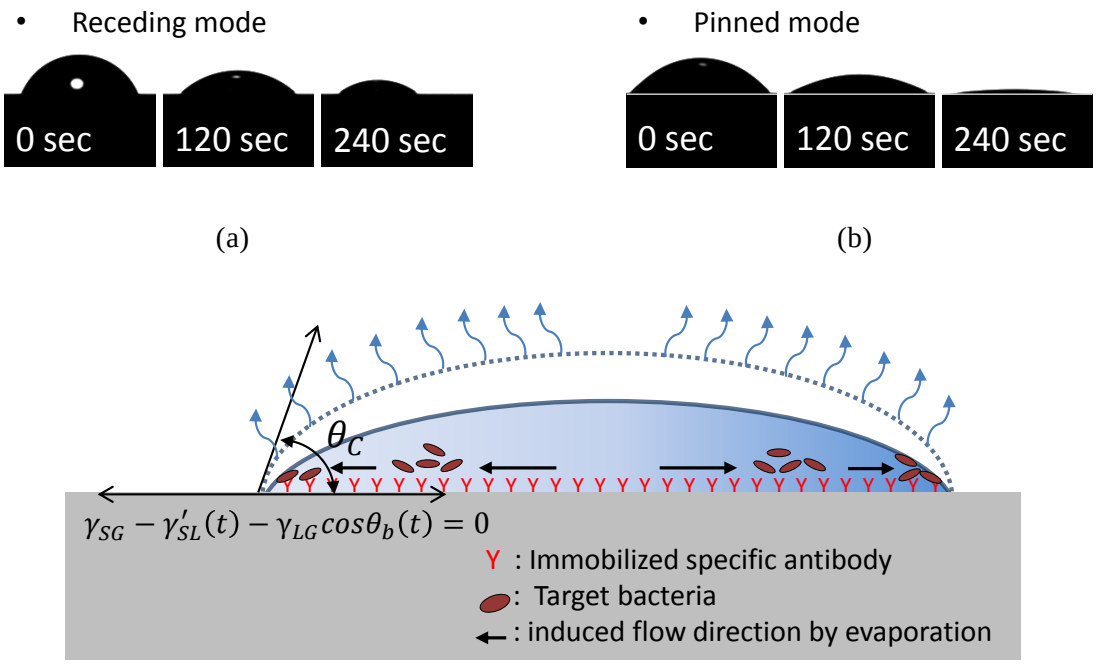


Fig. S4 (a) Side view of an evaporating droplet over time. Receding mode on the bare gold surface, the diameter of the wetting area decreases; (b) Pinned mode with antibody-coated surface: the contact angle decreases while the wetting area remains unchanged; (c) Young-Laplace equation combined with binding forces at steady state and during evaporation in pinned mode.

When a liquid drop containing bacterial cells is deposited on the substrate functionalized with antibodies, the contact angle of this liquid drop can vary due to the binding activity. Without bacteria, the contact angle of the liquid drop on the surface immobilized with antibodies is governed by the Young-Laplace equation:

$$\gamma_{SG} - \gamma_{SL} - \gamma_{LG} \cos\theta_0 = 0, \quad \text{(s1)}$$

where γ_{SG} is the solid–vapor interfacial energy, γ_{SL} is the solid–liquid interfacial energy, and γ_{LG} is the liquid–vapor interfacial energy.

When bacterial cells are present in the liquid drop, the binding forces at the steady state (f_b) and during the evaporation ($f'(t)$) are added to the Young-Laplace equation as illustrated in Fig. S4 c. The total solid-liquid interfacial energy ($\gamma'_{SL}(t)$) modified by the binding affinity is:

$$\gamma'_{SL}(t) = \gamma_{SL} - f_b - f'(t) \tag{s2}$$

Due to the modified γ_{SL}, the contact angle (θ_0) without bacteria is changed into θ_b with the presence of bacteria:

$$\gamma_{SG} - \gamma'_{SL}(t) - \gamma_{LG}\cos\theta_b(t) = 0 \tag{s3}$$

By combining equations (s2) and (s3), the contact angle (θ_b) is given by:

$$\begin{aligned}\cos\theta_b(t) &= (\gamma_{SG} - \gamma_{SL} + f_b)/\gamma_{LG} + f'(t)/\gamma_{LG} \\ &= (\gamma_{SG} - \gamma_{SL})/\gamma_{LG} + (f_b + f'(t))/\gamma_{LG} \\ &= \cos\theta_0 + f_b/\gamma_{LG} + f'(t)/\gamma_{LG}\end{aligned} \tag{s4}$$

In equation (s4), the contact angle (θ_b) of the solution is modified by the binding forces between cells and antibodies. In particular, $\cos\theta_b(t)$ consists of 3 parts: the first term captures the initial contact angle of the pure liquid on the functionalized surface, the second term is related to the binding force contribution, and the third term is related to the evaporation effect. The right hand side increases with specific binding force contribution, hence yields the smaller contact angle for the specific binding cases.

The equation (s4) can be modified to:

$$f_b = \gamma_{LG}[cos\theta_b(t) - cos\theta_0] - f'(t) \quad \text{(s5)}$$

where $f'(t)$=0 at t=0. Therefore, f_b can be estimated by equation (s5) with the measurement of $\theta_b(0)$ and θ_0 at t=0. The unit of f_b is force per unit length.

5. Transport of bacteria cells to the edge of a liquid drop on the antibody-coated surface

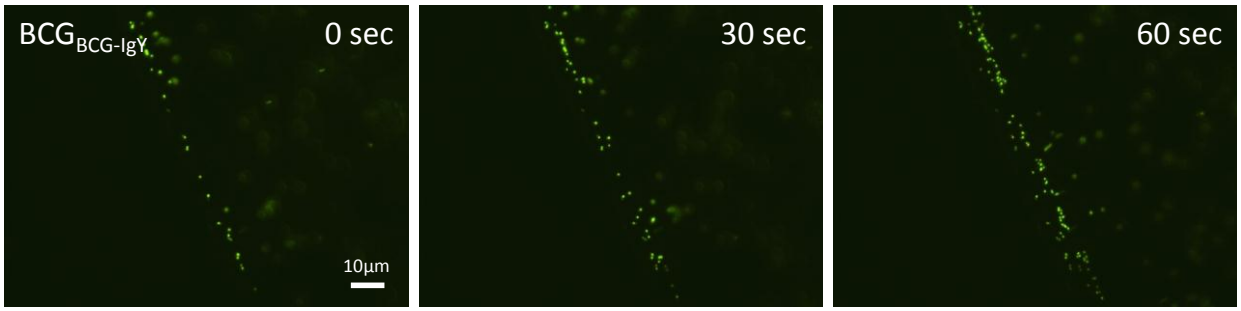


Fig. S5 Still images captured every 30 seconds. The binding force for specific binding of BCG cells on BCG-IgY antibodies held the contact line of the liquid drop, which transported target bacteria to the edge of the droplet with evaporation of the liquid drop.

To observe the behavior of bacteria cells on the antibody-coated surface, BCG cells at 10^7 cfu/mL in 1xPBS buffer were stained with an intercalating dye (SYTO 9® green fluorescent nucleic acid stain; Molecular Probes L7007, Invitrogen, Carlsbad, CA). The solution was then centrifuged to eliminate unbound staining dye. The collected pellets were re-suspended in PBS. The substrate was immobilized with anti-BCG IgY. A 0.5μL-droplet of stained BCG cells was placed on the functionalized substrates. During the evaporation, the motion of bacterial cells at the edge of the droplet was recorded under an epi-fluorescence microscope (Nikon Eclipse 55i). As evaporation continues, BCG cells are transported to the contact line due to the outward capillary flow as shown in Fig. S1.