Supporting Information

Study on the Attachment of *Escherichia coli* to Sediment Particles at a Single-Cell Level: the Effect of Particle Size

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1. *E. coli* Concentration Validation through Estimation of *E. coli* Flux in Microfluidic Devices

In the flow cells we choose observation fields at different positions, and the dimensions of each observation field were 67.5 μm × 67.5 μm × 20~30 μm. Videos of the observation fields were recorded using the microscope and camera, and then the number of *E. coli* passed through was counted and the flux at the positions was calculated according to Equation (1).

\[
\Phi_p(t) = \frac{N_{\text{count}}(t)}{t_{bf} h_{bf}}
\]  

(1)

where \(N_{\text{count}}\) = the total number of bacteria passing through the boundary for time duration \(t\); \(l_{bf}\) = the width of the observation field, μm; \(h_{bf}\) = the thickness of the counting area, μm.

Due to the limited depth of focus of the microscope, z-scan was performed in a loop manner during the video recording. In order to improve the statistics of results, a rectangular block region was chosen to record the bacterial passage through a big volume. Thus the height of the rectangular block should be chosen to be as large as possible to include more volume under the constraint that there is no cell missed during the bottom-to-top scan period. In other words, the bottom-to-top scan should be quick enough so that during one bottom-to-top scan, the moving distance of a cell would not exceed the length of the rectangular block region along the flow direction. In the experiments, each recording lasted for 15 minutes. The length and width of the rectangular block for observation are both 67.5 μm, and the height is 20 μm to 30 μm. The step size of z-scan was set to 0.9 μm at minimum. It took 7.5 s to finish one bottom-to-top scan for a block with 30 μm height and 4.7 s for a block with 20 μm height. Under this set-up, no cell would be missed during a z-scan, and the number of *E. coli* can be counted accurately.

*E. coli* fluxes at different positions (Figure S1a) were measured as described above. Among the selected positions, \(L_a\), \(L_b\) and \(L_c\) have the same x and y coordinates but different z coordinates (\(z_a\): 0~20 μm; \(z_b\): 20~50 μm and \(z_c\): 100~130 μm). \(L_d\), \(L_e\) and \(L_f\) have the same x and z coordinates but different y coordinates (\(y_a\): 0 μm; \(y_b\): 1000 μm; \(y_c\): 2000 μm). \(L_e\) is near the side wall of the channel.

\(L_a\) and \(L_b\) are typical locations where particles are picked for attachment measurements. All other positions are only for the purpose of uniformity assessment of bacterial flux.

The obtained curves exhibited fluctuation at early stage due to small sample numbers but approached to a constant at long times given enough statistical events. Figure S1b showed that all curves approached to a constant starting at about 900 s. Then the cumulative average value at 900 s was used as the average *E. coli* flux \(\Phi_p\) at the corresponding position.
The measured *E. coli* fluxes showed variations at different positions. At *L* that is close to the side wall, the *E. coli* flux is the highest. This result may be induced by ‘shear-trapping’. Some motile cells like *E. coli* tend to be depleted in low shear region and accumulate in high shear region, owing to the alignment of swimming direction with fluid streamlines caused by shear forces [1].

![Diagram](image)

**Figure S1.** *E. coli* flux measurement and results. (a) Picked positions for measuring *E. coli* flux in the cross-section of the channel. (b) The cumulative average of *E. coli* flux at selected positions.

The average *E. coli* flux of the entire flow-cell $\Phi$ could be calculated as the mean value of bacterial fluxes at selected locations, $\Phi_a$, $\Phi_b$, $\Phi_c$, $\Phi_d$ and $\Phi_e$. Then the average bacterial concentration $C_w$ (at OD = 0.01) can be obtained from Equation (2):

$$\overline{\Phi} = \frac{QC_w}{S_{channel}}$$  \hspace{1cm} (2)

where $Q$ = the flow rate, mL/s; $S_{channel}$ = the cross-section area of the channel, $\mu$m$^2$. The calculated $C_w$ was $5.54 \times 10^9$ total cell count/L. On the other hand, the concentration of the *E. coli* suspension was also measured using the membrane filtration method. And the measured result was $5.4 \times 10^9$ cfu/L. The concentrations measured by two different methods agreed. So *E. coli* die-off in our microfluidic experiments was negligible, which meant that total cell count in the micro level can be considered numerically equivalent to colony forming units in the macro level.

2. Surface Morphology of Particles
Figure S2. SEM micrographs of glass microspheres. (a) 10 μm microspheres at low magnification (× 10k); (b) 10 μm microspheres at high magnification (× 30k); (c) 20 μm microspheres at low magnification (× 10k); (d) 20 μm microspheres at high magnification (× 30k); (e) 50 μm microspheres at low magnification (× 3.6k); (f) 50 μm microspheres at high magnification (× 20k).
Figure S3. SEM micrographs of sediment particles. (a) 10 μm sediment particles at low magnification (× 10k); (b) 10 μm sediment particles at high magnification (× 30k); (c) 20 μm sediment particles at low magnification (× 3k); (d) 20 μm sediment particles at high magnification (× 10k); (e) 50 μm sediment particles at low magnification (× 3k); (f) 50 μm sediment particles at high magnification (× 10k).

References