Gradient Microfluidics Enables Rapid Bacterial Growth Inhibition Testing

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Supplementary Information:

Figure S1: Timelapse fluorescence images of fluorescein solution for a period of 6 h;
Figure S2: Calibration curve of fluorescein concentrations vs. fluorescence intensity;
Figure S3: Growth curves for on-chip and 96-well plate culture;
Figure S4: Growth curves for *E.coli* at different amoxicillin concentrations in 96-well plates;
Figure S5: Optical and SEM images showing morphology variations in E. coli exposed to different amoxicillin concentrations;
Figure S6: Time-lapse images of Nitrosomonas europaea growth on-chip for 2 days.

Protocols for bacteria culture, image analysis and SEM preparation;
Figure S7: Illustrative images from the image processing routine.

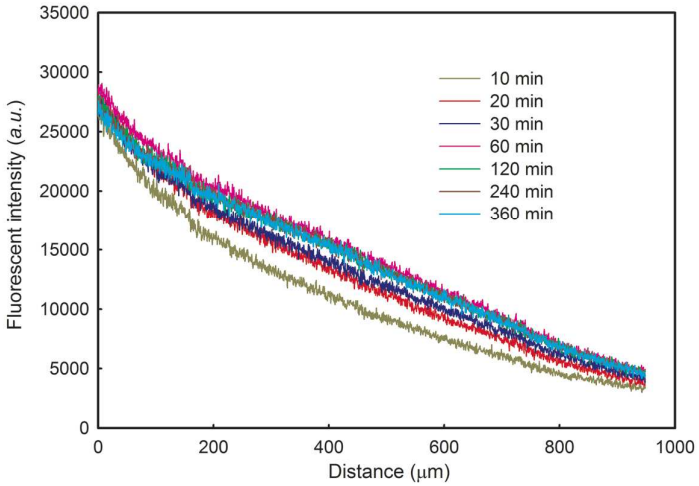

Figure S1. Timelapse fluorescence images of fluorescein solution for a period of 6 h.

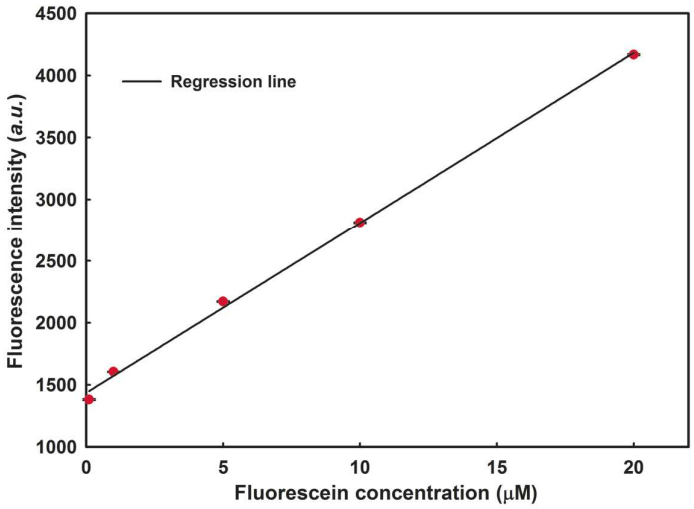

Figure S2. The calibration curve of fluorescein concentrations against its fluorescence intensity (n=4).

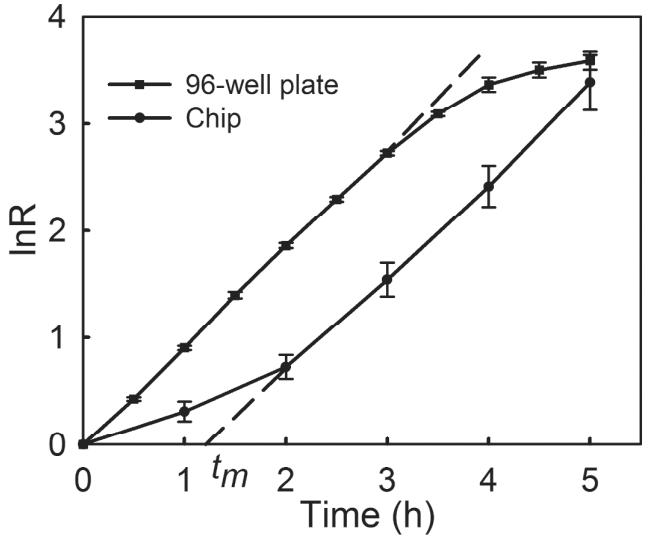

Figure S3. The growth curves for on-chip culture (n=11 randomly selected colonies under the sink channel) and for in suspension culture in a 96-well plate (n=3). The dashed lines represent exponential growth curves obtained by fitting to the experimental data. The x-intercept of the dashed line gives the lag time (t_m). The error bars represent standard deviations.

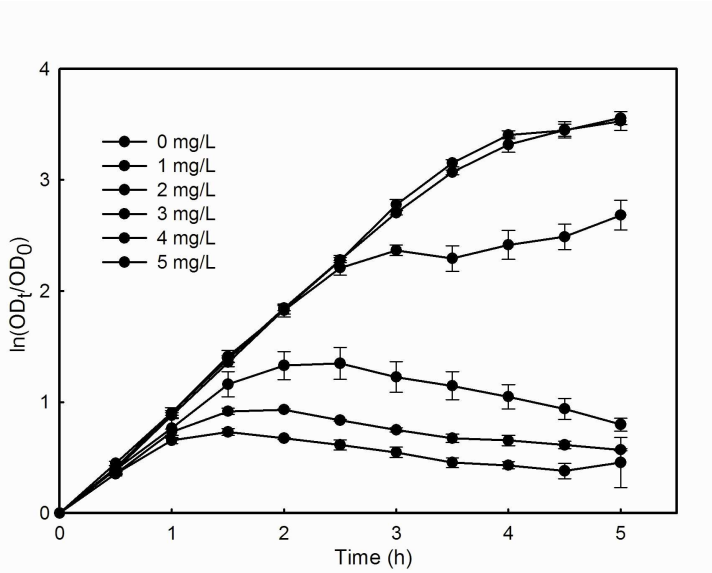

Figure S4. The growth curves of *E.coli* under different concentrations of amoxicillin in 96-well plates (n =3).

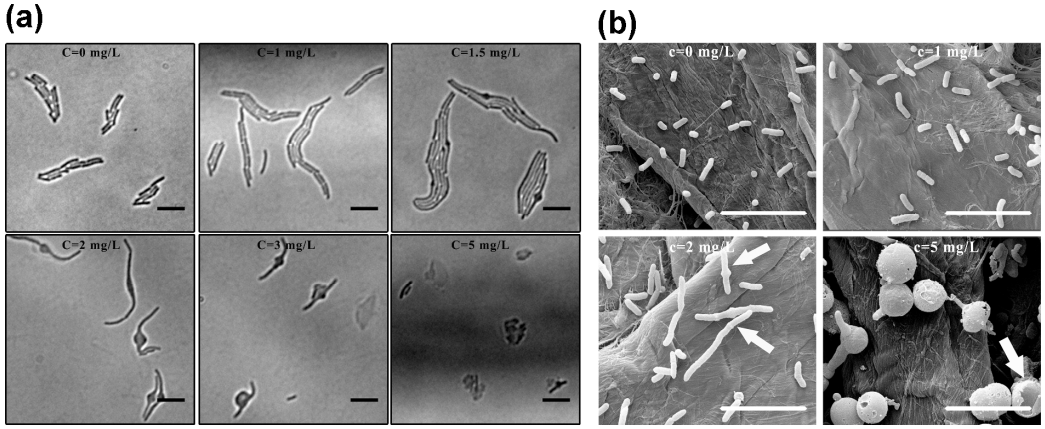

Figure S5. Morphology variations in *E. coli* exposed to different amoxicillin concentration at time 4 h: (a) Optical images of a monolayer of bacteria on chip at time 4 h; (b) SEM images of bacteria in well plates under the indicated amoxicillin concentrations. Scale bar 10 μm. The morphological variations of *E.coli* were further confirmed with high-resolution SEM images of cells exposed to 0, 1, 2 and 5 mg/L of amoxicillin solutions at time 3 h. Cells treated with 1 or 2 mg/L of amoxicillin solutions were significantly longer that those in the control (c_{AM} = 0 mg/L). At 5.0 mg/L, cells shrunk into small balls with damaged cell wall, which appeared as faint residue in the optical images.

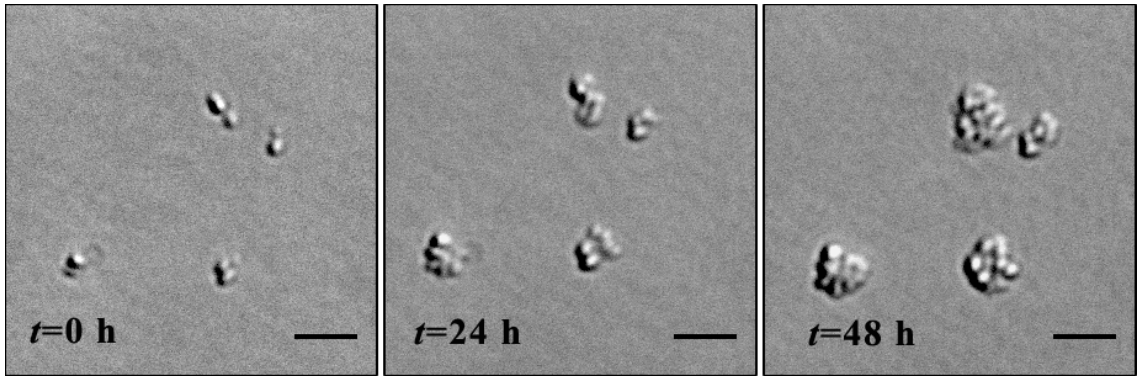

Figure S6. Time-lapse images of Nitrosomonas europaea growth on-chip for 2 days. Scale bar 5 μm.

PROTOCOLS

Culture of the Bacterial Cells and Chemicals. *Escherichia coli* (ATCC 25922) were cultured in Luria-Bertani (LB) broth on a shaker (150 rpm, 37^{o}C). When the optical density of a bacteria solution at 600 nm (OD_{600}) reached 0.6 ~ 0.8, indicating the exponential growth stage, bacteria were harvested by centrifugation (5000 rpm, 1 min), washed three times with phosphate buffer (PBS) and re-suspended in fresh LB broth. The bacterial suspension was diluted to reach an OD_{600} value of 0.05 ~ 0.08 for inhibition tests using the microfluidic device. Amoxicillin (MW = 419.46 Da, Sigma-Aldrich) was prepared as a 2.0 g/L stock solution in RO water and then diluted with LB broth to the required concentration.

Nitrosomonas europaea (ATCC25978) grows very slowly and its enrichment is difficult. The growth medium for N. europaea contains 25 mM $(NH_4)_2SO_4$, 43 mM KH_2PO_4, 3.92 mM NaH_2PO_4, 3.74 mM Na_2CO_3, 0.75 mM $MgSO_4$, 0.18 mM $CaCl_2$, 0.01 mM $FeSO_4$, 0.017 mM EDTA, and 0.5 μM $CuSO_4$. The medium was adjusted to pH 8.0 with NaOH. N. europaea was cultured on a shaker (150 rpm, 37 ^{o}C) for almost one month before its OD_{600} reached 0.2 ~ 0.3. Then the bacteria suspension was diluted to reach an OD_{600} value of ~0.08 for inhibition tests. For inhibition tests, amoxicillin was prepared as a 15 mg/L solution in the medium.

Scanning Electron Microscope (SEM) Imaging. SEM (FEI Quanta 200) imaging was used to obtain high-resolution images of *E. coli* cultured under a range of inhibitory concentrations in 96 well plates for 3 h. Cells were collected, fixed with 2.5% glutaraldehyde overnight at 4°C, and then washed with 0.1 M PBS prior to dehydrating stepwise with increasingly concentrated ethanol/water solutions (30, 50, 70, 85, 95 and 100%, for 15 min each). Finally, samples were critical point dried (BAL-TEC CPD030) and sputter coated with gold (BAL-TEC SCD005).

Image analysis. All the images were processed with Image J. For the cell number/cell mass calculations, first the inherent variations in the bright field illumination intensity due to the microscope optics were corrected by normalising using 'white light' images collected in the absence of the device and the dark counts on the CCD camera. Then the contrast of original images (e.g. Figure S7a) was enhanced by using the auto brightness and contrast adjusting function in Image J, Figure S7b. A level of background intensity (or threshold) was subtracted from the images (Figure S7c) so as to preserve both the individual and colony features. These were then converted into binary (black and white, as opposed to grey scale) formatted images (Fig. S7d). Finally, the areas of each colony were calculated using the analyzing particles function in Image J and the circularity parameter of 0-1 and particle size from 0-infinity, with the answers being given in terms of the number of pixels.

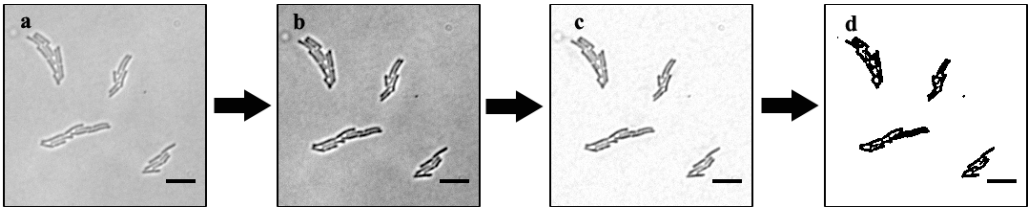

Figure S7. Image processing. (a) original image; (b) image after enhancing contrast; (c) image after substracting backgrond; (d) binary image.