Salt-induced Self-assembly of Bacteria on Nanowire Arrays

Kelsey K. Sakimoto,[†] *Chong Liu,*[†,‡] *Jongwoo Lim,*[†] *Peidong Yang*[†,‡,§,*]

[†] Department of Chemistry, University of California, Berkeley, CA 94720, USA
[‡] Materials Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA
[§] Kavli Energy Nanosciences Institute, Berkeley, CA 94720, USA

Supporting Information

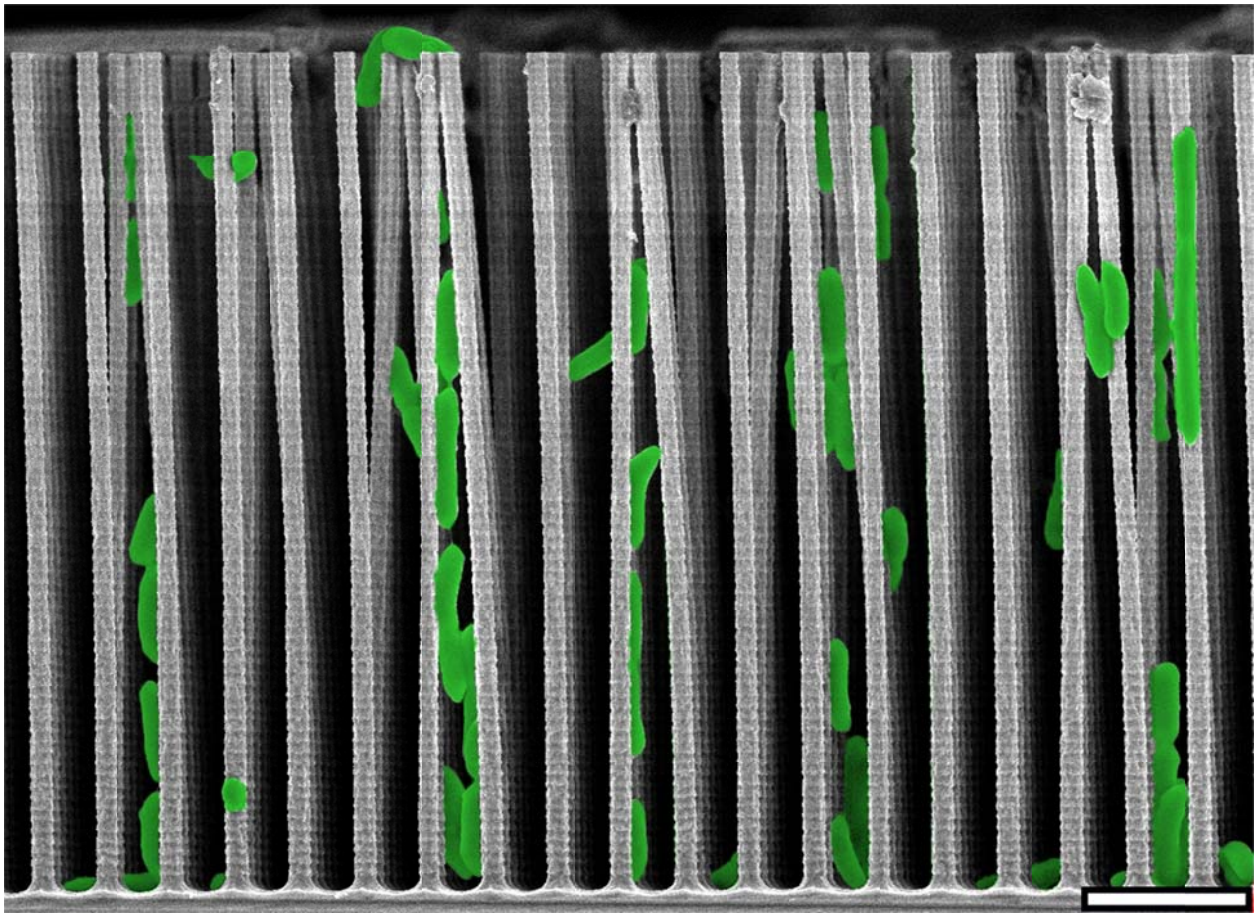

Figure S1. Scanning Electron Micrograph of Aligned Filamentous *S. ovata* on Silicon Nanowire Arrays. When grown under conditions of increased NaCl concentration and H_2:CO_2 pressure, *S. ovata* cells form multicellular filamentous cells. Such cells preferentially attach parallel to the

nanowire in their filamentous form, followed by eventual division into single cells. Scale bar 5 μm.

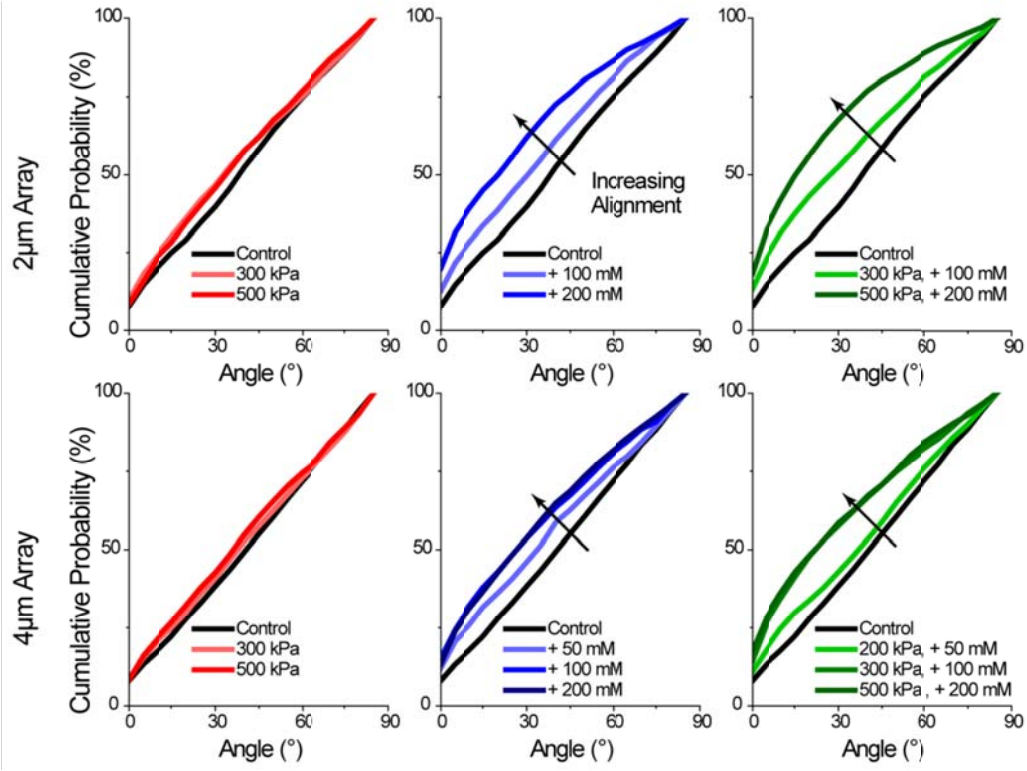

Figure S2. Cumulative Distribution Functions of Bacteria-Nanowire Angle. Bacteria-nanowire angles for various growth conditions (increasing NaCl concentration or H_2:CO_2 pressure) were measured by SEM for 2μm and 4μm periodicity arrays. For both periodicities, little change is observed for increasing pressure, while conditions of increasing NaCl concentration show gradually greater alignment.

Materials and Methods

Si Nanowire Array Fabrication[1]

Si p-type <100> oriented 4-6" wafers were patterned with a photoresist square dot array with a periodicity of 2 or 4 μm using a standard photolithography stepper. Nanowires were formed by inductive-coupled plasma deep reactive-ion etching (Surface Technology Systems, Inc.), followed by O_2 plasma to remove residual photoresist. Nanowire diameters were controlled by iterative cycles of dry thermal oxide growth (1050 °C, 40 min) and 10:1 buffered HF. Periodicity 2 μm arrays required critical point drying (Tousimis, Inc.) to prevent nanowire bundling. A final oxide outer coating was formed by atomic layer deposition of 30 nm conformal TiO_2 (300 °C,

$TiCl_4/H_2O$ precursors), or dry thermal oxidation (850 °C, 12 hours, ~50 nm SiO_2). Final dimensions of Si nanowire arrays were approximately 800 nm in diameter and 25-30 μm in length.

Bacterial Growth on Nanowire Arrays

Cryopreserved (10% DMSO) *Sporomusa ovata* (ATCC 35899) was inoculated 0.4% into aluminum crimped Balch tubes containing pre-reduced media (DSMZ 311) omitting betaine, casitone, and resazurin prepared under standard anaerobic technique (defined as the normal, control growth conditions). Up to 200 mM additional NaCl was added prior to autoclaving.

Nanowire arrays were cut into 0.5 cm x 0.5 cm chips and cleaned with O_2 plasma prior to sealing within sterile Balch tubes flushed with anoxic H_2:CO_2 (80:20). To each 20 mL Balch tube (23 mm x 75 mm, Chemglass Life Sciences) was added 2.5 mL of inoculated media, giving approximately 1mm of media above the surface of the nanowire array. The headspace of each tube was pressurized with anoxic H_2:CO_2 (80:20) up to 500 kPa (100 kPa defined as the normal growth conditions). As H_2:CO_2 was consumed, the culture tubes were repressurized every 24 hours, or connected via gas pressure regulator to a gas cylinder to maintain constant pressure. Inoculated tubes were incubated at 30 °C for 6 days with minimal agitation.

Bacteria Growth Kinetics and Cell Length

Planktonic bacteria were grown in suspension as per the protocol above. To test for substrate dependence, culture tubes were optionally supplemented with 50 mM betaine or sodium lactate, flushed with anoxic N_2 and pressurized up to 400 kPa. For the first 3 days of growth, cultures

were sampled every 6 hours. OD_{600} measurements (SpectroVis, Vernier) were used to determine growth rate under varying concentrations of NaCl and H_2:CO_2 pressures based on standard Monod kinetics. Optical measurements were confirmed with cell counting in a Petroff-Hausser counting chamber. Cultures were then processed for scanning electron microscopy (SEM) as detailed below.

Fluorescence Microscopy

After bacterial growth on nanowire arrays, the chips were removed from media and gently washed with isotonic phosphate buffered saline (PBS) to remove planktonic cells. Bacteria were stained with a fluorescent dye (BacLight Green, Invitrogen) in PBS as per the manufacturer suggested protocol. Stained bacteria-nanowire chips were imaged with an upright microscope (Eclipse LV100D-U, Nikon) outfitted with filter sets appropriate for FITC imaging (B-2E/C FITC, Nikon). Images were captured by a microscope mounted camera (Moticam Pro, Motic) at 20X, 50X magnification. All steps were conducted in an anaerobic chamber (Coy Labs).

Scanning Electron Microscopy

Bacteria on nanowire arrays were prepared for SEM by overnight fixation with 2.5% glutaraldehyde added directly to the growth media. Chips were washed with isotonic deionized water followed by dehydration in increasing concentrations of ethanol (25%, 50%, 75%, 90%, 100%, 10 min each), and then transferred into hexamethyldisilazane (HMDS) (Aldrich) for 1 hour.[2] Chips were air dried overnight. Prior to imaging, chips were cleaved along the middle and sputtered with ~3 nm of Au (Denton Vacuum, LLC). Bacteria on nanowire arrays were imaged at 5 keV/12 μA by field emission SEM (JEOL FSM6430).

Planktonic bacteria collected from the bulk solution for cell length measurement were fixed as above. Prior to dehydration, fixed cell suspensions were vacuum filtered onto 0.1 μm polycarbonate membranes (Isopore Membrane Filter, EMD Millipore) sputtered with ~10 nm of Au, followed by dehydration and HMDS treatment, and another 3 nm of sputtered Au. SEM images were adjusted for brightness, contrast, and false colored in Adobe Photoshop.

Cell Length and Bacteria-Nanowire Angle Measurement

All images were manually processed in ImageJ for cell length and angle measurements. Cell length histograms were generated for varying conditions. To determine the statistical significance of changes in each cell length distribution histogram, a cumulative distribution curve was constructed and the two-sample modified Kolmogorov-Smirnov test statistic and accompanying significance level, α, for each growth condition relative to normal conditions was calculated.[3] Histograms of same growth conditions with different nanowire array periodicities, same NaCl concentration with different pressures, and similar pressures with different NaCl concentrations were also evaluated for statistically significant differences.

Derjaguin-Landau-Verwey-Overbeek Modelling

To model the colloidal interaction between bacteria and nanowire, free energy curves were generated based on Derjaguin-Landau-Verwey-Overbeek (DLVO) theory, considering the total interaction energy as the sum of a van der Waals attractive potential and an electrostatic double layer repulsive potential. For this model, though the surface properties of *S. ovata* are unknown, parameters typical of bacteria cells were selected to illustrate general trends in bacteria-nanowire

interactions. The zeta potential for bacteria and metal oxide surfaces were chosen as -20 mV and -40 mV, respectively. The Hamaker constant was selected as 6.5×10^{-21} J. The minimum separation distance (where the primary minimum was defined) was selected at 0.158 nm, corresponding to the typical van der Waals radius of bacteria outer membrane surface.[4–6]

For the unaligned attachment scheme (bacteria perpendicular to nanowire), bacteria and nanowires were treated as spheres of radius 250 nm. To provide a first order approximation of the aligned attachment scheme (bacteria parallel to nanowire), an aspect ratio of 4:1 was assumed, resulting in interaction energy curves 4 times that of the perpendicular attachment scheme, scaling the interaction energy with surface area. Curves were generated for an ionic concentration of 100 mM and 300 mM. Kinetic activation energy barrier for attachment and detachment were calculated by the difference between the primary maximum and the secondary minimum, and the primary maximum and primary minimum, respectively.

References

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