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Supplementary Information for

Detection of bacterial cells by impedance spectra via fluidic electrodes in microfluidic device

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This supplement contains:
Materials and Methods
Figure S1

Materials and methods

Chemicals

Peptone and ampicillin were purchased from Sigma Aldrich, USA. Tryptone and Yeast extract were from Oxoid, UK. PDMS monomer and curing agents Sylgard 184 were purchased from Dow Corning; SU-8 2025 photoresist and developer from MicroChem Corp., USA; other chemicals were obtained from the Department of Chemistry, Peking University, China; Deionized water was produced with a Milli-Q SP system. The conductivity of KCl (3 M) solution is 281 $mS \cdot cm^{-1}$ and the conductivity of DI water is 3 $\mu S \cdot cm^{-1}$, measured by a conductivity meter.

Microchip fabrication

The microfluidic chips were fabricated by standard soft-lithography. The mask was printed on a plastic film with a resolution of 3600 dpi. The width of the inlet channels is 150µm, and that for the confluent channel is 100µm, and the widths of the outlet channels are 50 and 150µm for bacterial suspension and KCl solution, respectively. The channel structure on silicon wafer was fabricated by SU-8 2025 photoresist with a thickness of 30 µm. The PDMS (10:1) was poured onto the silicon curing at 80℃ for 25 min. After the cured PDMS was peeled off from the silicon master mold, holes were punched with Harris Uni-cores for the inlets, outlets and electrode connections. Finally, the PDMS channels were bonded to a glass slide by using oxygen plasma and the Ag wires were punched into the chip. The chip sterilization was done by injecting 75% ethanol and exposing to UV light for 1 h before use.

Bacterial culture and media

Porphyromonas gingivalis ATCC33277, provided by Beijing Stomatological Research Center, was cultured on chocolate agar (5% sheep blood with 5mg·mL hemin and 1.0$mg \cdot L^{-1}$ vitamin K1) incubated at 37℃ in an anaerobic chamber (Anoxomat™ MarkⅡ, Mart® Microbiology, Netherlands) with an atmosphere of 80%N_2-10%CO_2-10%H_2. Culture purity was routinely checked by Gram staining and by colony morphology. Four-day-old *P. gingivalis* ATCC33277 cells were used to inoculate in Trypticase soy broth supplemented with yeast extract (1$mg \cdot mL^{-1}$), hemin(5$\mu g \cdot mL^{-1}$) and cysteine(0.5$\mu g \cdot mL^{-1}$) were cultured anaerobically to late log phase at 37℃. *Escherichia coli* RP437 was cultured in a shaking incubator (30 ℃, 200 rpm, 12 h). Then the fresh culture media was added with an volume ratio of 1:20 (30 ℃, 200 rpm, 2 h). The bacterial cells were resuspended in centrifugal tubes with culture media and counted on an ordinary counting chamber. A series of dilutions were then created with concentrations ranging from 10^3 to 10^9 $cells \cdot mL^{-1}$. After that, these bacterial cells were centrifuged at 8000 × g for 3 mins and the supernatant was carefully aspirated. The mannitol was added to wash the sample followed by centrifuging and removing of the supernatant for two times to remove the solution ion. DI water was then added to the bacteria suspensions and laid at room temperature for 30 mins before tests.

Devices and electrical impedance spectroscopy (EIS)

Figure S1 illustrates the experimental setup for hydrodynamical manipulation and electrical interrogation of the bacteria suspension in DI water. The two Ag wires punched to the inlets of the KCl solution channels were connected with an LCR meter (Agilent 4980A, Agilent Technologies Inc.), to measure the impedance of the focused central layer with corresponding bacterial concentration. The measurement was an automatic process controlled by an acquisition program through an USB interface provided by Agilent. Impedance spectra were recorded in a sweep frequency range from 100 Hz to 1 MHz, with amplitude of 500 mV and 33 sampling data uniformly distributed on the logarithmic coordinates. The impedances at a fixed frequency of 1.2 kHz were also measured for detecting the bacterial concentrations with the same amplitude. All tests were performed at room temperature.

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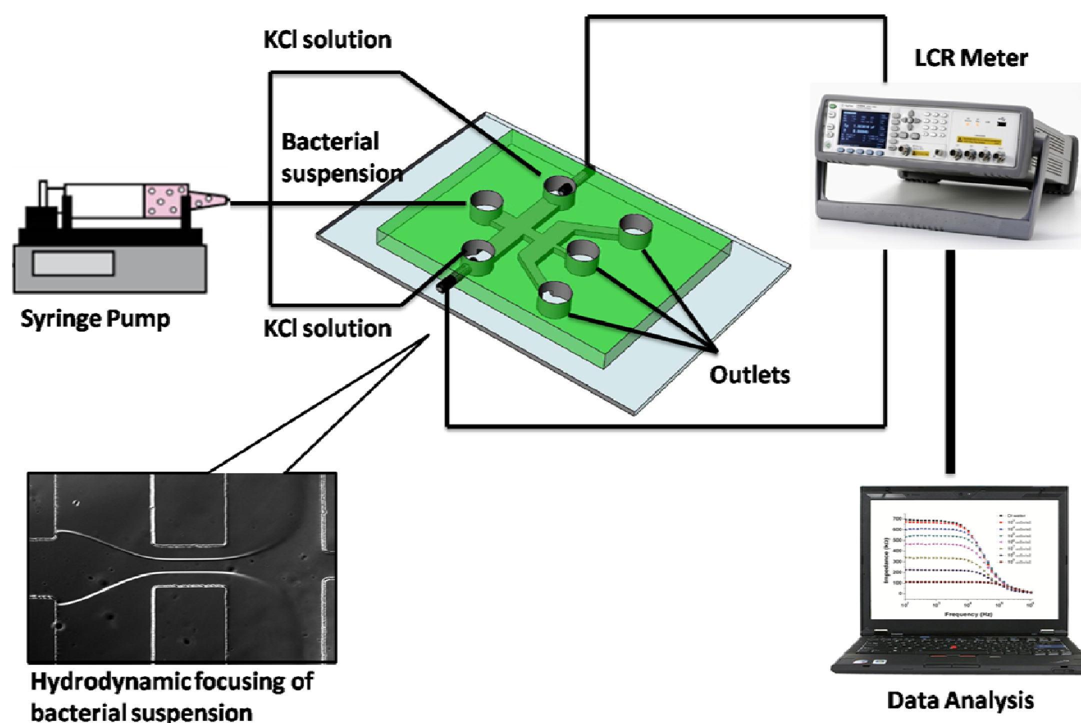

Figure S1. Experimental setup: a syringe pump controls the feeding speeds of the sample supply and the sheath flows to form hydrodynamic focusing; an impedance analyzer is connected with fluidic electrodes of KCl solution to record impedance spectra of the suspensions with different cell concentrations.