

Construction of artificially structured microbial consortia (ASMC) using dielectrophoresis: Examining bacterial interactions via metabolic intermediates within environmental biofilms

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Abstract

The construction of artificial biofilms with defined internal architectures is described. Bacterial cells are suspended in a low conductivity medium, guided to specific areas in a microelectrode array by dielectrophoresis (DEP), and then immobilised using the flocculating agent poly(ethylenimine). Multispecies biofilms can be constructed by introducing different species at different times. The rapid construction of such biofilms with defined internal architectures provides, when combined with visual reporters of gene activity, a powerful new method for the investigation of the effects of the spatial organisation on interactions between bacterial species in biofilms. To demonstrate the utility of the technique as a method for investigating metabolic interactions in biofilms, aggregates were constructed from *Acinetobacter* sp. C6 and *Pseudomonas putida*::gfp. The *Acinetobacter* degrades benzyl alcohol, overproducing benzoate, which in turn is consumed by the *Pseudomonas* strain. The *P. putida* has a chromosomally expressed cassette encoding a gfp downstream of the promoter which controls degradation of benzoate, making the interaction between the two strains in the metabolism of benzyl alcohol visible by the production of green fluorescent protein (GFP). Microscopic observation of the biofilms, including the use of confocal laser scanning microscopy (CLSM), confirmed that metabolic exchange occurred. In addition, it was observed that the bacteria appear to have a preferred biofilm architecture, with *P. putida* in the bottom layer, and *Acinetobacter* at the top.

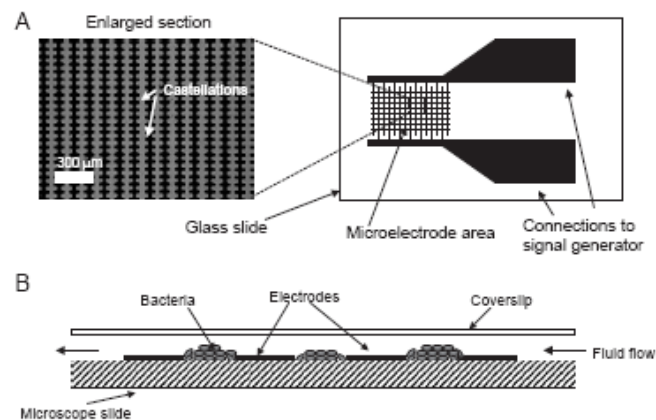


Fig. 1. A schematic diagram of the experimental set up (not to scale). (A) shows a top view of the glass slide with the electrodes. The electrodes, which consist of a 100-nm-thick layer of chromium deposited onto a standard glass microscope slide design, were made using photolithography and appear dark in the photograph. The electrodes were of the interdigitated-castellated design. Castellations, of size $30\ \mu\text{m} \times 30\ \mu\text{m}$, are indicated by the arrows in the photograph. When an electric signal is applied to the electrodes high electric field regions are created between the castellations, to which suspended bacterial cells can be attracted. (B) shows a stylised cross-section of a completed chamber, made from a microscope slide with microelectrodes and a cover slip, using insulating tape ($100\ \mu\text{m}$ thickness) as a spacer.

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