Microwell arrays for high-throughput investigation of microbial interactions

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Bacteria often function within diverse communities where different species interact through diffusive chemical signaling, metabolite transfer, and through contact-mediated interactions. Many inter-bacterial interactions that occur in nature are poorly understood, largely because traditional microbiological assays probe these interactions using co-culturing methods that are qualitative, low-throughput, and do not re-create the physical and chemical complexities of the natural environment. In addition, these methods typically measure interactions from bulk cell populations, overlooking heterogeneity existing within cell sub-populations that can play crucial roles in community function. Given these limitations, there is considerable interest in developing high-throughput, single cell approaches to monitoring inter-bacterial interactions in a tailored physical and chemical environment.

In order to address these limitations, we have developed a micro and nanostructured interface that screens a large number of bacterial populations for growth in a tailored physical and chemical environment. This is achieved by assembling bacterial solutions over surfaces containing arrays of etched silicon wells and a parylene mask. Upon removing the mask, cell populations become trapped within their microwell environment (Figure 1A). By varying well geometries, cell populations can be assembled into wells in either a controlled manner, where each well population is similar, or in a stochastic manner, where each well contains a dramatically different population (Figure 1B).

Using stochastic assembly, the evolution of thousands of different combinations of bacterial cells can be monitored using time-lapse fluorescent microscopy, allowing for a high-throughput screening approach to identify populations and environmental conditions that promote or inhibit microbe colonization. We have currently used this approach to investigate populations and microscale environments that promote or inhibit the colonization of *Pseudomonas aeruginosa* PA01, an opportunistic pathogen common in many clinical infections (Figure 2). The continued refinement of this approach will allow for the screening of inter-species interactions in order to identify symbiotic and pathogenic relationships and physical and chemical environments that are relevant to bacterial disease pathology.

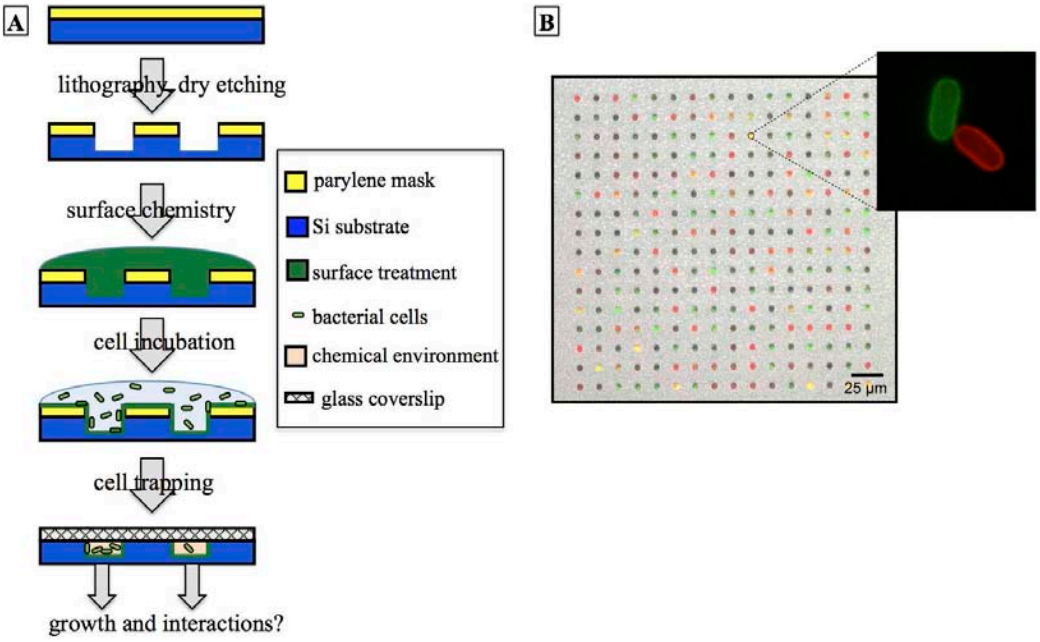

Figure 1: Isolation of bacterial populations in microwell arrays: A) Experimental approach for isolating and monitoring growth and interactions of multiple bacterial populations. B) Fluorescent-brightfield image of a microbe array after stochastic assembly of GFP and RFP-expressing microbes. Each well contains a unique fluorescence signal, indicating different combinations of microbes.

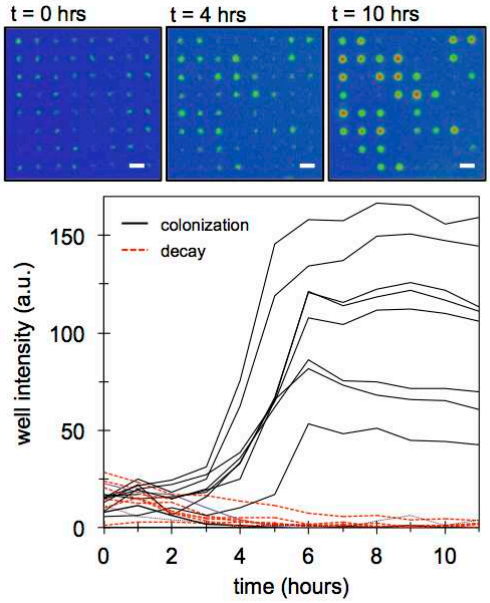

Figure 2: Growth or decay of P.aeruginosa populations in stochastically seeded microwell arrays. Top: False color fluorescent images of microwells arrays at different incubation times (scale bar = 10 µm). Bottom: Growth trajectories of sixteen representative wells.