Microwell arrays for high-throughput investigation of multi-species biofilm formation

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Statement of Purpose: The development of surface-associated bacterial communities into biofilms is influenced by the properties of the surface, the surrounding chemical and physical environment, and by inter-bacterial interactions. For example, biofilms often form in spatially-confined environments, where the restricted diffusion of chemical signaling molecules can induce quorum-mediated behavior, such as increased exopolysaccharide production. In this work, we develop a high-throughput experimental platform that simultaneously quantifies cell growth or lysis events occurring in hundreds of micro-scale wells, each containing unique bacterial communities. This approach allows for the screening and identification of bacterial populations that promote or inhibit community growth in spatially-confined environments, and will inform strategies for inhibiting the proliferation of pathogenic communities on medically-relevant surfaces.

Methods: Substrates were designed to contain arrays of micron-scale wells for the capture and growth of bacterial cells. To achieve this, silicon wafers were first coated with parlyene films. Reactive ion etching methods were then used to etch wells to the desired dimensions. Surfaces were coated with a bio-attachement layer, then seeded with solutions containing a binary population of microbes. Finally, the parylene film was removed, a membrane was attached, and the substrate was incubated in growth media. Time - lapse fluorescence microscopy was then used to monitor cell growth or cell lysis events occurring in each well (Fig. 1).

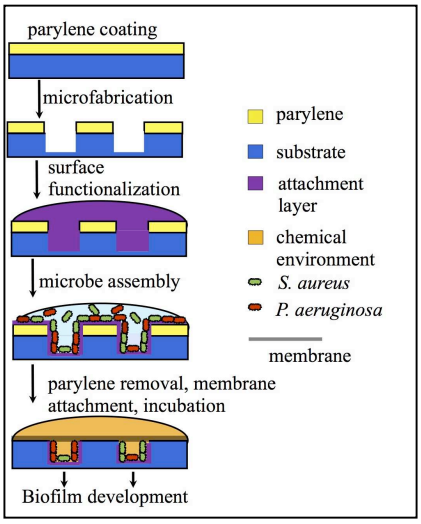

Fig. 1. General approach for assembling bacterial populations into microwell arrays.

Results: Binary populations of *E.coli* cells modified to express GFP or mCherry fluorescent reporter proteins were used to characterize the seeding process over wells of different sizes (2-20 μm diameter). Larger wells favored the seeding of reproducible cell populations, while smaller wells with diameters approaching the size of individual cells favored the formation of highly variable populations due to the increasingly stochastic nature of cell-well interactions. This allowed for the assembly of ~10^2 unique populations within one array of microwells (Fig. 2).

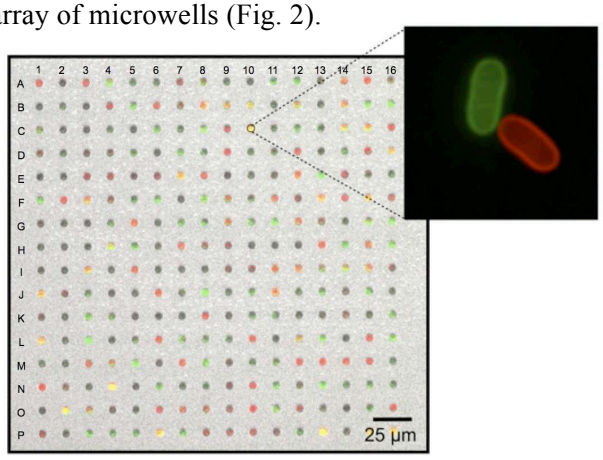

Fig. 2. Brightfield-fluorescent image of an array of 5 μm diameter wells after seeding a population of GFP and mCherry expressing *E. coli* cells.

After characterization of the seeding process, the growth and biofilm formation of populations containing *P. aeruginosa* PA01 strains stochastically assembled into the wells was monitored. Wells initially containing intermediate numbers of PA01 cells proliferated while wells initially containing high or low numbers did not, suggesting that there is a "sweet-spot" in terms of initial community composition that exists for proliferation in spatially-confined systems.

Conclusions: The microwell array allows for a high-throughput screening approach to monitoring the growth of bacterial communities in spatially-confined environments. The continued refinement of this approach will allow for the identification of both the community structures and the physical and chemical parameters that promote or inhibit bacterial proliferation. These findings will inform the design of medical devices that inhibit biofilm formation.