Three-Dimensional Biomolecular Architectures for Characterizing Bacterial Sociomicrobiology

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Statement of Purpose: Bacteria are social organisms, displaying a range of phenotypes that depend on the community of cells in which they live. At sufficient cell densities, small clusters of bacteria display the capacity to undergo quorum sensing, a process in which gene transcription patterns are modified in response to small signaling molecules, and to construct antibiotic-resistant sessile communities known as biofilms. Moreover, within native environments such as the oral cavity and puncture wounds, bacteria integrate into polymicrobial communities in which viability is impacted by both synergistic and antagonistic relationships. Despite the critical importance of cell-cell relationships, adequate tools have not existed to systematically assess how organization impacts mono- and polymicrobial cultures on a microscopic scale. Here, we present development of a new methodology for organizing bacterial communities within microclusters of essentially any desired shape on microscopic dimensions relevant to transmission of many pathogens. This approach, based on laser microfabrication of protein-based cellular containers, offers the capacity to evaluate the interplay of key parameters affecting cell phenotype, including colony size, geometry, and the flow rate of surrounding media. The high porosity of the protein-based materials used to organize bacterial populations are permeable to nutrients, waste products, and signaling molecules, providing containment environments that are capable of supporting normal growth and communication between physically sequestered populations. The applicability of this microfabrication strategy to the study of sociomicrobiology is demonstrated for isolated populations of the opportunistic pathogen, *Pseudomonas aeruginosa*, and in polymicrobial cultures of *P. aeruginosa* and *Staphylococcus aureus*.

Methods: A dynamic-mask based strategy[1] is used to direct fabrication of three-dimensional (3D) microcontainers capable of containing and organizing multiple populations of bacteria in defined spatial relationships to one another. In this approach, a pulsed femtosecond titanium-sapphire laser beam is focused onto a digital micromirror device (DMD) that displays a mask pattern for directing fabrication at a conjugate plane within reagent solution. Multiphoton excitation of a photosensitizer (Rose Bengal, methylene blue) is used to promote photocrosslinking of proteins within a three-dimensionally resolved voxel, creating a solid matrix of highly porous material. By raster scanning the laser beam across the DMD, this protein-based material can be patterned within an individual plane. By shifting the focus through different planes within the reagent solution in synch with display of a defined sequence of masks, essentially any desired microform can be fabricated efficiently with submicrometer resolution. Various highly soluble proteins can be used as the building block for solid matrices, including bovine serum albumin (BSA), avidin, and lysozyme. In addition, the protein-photosensitizer reagent mixture can be induced to undergo gellation through various approaches, some of which provide the means to fabricate compartments around suspended bacteria. In this way, multiple populations of cell types can be efficiently organized into biologically relevant arrangements, including core-shell structures in which one population of cells fully encapsulates a second population.

Results: We demonstrate that individual cells can be contained with microcavities and grown to high-density populations ranging in size from a few dozen cells to thousands of cells.[2,3] Importantly, the generation times of trapped cells are indistinguishable from those grown in batch cultures. The methods are used to study phenotypic changes that occur in microcolonies of common pathogenic Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Pseudomonas aeruginosa*) bacteria, demonstrating that quorum sensing is dependent on population size, population density, and the flow rate of surrounding media. In addition, we show that populations of cells containing as few as approximately 100 individuals can develop phenotypic resistance to antibiotics similar to that found within biofilms, and that the antibiotic resistance of one isolated population can influence resistance within a second, proximal population.

Conclusions: Multiphoton fabrication of 3D protein-based microstructures is shown to be a valuable approach for organizing small populations of bacteria, allowing populations to be defined at densities and on size scales believed to be relevant to the transmission of many infections. The spatial, chemical, and mechanical control provided by this approach should open opportunities for clarifying mechanisms involved various disease conditions, including chronic infections in the cystic fibrosis lung.

References:

1. Nielson R. et al. Small, 2009; 5: 20-125.
2. Connell JL et al. mBio, 2010; 1: e00202-10.
3. Connell JL et al. Nat. Chem. Biol., 2012; 8: 10-13.