Biotic/Abiotic Materials: Behavior of Cells in Nanostructural Isolation

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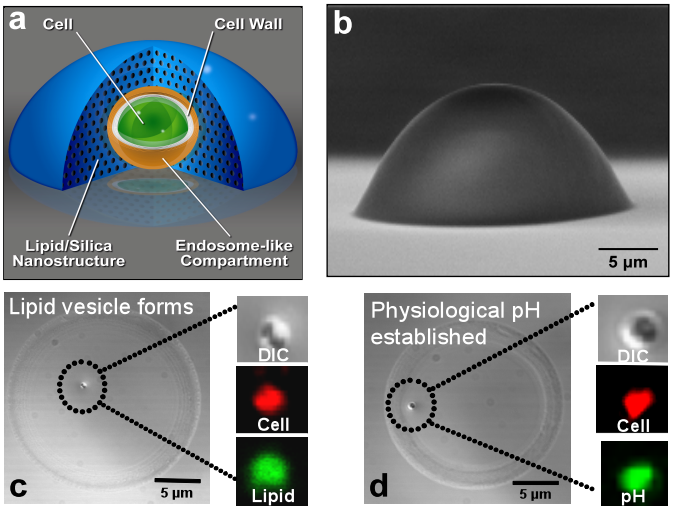

Figure 1. Isolation of individual *S. aureus* within a nanostructured droplet. (**a**) schematic of physical system (not to scale) showing a cell incorporated in an endosome-like lipid vesicle within a surrounding nanostructured lipid/silica droplet deposited on glass substrate and (**b**) SEM image of physical system. The nanostructure maintains cell viability under dry external conditions and allows complete chemical and physical isolation of one cell from all others. **c** and **d** show plan-view optical microscope images of individual cells in droplets (large outer circular areas). Magnified areas show differential interference contrast image and red fluorescence image of individual stained, isolated cells (both **c** and **d**) and green fluorescence image of NBD-labeled lipid localization at cell surface (**c**) or localized pH (**d**), using Oregon Green pH-sensitive dye). We find that, within the droplet, the cells become enveloped in an endosome-like lipid vesicle (**c**), and establish a localized pH consistent with physiological early endosomal conditions (~5.5) (**d**).

Many bacteria emit and sense small, diffusible 'signaling' molecules (autoinducers) whose extracellular concentration regulates gene expression through a positive feedback system, controlling important functions including virulence and biofilm formation. The prevailing view of why this signaling takes place is that it allows populations of cells to assess their density. If a 'quorum' exists, bacteria coordinate their gene expression to function as a community, thereby providing group benefits exceeding those of individual cells. This idea that bacteria act cooperatively for the social good is so appealing that the potential benefits of quorum sensing at the individual cell level have not yet been fully explored. We used *cell-directed assembly* (*Science* 2006) to develop a physical system that simulates endosomal or phagosomal bacterial entrapment during infection and maintains cell viability under conditions of complete chemical and physical isolation. *S. aureus* were immobilized, individually within a matrix fabricated at a sufficiently small physical scale (~20 µm diameter, physically isolated hemispherical droplets, see Fig. 1a-b) so that the overall cell density exceeded the reported QS threshold (10^7 – 10^9 cells mL^{-1}). The matrix was formed by adaptation of our cell-directed assembly approach to an aerosol procedure we developed previously to form ordered porous silica nanospheres. It results in cells incorporated within a dihexanoylphosphatidylcholine (*di*C_6PC) lipid vesicle (Fig. 1c) maintained at a pH of ~5.5 (Fig. 1d), approximating that of the early endosome, and surrounded by an ordered silicon dioxide nanostructure (Fig. 1a and b) that serves as a reservoir for any added buffer and media. This construct mimics some of the physical and chemical features of a bacterium entrapped within an intracellular membrane-bound compartment (endosome or phagosome). Importantly, this architecture, *viz* a vesicle-enveloped cell incorporated in a much larger nanostructured silica bead (Fig. 1a-b), allows individual cells to be maintained in a viable state under externally dry conditions that establish complete physical and chemical isolation of one cell from all others. This reduced physical system is biologically relevant, because *Staphylococcus aureus* is known to become trapped in such intracellular compartments, and it is proposed that they employ a QS strategy to induce new gene expression, promoting intracellular survival and/or escape. However it is presently unknown whether

confinement alone can promote QS or whether other factors within the endosomal organelle are required. We use our system to test confinement alone as a mechanism for inducing QS. To optically monitor the onset and kinetics of auto-induced QS, we used *S. aureus* strains containing reporters of quorum sensing-dependent *agr* P3-promoter activation and QS-mediated downstream synthesis of the pore-forming toxin, α-hemolysin. Progressively increasing GFP expression over 10 hours provided the first proof of auto-induction of an individual, physically and chemically isolated organism. Additionally these data provided the first evaluation of gene expression kinetics for a large population of isolated individual cells. We postulate that quorum sensing allows isolated *S. aureus* to sense confinement through increased extracellular concentration of autoinducer and to activate virulence factor pathways and initiate new gene expression needed to adapt and survive in such confined environments.

Implications for Induced Dormancy and Drug Resistance – Beyond QS, there is now overwhelming evidence of environmental influences on cellular behavior, and these epigenetic effects are currently being recognized as crucial to the understanding of a diverse spectrum of problems including cancer metastasis, drug resistance, TB dormancy, and nanoparticle toxicology. For example, it has recently been proposed that cancer cells may use a quorum sensing mechanism, similar to bacteria, to regulate gene expression and control steps in metastatic colonization. Progress on addressing these problems, however, is currently hindered by an inability to incorporate cells into three-dimensional architectures that better represent the nanostructured extracellular matrix (ECM), tissues, or niches (e.g. capillaries), where cells may reside *in vivo*. Using a derivative of the cell-directed assembly approach developed by our team, we immobilized Human hepatocarcinoma (Hep3B) cells within a coherent 3D lipid/silica matrix qualitatively similar to that used in the bacterial entrapment studies discussed above. Our initial results demonstrate that integration of Hep3B within a silica matrix induces cellular dormancy within four hours and that dormant cells re-enter the cell cycle as a homogeneous, synchronized population, once the matrix is suspended in serum-containing growth medium and begins to dissolve. We find that, by simply controlling the amount of time that cells remain integrated within the silica film, they become arrested at various points in the cell cycle and can be maintained under 'normal' growth conditions with minimal loss of viability for several weeks. Additionally, we observe that confinement of individual Hep3B induces resistance to chemotherapeutic agents (e.g. doxorubicin and camptothecin) that interfere in DNA replication and, therefore, normally target proliferating cells during the S-phase of the cell cycle. Integrated Hep3B cells can be exposed to a high concentration of doxorubicin (~70 μM, 1000 times the IC_{50} value for DOX-sensitive cells) for 7 days without induction of apoptosis, suggesting that confinement within our nanostructure can induce and preserve drug resistance and other specific cellular states not accessible in tissue culture or *in vivo*.