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Selectively Permeable Microbial Nanoculture System as Artificial Microniches

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Microfluidics-based approaches for isolating microbes, replicating their microenvironments and growth conditions can provide new platforms to elucidate many fundamental biological questions. For instance, the spatial confinement of microorganisms in microcompartments can spawn new insights on the inherent advantages of cell clustering and biofilm formation in microbial survival and virulence mechanisms. As the worldwide antibiotic crisis persists, there is an urgent need for emerging technologies that enable the scrutiny of the evolution of multidrug resistance, and facilitate the study of cooperative or antagonistic microbial interactions. Here we describe a method to generate artificial multifunctional microniches in order to encapsulate and interrogate microbes. Using a flow-focusing microfluidic device, microorganisms including *Pseudomonas aeruginosa* and *Escherichia coli* (Gram-negative bacteria), *Streptococcus mutans* (Gram-positive bacterium) and *Candida albicans* (fungus) were grown in a polydimethylsiloxane (PDMS)-based nanoculture system. The selective permeability of the nanocultures to small molecules including antibiotics, signaling molecules and fluorescent dyes allows assessing essential cell functionalities (e.g. growth kinetics, cell vitality, pH change), as well as characterize inter-species or inter-kingdom interactions, and microbial dynamics in response to antibiotic attacks. The multifunctional nanoculture system has broad potential for studying microbiome dynamics, and identifying new bioactive molecules such as antimicrobials from these defined microcosms.

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