Microbes self-organize in microcolonies while transitioning to a sessile form within a protective biofilm matrix. This stage of development has received scant attention, in spite of the importance of community dynamics in determining microbe fate. To enable the detailed study of microbial dynamics within microcolonies, new sessile culture systems are needed that sequester cells and mimic their complex growth conditions. We have developed a nanoliter-scale sessile culture system that is easily implemented via microfluidics-enabled fabrication. Hundreds of thousands of these nanocultures can be easily generated and imaged using conventional or confocal microscopy. Each nanoculture begins as a several nanoliter droplet of suspended cells, encapsulated by a polydimethylsiloxane (PDMS) membrane. The PDMS nanocultures provide long-lasting mechanical support, enabling long-term study. The PDMS membrane is selectively permeable to small molecules including antibiotics, signaling molecules and functional fluorescent probes. As microcolonies mature, they can be stressed or interrogated using selected probes to characterize cell physiological properties, antibiotic susceptibilities, and antagonistic interactions. We demonstrate this platform by investigating broad ranges of microcolony dynamics, including direct and indirect bacterial-fungal interactions. This versatile new tool has broad potential for addressing biological questions associated with drug resistance, chronic infections, microbiome dynamics, and antibiotic discovery. It also sets a precedent for the creation of upstream bioprocessing technologies through functional membranes capable of encapsulating probiotics or unculturable microbes, which could facilitate high-throughput screening and characterization of bioactive molecules.

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