Multimodal microfluidic platform for controlled culture and analysis of unicellular organisms

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Modern live-cell imaging approaches permit real-time visualization of biological processes, yet limitations exist for unicellular organism isolation, culturing, and long-term imaging that preclude fully understanding how cells sense and respond to environmental perturbations and the link between single-cell variability and whole-population dynamics. Here, we present a microfluidic platform that provides fine control over the local environment with the capacity to replace media components at any experimental time point, and provides both perfused and compartmentalized cultivation conditions depending on the valve configuration. The functionality and flexibility of the platform were validated using both bacteria and yeast having different sizes, motility, and growth media. The demonstrated ability to track the growth and dynamics of both motile and non-motile prokaryotic and eukaryotic organisms emphasizes the versatility of the devices, which should enable studies in bioenergy and environmental research. Published by AIP Publishing. [<http://dx.doi.org/10.1063/1.4986533>]

INTRODUCTION

Long-term tracking of single cells in controllable environments is essential for gaining insight into the cell physiology, cell-to-cell heterogeneity, and dynamic responses to exogenous signals.^{1,2} Microfluidic platforms for analytical live-cell imaging can address these needs by providing precisely controlled fluidic and chemical environments, with both parallelized and long-term operation.^{2,3} Diverse microfluidic strategies have been reported for the tailored trapping and cultivation of specific types of mammalian cells,^{4–8} plant cells,^{9,10} filamentous fungi,^{11,12} yeasts,^{13–17} bacteria,^{8,13,18–24} and algae.^{25,26} A single platform having broad applicability to multiple cell types and growth modes should overcome the limitations of purpose-built microfluidic platforms to facilitate comparative studies of diverse species within a given microenvironment.

For microfluidics-based cell culture, the systems constructed to date operate in two primary working modes: (1) perfused or semi-continuous^{5,9,10,12,13,18,20,21} and (2) compartmentalized (i.e., batch).^{8,17,19,23,24,26} Perfused culture maintains a stable and constant environment by continuously replenishing fresh nutrients to growing cells while removing metabolic waste. This cultivation mode prevents the risk of nutrient exhaustion, and multiple culture niches can be supplied with a single stream of growth medium. In contrast, compartmentalization is achieved by physically isolating and confining cells into completely closed environments. The compartments are generally single-phase microchambers created by integrated pneumatic valves²⁷ or two-phase microdroplets generated by the surrounding aqueous solution with an immiscible carrier oil.²⁸ In

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both microfluidic cultivation modes, cells are trapped in volumes of typically a few picoliters to hundreds of nanoliters where they can be treated in a controlled manner for a defined period and analyzed by optical microscopy employing automated tracking and/or imaging. As microfluidic culture platforms developed to date have been designed for either compartmentalized or perfused growth, a direct comparison of these two culture modes within an identical chip architecture has not been possible, and their corresponding impact on cell growth and dynamics often go unmeasured. Also, while photolithographically patterned hydrodynamic cell traps have been widely used for mammalian cells, the much smaller average sizes, irregular shapes, and phenotypic heterogeneity of microbial systems or complex microbial communities pose a substantial challenge for such tailored traps. Alternate isolation methods that avoid the use of patterned traps while maintaining efficient isolation will therefore benefit extended studies of unicellular organisms.

Herein, we present a highly versatile microfluidic platform providing various functionalities (i.e., trapping, cultivation, and medium switching) for comparative cell growth analysis, which is suitable for diverse types of unicellular organisms. Cell loading is accomplished by directed flow through an array of 30 growth chambers using a series of castellated microvalves wherein the chambers occupy ~35% of the 10 nl flow path. After loading, the flow path is reconfigured and cells located outside of the growth chambers are flushed away. The percentage of chambers containing a single microorganism follows a Poisson distribution and depends upon the concentration of introduced cells. Rapid nutrient delivery is accomplished by a short diffusion path from the main delivery channels to the growth chambers. Alternatively, an immiscible oil may be infused through the main channels to surround the chambers for compartmentalized cultivation. To validate the overall platform versatility, we have probed single-cell growth kinetics and long-term cellular responses to nutrient switching using two model organisms (i.e., *Yarrowia lipolytica* yeast cells and *Shewanella oneidensis* bacterial cells) with vastly different trapping and growth requirements. This is a highly versatile microfluidic platform for the entrapment and growth of both, motile and immotile, prokaryotic and eukaryotic cells with two cultivation modes within a single microfluidic design. As such, the platform described herein moves beyond purpose-built devices to a generalized format for dynamic imaging and analysis of single cells and multicellular communities.

METHODS

Cells growth and preparation

The *Yarrowia lipolytica* yeast strain FKP503 expresses a mitochondrial targeted green fluorescent protein (GFP) and FKP391 is a leu2 prototrophic strain generated from Polg.²⁹ FEB91 was generated as an endogenous GFP translational fusion construct with histone H1 (YALI0B16280g). Transformation was carried out as described previously.³⁰ Modifications and further details of all the above strains can be found elsewhere.³¹ Strains were stored at -80°C and 25% glycerol, and maintained in YPD medium containing 1% yeast extract (Sigma-Aldrich), 1% peptone (ThermoFisher Scientific), and 2% glucose (VWR) at 28°C . Cultures were grown for 16 to 18 h in YPD with shaking. For dimorphic growth experiments, YNB medium containing 1.7 g/l yeast nitrogen base without amino acids and ammonium sulfate (Thermo Fisher Scientific), and 20 g/l glucose was used. Cells were counted with a hemocytometer and diluted in YPD medium.

The *Shewanella oneidensis* strain MR-1 carrying p519nGFP³² was aerobically grown overnight in Tryptone Soya Broth without dextrose (TSB; Sigma-Aldrich) at 30°C and 100 rpm of shaking. To retain the GFP, kanamycin sulfate was added to the TSB medium to a concentration of 25 $\mu\text{g/ml}$. Cells were spectrophotometrically measured at OD600 and diluted in TSB medium.

Microchip fabrication

The microfluidic chips were fabricated by multilayer soft lithography²⁷ as demonstrated in our previous studies.¹² Chip designs were created with the AutoCAD software (Autodesk), and reproduced onto photoresist-coated chromium-on-glass photomask blanks (Telic) using an

Intelligent Micro Patterning SF-100 Xpress direct-write photolithography system. Master molds for the control layer (25 μm tall) and fluidic layer (5 μm tall) were made with a SU-8 25 negative photoresist (Microchem) and AZ P4260 positive photoresist (Capitol Scientific), respectively, on 4-inch silicon wafers (University Wafer). To create a rounded cross section for fluidic channels, the fluidic layer mold was thermally reflowed at 150 °C for 20 min. Chips were manufactured by pouring the polydimethylsiloxane (PDMS) prepolymer mixture comprising the base monomer and curing agent (Sylgard 184; Dow Corning) at a mass ratio of 10:1 onto the fluidic layer mold to ~ 5 mm thick, and spin-coating the control layer mold at 2000 rpm for 30 s to form a thin membrane (~ 50 μm thick). After baking both layers at 70 °C for 1 h, the fluidic layer was removed from the mold, precisely aligned onto the control layer under a VHX-600 digital microscope (Keyence), and bonded by treating in oxygen plasma and curing at 70 °C for 2 h. The assembled PDMS was then peeled, cut, and punched to generate inlets and outlets. The PDMS stamp was finally sealed with a clean glass coverslip (ThermoFisher Scientific) by plasma oxidation, followed by baking at 70 °C overnight.

Microfluidic cell trapping and cultivation

Prior to each experiment, the control and fluidic channels were filled with water and medium (yeast extract peptone dextrose (YPD) for yeast cells, tryptic soy broth (TSB) for bacterial cells, and tris acetate phosphate (TAP) with L-arginine for microalgal cells), respectively, to avoid the formation of bubbles inside the channels during experiments. The actuation of pneumatic microvalves was regulated with computer-controlled solenoid valve manifolds (Festo) by applying the pressure (i.e., 60 psi) from a nitrogen source, and the solenoid valves were controlled by the custom software as described previously.³³ The cells and media were flowed into the microfluidic chip *via* plastic tubing (IDEX Health & Science) using syringe pumps (Harvard Apparatus). Cells at appropriate concentrations were pumped into the device at a flow rate of 0.04 to 0.1 $\mu\text{l}/\text{min}$. Once cells were trapped, the medium was flushed at 0.2 $\mu\text{l}/\text{min}$ into the two main channels for 5 min to wash away any remaining cells.

For cell growth under perfused conditions, the medium was then constantly perfused into the main channels at 0.1 $\mu\text{l}/\text{min}$. In the compartmentalization cultivation format, cells were admixed with molten ultra-low gelling temperature agarose Type IX (Sigma-Aldrich) at the final concentration of 2% or 1% (w/v). Following loading cells and flushing the main channels, the device was placed on an ice block to sufficiently polymerize the agarose within the growth chambers and trap cells, during which FluorinertTM FC-40 oil was introduced into the main chip at 0.05 $\mu\text{l}/\text{min}$. After incubating for 5 min, the ice block was removed, and the oil flow rate was set to 0.02 $\mu\text{l}/\text{min}$.

Live-cell imaging

The microfluidic devices were mounted onto a PALM MicroBeam inverted fluorescence microscope system (ZEISS) equipped with a fluorescence filter cube optimized for GFP imaging. Images were acquired with a 40 $\times$ dry objective, an AxioCamMRm CCD camera, and AxioVision software (ZEISS).

Flow analysis

To better understand the fluidic profiles during cell cultivation, flow dynamics of the microchannels were numerically simulated with the commercial finite element analysis software, COMSOL Multiphysics (COMSOL), based on the incompressible momentum Navier–Stokes equation. The flow properties of water density, $\rho = 1000 \text{ kg}/\text{m}^3$, and water dynamic viscosity, $\eta = 8.9 \times 10^{-4} \text{ Pa s}$, were employed in the simulations. The inlet velocity was set to 6.7 mm/s (0.1 $\mu\text{l}/\text{min}$) for both main channels in Fig. 1(c). To experimentally track the molecular diffusion profile, the microfluidic device filled with 2% agarose in growth chambers was loaded with the fluorescent dye solution containing 100 μM of 2-deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-D-glucose (2-NBDG; Sigma-Aldrich), for 15 min, and subsequently pure water for 15 min. Images were captured each minute.

FIG. 1. Unicellular microfluidic chip for trapping, growth, imaging, and analysis. (a) Schematic of the chip consisting of a fluidic layer (green) and a control layer (blue) of channels. The fluidic layer primarily comprises sample delivery channels, two serpentine main channels, and 30 cell growth chambers which are interconnected to the main channels through an array of connecting channels. The control layer contains a pair of offset castellated loading valves, an isolation valve, three valves for controlling the delivery of cells, media, and oil, as well as two valves for controlling the waste output. (b) Micrographs showing the main channels and connecting channels precisely aligned to the valves. Due to their rounded cross sections, the fluidic channels can be fully closed when the valves are actuated. Scale bar, 100 μm . (c) Numerical simulation of flow profiles in the channels during medium infusion. The flow velocity along the central cross line from the two ends of the main channels (right panel) demonstrates that media movement across the growth chambers is absent, minimizing flow effects on cells during growth and *in situ* imaging.

RESULTS AND DISCUSSION

Microfluidic chip design

The microfluidic unicellular cultivation chip was manufactured from PDMS using multilayer soft lithography.²⁷ The chip comprised a layer of fluidic channels (5 μm tall; green) for transport of cells and solutions, and a layer of control channels (25 μm tall; blue) for pneumatic microvalve actuation (Fig. 1). The fluidic channels contained an array of 30 cell growth chambers (each was circular with a diameter of 150 μm unless otherwise specified), and a pair of parallel serpentine main channels (50 μm wide) for medium or oil infusion [Fig. 1(a)]. Each growth chamber was interconnected to the main channels through two narrow connecting channels (40 or 20 μm wide, 100 μm long) in between. In principle, the chamber number is scalable to meet the desired goals, but the design shown in Fig. 1(a) was convenient to fabricate and was fully contained on a standard 25 $\times$ 40 mm #1.5 coverslip, making it widely transferrable to common microscope platforms. Additionally, the microfluidic networks consisted of three inlets to input cells, various types of media and oil, one outlet for waste, and sample delivery channels connecting the access holes. Due to their rounded cross sections, the fluidic channels could be fully closed during valve actuation [Fig. 1(b)]. In the control layer, the design incorporated a total of eight integrated valves for controllable fluidic delivery and exchange. Apart from three valves to regulate the sample input and two for the sample output, a pair of offset castellated loading valves was accurately aligned to the serpentine main channels to facilitate cell trapping [Fig. 1(b)]. Moreover, an individual isolation valve composed of two parallel channels branching from

the same access port was precisely aligned to the two rows of connecting channels to create compartmentalized environments when actuated [Fig. 1(b)]. The serpentine geometry of the main channels facilitated alignment of the valves to the flow channels and served to minimize the distance from the main channels to the growth chambers for rapid nutrient exchange.

Cell trapping is the first critical step for microfluidic cultivation of living cells. While optoelectric and hydrodynamic trapping have both been successful for isolating small cells, the high laser power used for optoelectric trapping can cause laser-induced heating effects that perturb normal cell physiology and function,^{34,35} and hydrodynamic trapping requires sophisticated fabrication procedures with patterned features specifically tailored for each individual cell based on the size and shape.^{21,22} Random confinement of single cells within a small volume represents a simple way for entrapment of unicellular organisms without requiring major reengineering of the traps for each biological system under study, albeit with reduced efficiency relative to hydrodynamic trapping. Our microfluidic design incorporates 30 cell growth chambers occupying a common 2-dimensional footprint. The typical height used for the experiments detailed below was 5 μm and corresponded to a trapped volume of ~ 88 pl per chamber. Cell suspensions were injected into the chip *via* the cell inlet, during which only the cell inlet valve, the isolation valve, and the top outlet valve were open (Fig. 2). Taking advantage of the configuration of serpentine main channels actuated with offset castellated valves, cells were able to flow through all of the growth chambers along the defined fluidic path. When entering into the chambers, cells slowed down due to wider structures, and were retained in the chambers once all valves were actuated. Cells were stochastically distributed across the chambers following Poisson statistics, and the distribution was modulated by the varying cell density and flow rate. This design differed from the majority of microfluidic cell loading strategies relying on passive entrapment by actively introducing cells into trapping regions, therefore enhancing cell trapping efficiency, uniformity, and speed. Following cell trapping, cells remaining in main channels were washed out by flowing medium with the isolation valve closed and the loading and outlet valves open (Fig. 2). For motile cells (i.e., *S. Oneidensis* bacteria), we additionally incorporated gel polymerization-based immobilization to prevent the escape of cells from the growth chambers. Cell suspension admixed with molten agarose (2%, w/v) was loaded into the chip, and ultralow-gelling temperature agarose with a gel point of 8–17 °C and a remelting point of ≤ 50 °C was employed to avoid gelation during setup. Once cells were confined within the chambers, the agarose could rapidly transform into gels simply by incubating the device below the gelling temperature.²⁸ To eliminate potential fluidic clogging, unpolymerized agarose in channels other than the growth channels was flushed with the medium along with the removal of extra cells.

The platform is multipurpose for cell cultivation in both perfusion and compartmentalization modes, which can facilitate comparative studies and determine the time at which growth rates diverge for higher throughput compartmentalized studies using, e.g., droplet-based microfluidics. Perfused cultivation was enabled by flowing medium into the main channels with the loading and isolation valves open (Fig. 2). Numerical simulation of the flow profile indicates that growth chambers exhibited no fluidic flow, because both ends of the main channels were exposed to identical flow rates and balanced pressures determined by the symmetric channel configuration [Fig. 1(c)]. Gelled agarose in the growth chambers further provided a barrier to bulk flow when motile species were studied. Hence, fresh nutrients were constantly transported into the chambers solely based on diffusion. Such a flow pattern allowed cell growth in a steady environment without the perturbation of cell position caused by active flow across the chambers, which was critical for accurate long-term single-cell tracking and measurement. Nutrient supply was not limited due to the short diffusion distance. Assuming a typical diffusion coefficient for small molecules (e.g., glucose) of $D = 6 \times 10^{-10}$ m²/s, the solute diffusion time from the entrance of a connecting channel to the center of the circular chamber ($100 + 75 = 175$ μm) can be estimated as $\tau \approx d^2/2D = (175 \mu\text{m})^2/2 \times 6 \times 10^{-10}$ m²/s ≈ 25.5 s. Notably, the diffusion of low-molecular-weight molecules within the porous gel matrix containing low concentrations of agarose is not significantly restricted, as the diffusion coefficient was similar to that obtained in free solutions.³¹ The fast medium exchange was experimentally verified by loading a glucose fluorescent analogue, 2-NBDG, into chambers filled with agarose gel (Fig. 3).

FIG. 2. Workflow for single-cell trapping and multimodal cultivation. Cells in media are loaded into the device *via* the cell inlet channel, while both loading valves are closed to enable cells to flow through all of the growth chambers. Fresh media is then infused with the isolation valve closed to flush cells remaining in the main channels. Motile cells are admixed with agarose solution and immobilized by incubating at low temperature to gel the agarose within growth chambers. For cell cultivation, perfusion is achieved by introducing media into the main channels with the isolation valve open, while compartmentalized conditions are formed by infusing oil into the main channels and closing the isolation valve.

Compartmentalized growth is achieved by simultaneously closing the isolation valve and infusing fluorinated oil into the main channels (Fig. 2). Discrete water-in-oil compartments were rapidly and easily generated *in situ*, and single cells were trapped within the compartments. The compartment volume, shape and cell number per compartment were predefined during chip design and cell loading. Our strategy combined the benefits of single-phase microchambers created by integrated microvalves and two-phase microdroplets to form a closed environment for single-cell growth. The valves made it possible for the chambers to be easily separated into multiple compartments while mitigating liquid evaporation inside the devices. Furthermore, the compartments were completely isolated by the oil phase, eliminating the risk of cross-contamination and cell-to-cell communication among the different chambers.

Comparative cell growth and dimorphic yeast dynamics

Yarrowia lipolytica is a strictly aerobic yeast which has substantial potential for biotechnological applications in metabolite production.³⁶ We first assessed the trapping and growth of the

FIG. 3. Molecular diffusion of a chip filled with 2% agarose. (a) 100 μM 2-NBDG fluorescent dye solution was infused after agarose gelation, and the fluorescence intensity of the same chamber increased over time. (b) Water was subsequently infused, and the same chamber from (a) was imaged over time, showing the fast replacement of media as indicated by diminishing fluorescence. The scale bar represents 50 μm for all panels. The fast molecular diffusion of small molecules verifies the rapid exchange of media possible in these devices even through an agarose gel matrix used to immobilize cells.

Y. lipolytica strain FEB91 under both culture modes (Fig. 4). Chambers containing only one or two cells per chamber were tracked for this study, comprising 7–8 of the 30 total chambers. Cells were cultivated with nutrient-rich YPD medium in the array of 150- μm -diameter (~ 88 pl) circular chambers over 10 h. To determine cell growth, images were captured and cells were counted at regular intervals of 1 h after cell trapping. Since the channel depth was comparable to the size of a *Y. lipolytica* cell, the growing cells formed a cell monolayer which was mechanically clamped within a focal plane [Fig. 4(a)], simplifying imaging-based cell enumeration and analysis at single-cell resolution. Moreover, no drastic yeast morphology changes or abnormal growth patterns were detected, confirming that oxygen supply was not a limiting factor due to the gas permeability of the PDMS elastomer. Cells continuously proliferated with time, and perfused growth generally yielded more cells, especially after 6 h [Fig. 4(b)]. For each chamber in the same culture mode, cell number disparity at the endpoint was attributed to overall variations regarding the growth rates of individual clonal populations and cellular heterogeneity [Fig. 4(c)]. Note that the calculated growth rates accounted for variation in the initial number of trapped cells. Analysis of average growth rates for each condition reveals that the cells grew with linearly increasing growth rates in the first 9 h for perfusion but only during the first 6 h for compartmentalization [Fig. 4(d)]. After 6 h, the growth of compartmentalized cells slowed, suggesting that one or more nutrients originally trapped with the cells began to be depleted. After 10 h, cell growth decelerated for both culture modes, likely a result of increasing cell occupancy within the chambers, causing spatial hindrance that inhibited cell division and proliferation.

Y. lipolytica exhibits a dimorphic growth pattern as either oval single yeast cells or filamentous hyphae. The yeast-to-mycelium transition is associated with a defense mechanism to adverse environmental conditions including nutrient sources, oxygen, pH, and temperature.³⁷ Understanding the role of various environmental stimuli to the *Y. lipolytica* morphological shift at the single-cell level will greatly benefit industrial bioprocess implementations by promising to

FIG. 4. Yeast cell (*Yarrowia lipolytica*) trapping and growth on the microfluidic chips. (a) Representative time-lapse imaging of *Y. lipolytica* (FEB91 strain) cell monolayer growth under perfusion and compartmentalization conditions. The scale bar represents 50 μm for all panels. (b) Representative cell growth curves in 16 chambers (8 for perfusion in blue, and 8 for compartmentalization in orange). The majority of chambers are seeded with only one or two clonal cells, and the cell number is measured at regular intervals of 1 h after cell trapping. (c) Corresponding growth rates calculated as “(final cell number/initial cell number) per hour” of the same cells shown in (b). (d) Average cell growth rates for indicated conditions ($n \geq 15$) which are equivalent for the first 6 h then diverge. The inset shows the average cell doubling time for indicated conditions at the end of the 10 h experiment, which is calculated as “ $10 \text{ h} \times \log 2 / \log (\text{final cell number at } 10 \text{ h} / \text{initial cell number at } 0 \text{ h})$ ” ($n \geq 15$).

make single cells metabolically more active and easily manipulated in suspension fermentation.³⁸ Microfluidic systems are well suited for long-term monitoring of cellular dynamics and intracellular events under controlled environmental conditions, and thus we investigated the morphological transition of *Y. lipolytica* strain FEB91 in 150 μm circular chambers under nitrogen limitation. Cells were constantly perfused with the medium over 48 h without changes in cell positions (Fig. 5). Single yeast cells were initially cultured in nutrient-rich YPD medium for 2 h, and then the medium was changed to nitrogen-deficient YNB medium by the actuation of microvalves. Following 3 h of nitrogen deprivation, cell growth proceeded in all chambers, while the cell shape remained oval. After 6 h of nitrogen deprivation, short filamentous hyphae formed at the periphery of the microcolony shown in Fig. 4 Chambers 1 and 2 due to unipolar growth, whereas no hyphal generation was found in Chamber 3. Although cells continued to divide during that period, growth rates significantly slowed compared to cells cultured in YPD medium. The hyphae elongated over 22 h of cultivation in YNB medium in Chambers 1 and 2, but Chamber 3 continued to grow as a pure population of oval single cells. Notably, cell nuclei started to become more evident in all chambers after long-term nitrogen limitation, and cells continued to grow at much slower rates. After 46 h of nitrogen starvation, more mature long hyphae developed, especially in Chamber 2, but the hyphal formation was still limited to the clonal periphery. In contrast, the culture in Chamber 3 solely consisted of single oval cells during the entire experiment. While the sample size was far too small for a statistically powered study, this does highlight the ability of the platform to perform nutrient switching experiments and the potential for determining cell-to-cell variability in yeast dimorphic behaviors and growth rates. While compartmentalized growth could also potentially lead to formation of hyphae, the nutrient switching experiments enabled a single variable (nitrogen availability) to be isolated without other confounding factors such as the accumulation of metabolic byproducts in the growth chamber.

FIG. 5. On-chip *Y. lipolytica* strain FEB91 dimorphic dynamics in response to nitrogen deprivation. After initial trapping and 2 h of cultivation in nutrient-rich YPD media, the media was switched to nitrogen-deficient YNB. The oval cells at the periphery of the microcolony begin to transition to filamentous hyphae at 8 h, and elongate over time. Differential responses of single clones in the three chambers demonstrate cell-to-cell variability. The bottom images show the nuclear distribution at 48 h, and GFP fluorescence is displayed over bright field. The scale bar represents 50 μm for all panels.

Motile cell trapping

Trapping motile cells and observing their growth in controlled microfluidic systems presents both spatial and temporal challenges, especially for prokaryotic cells (e.g., bacterial

FIG. 6. Motile bacterial cell (*Shewanella oneidensis*) trapping and growth on the microfluidic chips. Representative time-lapse imaging of *S. oneidensis* (MR-1 strain expressing GFP) cell growth under nutrient perfusion and compartmentalization. GFP fluorescence is displayed over bright field. The scale bar represents $50\ \mu\text{m}$ for all panels. For perfused cultivation, single cells are immobilized with 2% agarose to prevent escape from the chambers and to mitigate motion during imaging. The white arrowheads indicate the location of initially trapped cells and early migration in the compartmentalization cultivation series.

cells) that are 10–1000 times smaller than typical eukaryotic cells.²⁶ To address this, agarose gel polymerization was coupled with active flow-through cell distribution to immobilize motile cells. *Shewanella* species are rod-shaped facultative anaerobic bacteria in aquatic and sedimentary environments, and are able to swim by a single polar flagellum.³² The ability to utilize a broad range of chemically diverse electron acceptors enables their implementations in microbial fuel cells and bioremediation.^{32,39} We loaded the mixture of GFP-expressing *Shewanella oneidensis* MR-1 cells and 2% agarose in TSB medium into the devices at the concentrations of one cell per $150\ \mu\text{m}$ circular chamber on average. After agarose gelation by cooling, medium or oil was infused to culture cells under perfusion or compartmentalization conditions. In both cases, no cell motility-induced escape was observed throughout the entire measurement processes owing to the combined effects of agarose gel matrix-based immobilization and balanced flow in the two main channels. Single cells could be efficiently trapped and cell division proceeded in the chambers for both conditions (Fig. 6). Cells exposed to continuous nutrient perfusion grew laterally in the agarose gel matrix with constant infusion of fresh media, whereas cells freely swam in the compartmentalized chambers with unreplenished media. Similar to yeast cells, perfused cultivation yielded higher total biomass than compartmentalization. Under conditions of nutrient perfusion, the chamber was completely confluent with bacteria at 24 h, and three-dimensional biofilm structures had begun to develop since the height of each channel was $5\ \mu\text{m}$. Contrarily, the compartmentalized bacteria did not cover the majority of chamber surfaces even after 48 h, and many cells were clustered and accumulated at the chamber wall. Note that the chip depth can be adjusted to accommodate bacterial monolayer growth to achieve single-cell resolution if desired as demonstrated for yeast cells above.

CONCLUSIONS

We have described a multimodal microfluidic platform that empowers live-cell dynamic observations and quantitative analysis in real time and under controllable local environments. Our platform offers efficient, active flow-through cell trapping, the capacity to create both perfused and compartmentalized environments with the same device configuration, dynamic medium delivery, robust operation for long periods, scalability, flexibility, and compatibility with automated imaging platforms. The examples described above showcase the flexibility of the devices for trapping both large and small non-motile and motile cell types spanning eukaryotic and prokaryotic organisms. This wide-ranging compatibility for trapping, growing, and imaging such diverse unicellular microorganisms suggests that the approach is immediately relevant to studying axenic and non-axenic microbial organisms, and can advance live-cell high-resolution

imaging of cellular function, biogenesis, and other morphological and physiological changes over time. In particular, the results demonstrate the ability to analyze population trends as well as resolve strain-specific cell-to-cell statistical variability under controlled environments. It should be noted that further scaling of the cell trapping array will be necessary for many applications. As the trapping mechanism relies on a Poisson distribution, only a fraction of the 30 traps will contain, e.g., a single cell or a desired combination of cells, which will be insufficient for most statistically powered studies. In this work, the focus has been to demonstrate the versatility of the platform with its multiple operational modes such that even those chambers that were appropriately populated were not all monitored during growth experiments. The further scale-up of these trapping arrays and automated data acquisition are currently in progress.

Although we focused on suspended unicellular organisms, looking to the future, the device could be readily extended to adherent microbial or mammalian cells to, e.g., visualize targeted delivery of nanoparticle-delivered drugs. Furthermore, by introducing two different solutions into the two main channels on opposite sides of the growth chambers, the device design should enable the creation of chemical gradients by molecular diffusion, providing an immediate platform for investigating gradient responses such as gene expression and other phenotypic changes in response to changing environments for both fast and slow growing microorganisms. Even in the course of compartmentalized cultivation, oil could be temporarily flushed out to intermittently infuse aqueous solutions containing biochemical stimuli or dyes to provide more sensitivity for imaging intracellular dynamics even for non-genetically tractable species. Finally, in combination with heating stages the platform could also enable thermal ramping to detect changes in biofouling rates or changes in chemical conversion efficiency for biofuel feedstocks or other applications as a function of temperature.

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