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Individually addressable arrays of replica microbial cultures enabled by splitting SlipChips†

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Isolating microbes carrying genes of interest from environmental samples is important for applications in biology and medicine. However, this involves the use of genetic assays that often require lysis of microbial cells, which is not compatible with the goal of obtaining live cells for isolation and culture. This paper describes the design, fabrication, biological validation, and underlying physics of a microfluidic SlipChip device that addresses this challenge. The device is composed of two conjoined plates containing 1000 microcompartments, each comprising two juxtaposed wells, one on each opposing plate. Single microbial cells are stochastically confined and subsequently cultured within the microcompartments. Then, we split each microcompartment into two replica droplets, both containing microbial culture, and then controllably separate the two plates while retaining each droplet within each well. We experimentally describe the droplet retention as a function of capillary pressure, viscous pressure, and viscosity of the aqueous phase. Within each pair of replicas, one can be used for genetic analysis, and the other preserves live cells for growth. This microfluidic approach provides a facile way to cultivate anaerobes from complex communities. We validate this method by targeting, isolating, and culturing *Bacteroides vulgatus*, a core gut anaerobe, from a clinical sample. To date, this methodology has enabled isolation of a novel microbial taxon, representing a new genus. This approach could also be extended to the study of other microorganisms and even mammalian systems, and may enable targeted retrieval of solutions in applications including digital PCR, sequencing, single cell analysis, and protein crystallization.

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Insight, innovation, integration

Isolating microbes carrying specific genotypes of interest from environmental samples is critical for fundamental studies of microbes and microbe–host interactions, as well as for developing therapeutic applications of microbes. Achieving this, however, remains challenging, laborious, and often unattainable, especially for anaerobes that dominate the microflora in many ecosystems. One key technical obstacle is to achieve two incompatible goals: using destructive gene-based analysis to identify colonies of interest and retrieving live cells from colonies. We report a microfluidic approach that enables cultivation of microbes on the microscale and from each colony creates two copies: one for destructive analysis and one for scale-up culture. In subsequent work, this method enabled isolation, from a human biopsy, of a microbe representing a new genus.

Introduction

Microbial communities play critical roles in a number of ecosystems and have significant consequences for climate change,[1] development of biofuels,[2,3] and human health.[4–6] For example, human-associated microbes such as those found in the gastrointestinal tract impact myriad physiological processes of the host, including metabolism,[7] immunity,[8] and behavior.[9] Recent developments in metagenomics,[10] the study of the total genetic material acquired directly from environmental samples, are beginning to reveal important microbial taxa[8,11–13] that may play a key role in various ecosystems. Isolating these microbial targets, having specific genotypes of interest, as pure cultures from environmental samples is critical for obtaining high-quality microbial genomes, elucidating microbial functions, understanding how they impact the health and disease state of the host, and potentially leveraging microbes for therapeutics. However, genetically targeted cultivation is challenging using conventional approaches. The microbial targets are often "unculturable,"[14] because bulk culture conditions frequently cannot recapitulate the microbes' natural environments. Moreover, genetically targeted cultivation requires gene-specific assays, such as PCR or fluorescence

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in situ hybridization (FISH), to be performed on each microbial colony; in addition, such assays frequently involve processes, such as thermocycling and fixation, which can damage or even destroy microbial cells. This can prevent the microbes being analyzed from being preserved for subsequent cultivation and requires additional laborious and time-consuming liquid handling. These problems are exacerbated for the prevalent case of anaerobes, whose growth requires a carefully controlled gas environment—for example, by using an anaerobic chamber or a Hungate roll tube, which further increases the complexity of the workflow. While robotic systems are sometimes used to process aerobes, such as *E. coli* and yeast, their bulky volume, high cost, maintenance and operation constraints, and limited ability to image and isolate heterogeneous colonies from complex microbial communities restrict their applicability to isolating and culturing microbes from environmental samples under anaerobic conditions.

Microfluidic technologies[15–17] offer unique features for overcoming these problems: they allow superior control of the cellular microenvironment;[18–21] enable the use of growth substrates available only in small quantities, as described in an accompanying study;[22] facilitate sensitive detection of microbes,[23,24] also explored in an accompanying study;[22] enable the retrieval of valuable reagents;[24,25] and streamline the workflow to increase throughput, such as through parallelization.[16,26] While microfluidics has been used for the cultivation of aerobic microbes,[19,27,28] many real-world ecosystems are dominated by anaerobes, whose cultivation is more challenging because the microfluidic chip may need to be designed to both enable control over the gaseous microenvironment and also allow facile handling inside an anaerobic chamber. Recent work has also demonstrated the ability of some microfluidic systems to perform on-chip genetic assays;[29,30] this is crucial for the genetically targeted cultivation of microbes from environmental samples. These systems do not provide live cells post-analysis, however, due to the harsh processing employed in such assays. We thus envision that the ideal microfluidic platform for performing genetically targeted cultivation would integrate three additional essential functionalities: (i) on-chip cultivation of anaerobic, as well as aerobic, microbes from environmental samples; (ii) splitting of each of the microbial microcolonies into two addressable, replica copies, one of which can be used for potentially destructive genetic assays, the other of which can be used to preserve cells in a viable state for future use; and (iii) retrieval and scaling up of target colonies to obtain enough biomass for further characterization and use.

In this paper, we describe a microfluidic device design for creating individually addressable arrays of replica microbial cultures. This design integrates these three crucial functionalities within a single platform. This current paper focuses on the design, fabrication, underlying physics, and operating principles of the microfluidic device; in a separate, complementary paper,[22] we describe how this device, combined with strategies of genetically targeted isolation and cultivation, enabled isolation of a microbial taxon from the NIH Human Microbiome Project's "Most Wanted" list.[31] Our approach relies on the SlipChip,[32] a microfluidic device consisting of two plates etched with wells, which act as individual microcompartments, and ducts, which act as fluid conduits. Relative motion of the two plates along the in-plane direction ("slipping") is used to create and manipulate droplets, which we use here to confine[18] and cultivate microbes at the single-cell level. We further expand the capability of the SlipChip to enable these droplets to be divided into identical replicas, and develop and characterize the physics of the process of separating the resulting plates containing these replica droplets ("splitting"). One plate can then be analyzed using a range of methods or exposed to a variety of environmental conditions, such as thermocycling, drying, or fixation, thus enabling the replicas to be further analyzed or assayed without constraining them to the conditions required for preservation of live cells. The other plate, which contains the replica droplets, can be used to preserve colonies for subsequent cultivation. Importantly, this approach is promising for use with anaerobic samples: the device is made of glass, which restricts gas diffusion, and does not require complicated equipment such as pumps and valves, thus allowing it to be used in space-limited anaerobic chambers. Moreover, the SlipChip[33] is compatible with solutions of a wide range of viscosities and surface tensions, and we adapt it here for handling cultivation medium with various ingredients. Furthermore, tunable surface chemistry, flexible device architecture, and simple operation make it ideal for performing on-chip assays ranging from nucleic acid amplification and analysis,[29] to proteomics[34] and immunoassays.[35,36] In addition, the splitting capability developed here provides a direct and convenient means of retrieving individual droplets.

Results and discussion

The vast library of metagenomic data offers a promising avenue for streamlining cultivation of microorganisms, as knowledge gained from metagenomic studies could be used to facilitate the selective isolation of microorganisms of high importance or biomedical interest. To enable this gene-targeted approach, we designed a microfluidic device, based on SlipChip technology,[32] to cultivate microbial cells and to split and retrieve the microbial culture. On this device, the microbial suspension can first be separated into many droplets, each having a small volume such that the number of droplets is larger than the number of microbial cells *via* a "stochastic confinement."[18,23,24,37] The confined microbial cells are then incubated to allow growth of microcolonies within each droplet. The device is composed of a pair of two plates, which can then be split for the dual purpose of performing destructive assays on one plate, and targeted scale-up culture on identical copies of the same microcolony on the other plate (Fig. 1).

Device design and operation for microbial cultivation

To achieve stochastic confinement and microbial cultivation, we designed a "replica-SlipChip" device containing 1000

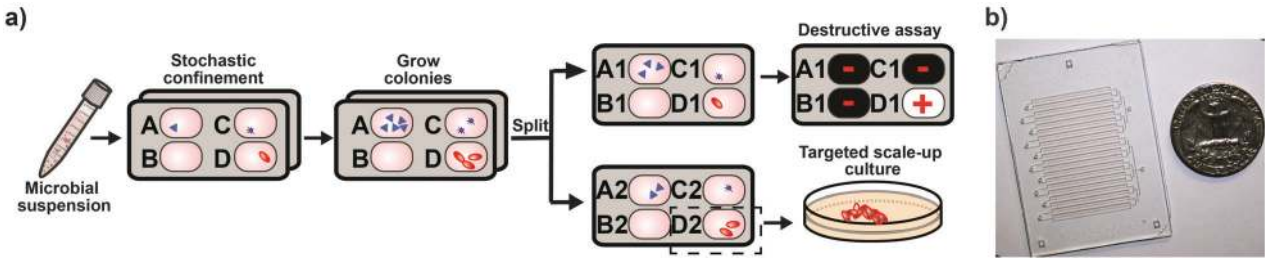

Fig. 1 (a) Illustration representing a microfluidic device designed to create individually addressable arrays of replica microbial cultures. A suspension of a diverse community of species, represented by different shapes and colors, is loaded onto a replica-SlipChip. Single microbial cells can be stochastically confined and cultivated to grow microcolonies. The chip is then split to make two copies of each colony. The first copy can be used for performing destructive assays; the second copy can be used for preserving viable cells for subsequent scale-up culture. (b) A photograph of 1000 microcompartments generated and stored on a replica-SlipChip, shown next to a U.S. quarter.

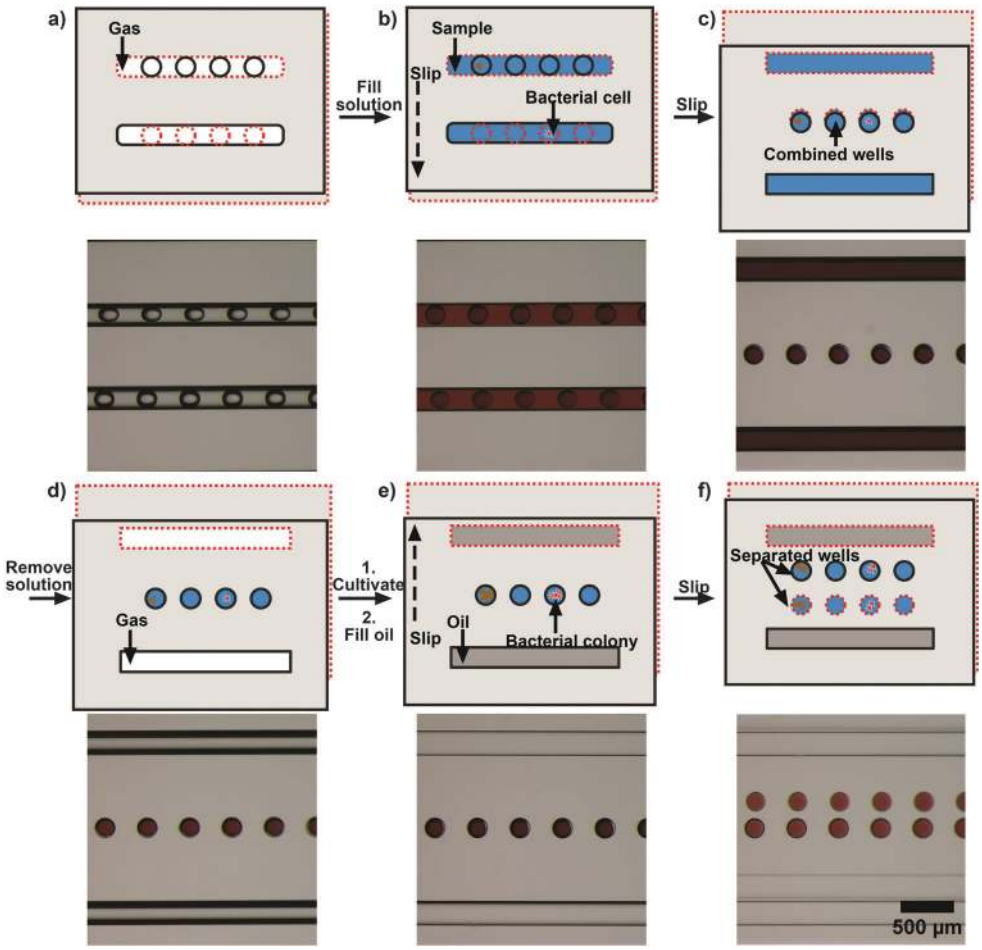

Fig. 2 Schematic drawings (top rows) and photographs (bottom rows) of a replica-SlipChip, illustrating device design for microbial culture and its operation visualized with red dye experiments. Top rows show illustrations of device operation with a sample containing a suspension of cells; bottom rows show representative photographs of the chip loaded with a red dye solution. (a) An empty chip was aligned so that wells and channels overlapped. The oil in the channel was removed by applying a vacuum to the inlets. (b) Sample was loaded. (c) The chip was slipped to overlap the wells in the two plates. (d) Channels were purged with a vacuum so that air was introduced through the inlets. The device was then incubated to promote microbial growth. (e) Channels were flushed with oil. (f) The chip was slipped to separate the overlapping wells and prepare their contents for splitting.

microcompartments (Fig. 1(b) and 2). Each microcompartment is composed of one well on the first plate and a paired well on the second (opposing) plate (Fig. 2(a); solid and dashed lines indicate different plates). The identical wells on the opposing plates can be combined, forming a single microcompartment and enabling cultivation (Fig. 2(c)). After growth of microbial colonies, the two plates are then slipped, creating an identical copy of each colony array within the wells on both opposing plates of the chip (Fig. 2(f)). The device incorporates continuous channels for filling reagents into the microcompartments

or promoting rapid gas exchange during cultivation (Fig. 2(b) and (d)).

To illustrate the operation of the replica-SlipChip, we loaded it with an aqueous phase, dyed red to facilitate visualization (Fig. 2). For clarity, the following narrative both describes what happens to cells and colonies during the operation of the replica-SlipChip, and also points out the corresponding images of the red dye experiments. The device was designed so that wells on one side of the chip overlap with channels on the other plate, and so that each plate contains both wells and channels (Fig. 2(a)). First, we load the suspension containing cells of interest into the channels and wells. This loading is shown as the loading of red dye in Fig. 2(b). Then, the loading channels and wells are separated by slipping, and single microbial cells are stochastically confined in wells. Paired wells on either side of the chip are combined as one microcompartment. This step is shown as the formation of droplets of red dye solution (Fig. 2(c)). Here, we use the word ''droplet'' imprecisely to refer to a small volume of aqueous fluid. Next, the sample in the loading channel is removed by purging with a vacuum so that gas can fill the channel to support microbial growth (Fig. 2(d)). We observed that the solution trapped in the channel was removed, gas could be introduced into channels, and that the aqueous solutions (*e.g.*, of red dye) remained in the wells and were not removed by the vacuum. The device is then incubated to grow microbial colonies (not performed in the visualization experiment with red dye solution). To minimize loss of oil and water during incubation, we place the device in a Petri dish saturated with the vapor of oil and water. The next step is to generate two copies of each droplet. To enable this, lubricating oil is loaded into the device channels to replace gas (Fig. 2(e)). The two plates are then slipped apart to separate the two wells that made up each microcompartment containing a droplet (Fig. 2(f)).

This device is specifically designed to generate addressable arrays of replica microbial cultures; distinguishing it from the previous designs created for protein crystallization,[38] nucleic acid amplification,[29] and immunoassays.[35] To accomplish this, it relies on the use of continuous channels to load reagents into the wells, which are useful in two ways. First, it provides a straightforward way to deliver sample fluids to the wells, and then cleanly remove them from the channel to avoid bacterial overgrowth outside of the wells and to facilitate subsequent splitting. During splitting, the volume of aqueous fluid left in the channel is likely to dewet from the channel and form a droplet with cross-sectional dimension larger than that of the channel,[39] and therefore is likely to come in contact with and interfere with the droplets inside the wells. Second, during the removal of the sample fluid, this channel can be filled with a gas of controlled composition, essential for microbial cultivation. Furthermore, the channel promotes rapid gas exchange in and out of the microwells. We fabricated the replica-SlipChip in glass because it is compatible with imaging and can be incubated at 37 °C for without significant evaporation. Controlling the gas environment is important for cultivating microbes.[40,41] As glass is not gas permeable, the transport of gas molecules around the trapped fluid is achieved by diffusion through the lubricating oil in the gap between the two plates, which can be tuned by the design of the chip.[38] Without this channel, the diffusion length scale for the gas could be tens of millimeters. In this design, the diffusion path is short for every well, as each well is a small (hundreds of micrometers) distance away from the gas-filled channel (see Fig. 2(d), Fig. S3 and the text in the ESI†).

Characterizing the process of replica-SlipChip splitting

Next, we tested if the replica-SlipChip can be split into two separate pieces without cross-contamination of the liquid droplets. We designed a chip holder with alignment pins (Fig. 3(a) and Fig. S4, ESI†) to keep the top and bottom plate from shifting horizontally during separation. The chips were designed so that at the configuration shown in Fig. 2(f), the through-holes on the top and bottom plates were aligned so the device could be placed through the alignment pins and onto the holder for controlled splitting. The replica-SlipChip was separated under a layer of tetradecane to prevent evaporation, and separation of the two plates was achieved by gravity.

Robust splitting of the droplets requires careful tuning of the experimental parameters: in preliminary experiments, we observed that droplets frequently fell out of their wells or moved laterally during splitting, as illustrated in Fig. 3(a)(ii). These issues precluded the consistent identification and containment of replica droplets. This movement can be minimized by choosing aqueous-phase or lubricating fluids with closely matched densities. We hypothesized that the lateral motion of the droplets out of the wells was due to the flow of the lubricating oil that arises as the two halves of the device are separated, as illustrated in Fig. 3(a)(iii). In particular, a droplet can be pushed out of its well if the viscous pressure drop across the droplet is larger than the threshold capillary pressure holding it in place.[38,42,43]

To test this hypothesis, we experimentally modeled the effect of the oil flow using a simplified microfluidic device, fabricated using soft lithography of polydimethylsiloxane (PDMS), as illustrated in Fig. 3(b)(i). The geometry of this device closely resembled the geometry within the replica-SlipChip, as it was split; however, the simplified PDMS device enabled us to quantitatively determine the conditions for a droplet to be laterally pushed out of the well. We used three different devices, characterized by three different values of the channel height h, and thus, three different values of the capillary pressure associated with pushing liquid from the well into the channel, which we approximate as $P_{\mathrm{cap}} \approx \gamma \cos\theta \left(\frac{1}{H} - \frac{1}{h}\right)$; here γ = 52 mN m^{-1} is the interfacial tension between the dyed droplet phase and the lubricating oil, H = 180 μm is the height of the well containing the droplet, and θ = 138° is the average three-phase contact angle measured on nine static droplets at different locations on the device, respectively. We varied the imposed oil flow rate, Q, thereby varying the viscous pressure drop across the droplet, P_{visc}, and monitored the corresponding droplet morphology using an optical microscope; we numerically simulated flow through the exact channel geometry used, and used the simulation to calculate P_{visc} for each value of Q explored

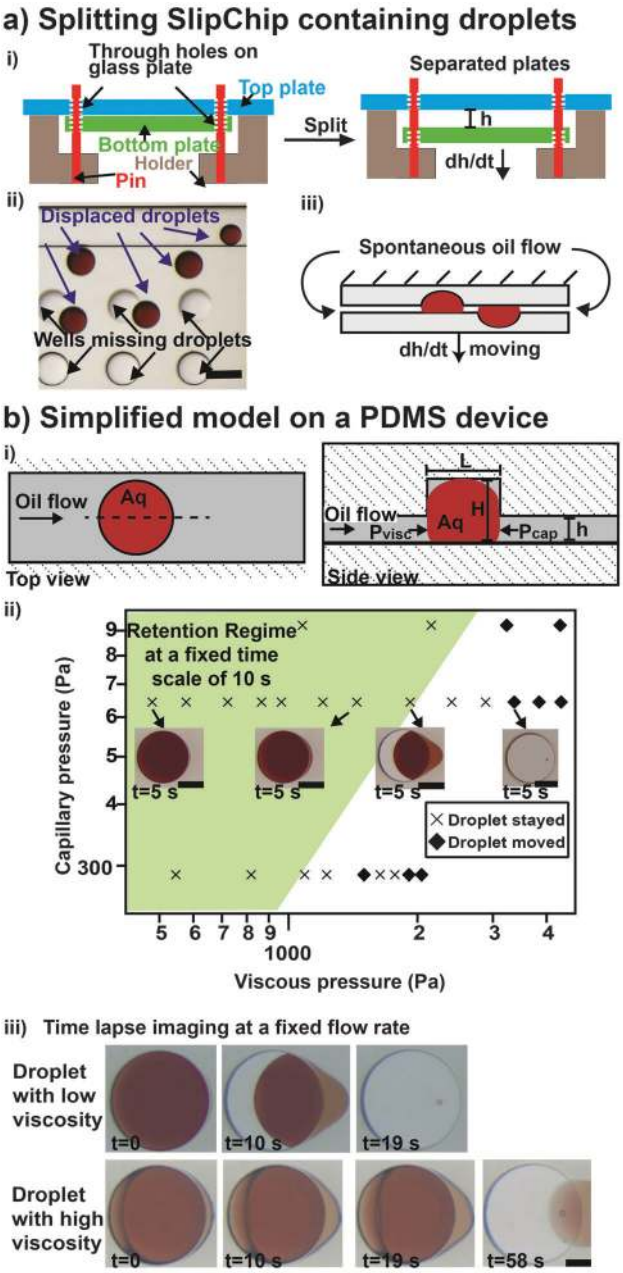

Fig. 3 (a) Splitting a replica-SlipChip while maintaining droplets in wells. (i) Illustration of SlipChip holder design. The corner of the bottom plate (green) was cut to fit into the holder. The top plate was designed to be suspended above the bottom of the holder. (ii) Microphotograph showing displacement of droplets after splitting the chip without the addition of agarose. Scale bar is 200 μm. (iii) Side-view schematic of the splitting process with droplets shown in (b). (b) Understanding the splitting process with a simplified model system. (i) Schematic of the device design. A droplet is anchored in a microwell and oil is introduced into the microchannel at a controlled flow rate. (ii) Regime of droplet retention (green) as a function of both the viscous pressure from oil flow and the capillary pressure. Each point on the phase diagram represents one experiment. Snapshots of droplets at 5 seconds (total duration of the imposed oil flow is 10 seconds) are shown as insets. The scale bar represents 200 μm. (iii) Time lapse photographs of droplets with different viscosities, showing that viscosity of the aqueous phase delayed the loss of the droplet. The scale bar represents 100 μm.

(see ESI†). The results of these experiments are summarized in the state diagram presented in Fig. 3(b)(ii). For all values of the threshold capillary pressure, we found that, for sufficiently low values of the viscous pressure, the droplet remained trapped within the well containing it, denoted by the X's in Fig. 3(b)(ii). However, within an experimental time scale of 10 seconds, similar to the time required to split the replica-SlipChip device, the droplet was pushed out when the viscous pressure drop across it was sufficiently large, approximately four times P_{cap}, denoted by the solid symbols in Fig. 3(b)(ii), consistent with our expectation; we note that the exact value of this threshold will likely depend on the time scale explored. Representative micrographs at five seconds are shown in the inset. Finally, we investigated the role of the droplet viscosity; even if the lubricating oil flow was sufficient to push a droplet out of its well, we expected that its motion would be slowed if it was composed of a high-viscosity fluid.[44–46] To test this hypothesis, we repeated this experiment in the same channel, using two different droplets composed of fluids that differed in their viscosity by a factor of 80. At a constant oil flow rate of $Q = 4$ mL h^{-1}, both droplets ultimately were pushed out of the well, as expected; however, this process took a longer time in the case of the high-viscosity droplet, as shown in Fig. 3(b)(iii), consistent with our expectation. These results thus demonstrate that droplets do not stably remain in their wells if the viscous pressure due to the flow of the lubricating oil exceeds a threshold, proportional to the capillary pressure. Even above this threshold, however, the droplet motion can be suppressed by using a high-viscosity dispersed phase. This result thus provides a guide for the robust operation of our device.

Separating a replica-SlipChip into two separate plates

To hold the droplets in the microwells during splitting, an ultra-low gelling temperature agarose was added to increase the viscosity of the droplet. To test if this setup could keep the droplets in microwells during splitting, 1% agarose aqueous solution was loaded onto replica-SlipChips while warm (~37 °C). The device was then incubated on a 10 °C chilling plate to gellify the agarose while remaining above the melting point of tetradecane (8 °C). The device was split on the holder under a layer of tetradecane. We observed that the shape of droplets changed during splitting, indicating that the droplet was partially released from the micro-structure (Fig. 4(a) and (b)). This shape change was not due to evaporation of droplets because the droplet shape could be restored by clamping the two plates back together. We analyzed 2000 wells on the whole device with a stereoscope (Fig. 4(c) shows a section of the device) and did not observe missing droplets or cross-contamination among wells during splitting. The droplets on the top plate did not fall onto the bottom plate, presumably due to the pinning of agarose gel (Fig. S5, ESI†). We varied the concentration of agarose from 0.3 to 2%. While 1% agarose was used for some preliminary experiments, we found that 0.5% was the minimum concentration that gave reliable results, and this concentration was used for all cultivation experiments.

On-chip cultivation of anaerobes and slowly growing microbes

Having shown the technical operation of the replica-SlipChip, we validated its functionality by growing a model anaerobic

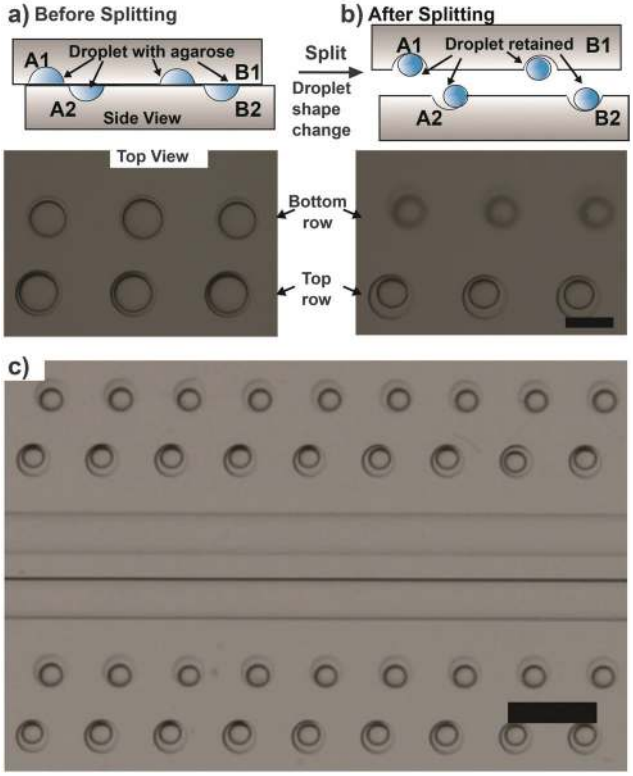

Fig. 4 Splitting SlipChip with 1% ultra-low gelling temperature agarose in water. (a) Top: side-view schematic of wells separated by slipping, as described in Fig. 2(f). The droplets adopted the shape of the microwell. Bottom: representative top-view photograph showing that the shape of the droplets conformed to that of the micro-well. Both top and bottom wells in the schematic are displayed. (b) Top: side-view schematic showing that the shape of droplets changed when the replica-SlipChip was split into two halves. Bottom: representative top-view photograph showing that the droplets were partially released from the micro-wells; the top wells were in focus and bottom wells were out of focus. Both indicated successful splitting. (c) A photograph of a section of the device after splitting. All droplets stayed in their respective wells. No missing droplet was observed. Scale bar is 200 μm for B and 500 μm for C.

microorganism as well as anaerobes from a clinical sample. We worked with replica-SlipChips inside an anaerobic chamber with the aid of a small dissecting stereoscope. To evaluate if the replica-SlipChip is compatible with culturing anaerobes, we used *Bacteroides thetaiotaomicron (B. theta)* as a model organism and loaded the cells in Cooked Meat Medium onto the device following the operation described in Fig. 2. After incubating at 37 °C for 8 h, *B. theta* cells grew to a dense microcolony, showing that the current design of replica-SlipChips is compatible with cultivating anaerobes (Fig. 5(a)).

Having demonstrated that a model anaerobe can be cultivated on a replica-SlipChip, we evaluated whether it could be used to grow samples from a diverse microbial community of anaerobes using microbes from a microbial suspension obtained from a mucosal biopsy from the colon of a healthy human volunteer. Cultivating members of a complex community from an environmental sample is challenging because different species grow at different rates, and slowly growing strains are often outcompeted by overgrowth of rapidly growing strains. Confinement can be used to prevent competition for nutrients by "weed" cells with a two-species model system of aerobes,[24] and here, we tested whether this practice would succeed with using a clinical sample. Further, to test if this approach is compatible with non-motile cells, we performed this experiment with a biopsy from the gut microbiome, species of which are often non-motile. We set up the cultivation experiment in an anaerobic chamber using a homemade AM2 medium[47] supplemented with 0.5% ultra-low gelling temperature agarose. The devices were then incubated at 37 °C in an anaerobic chamber for 8 days. Afterward, devices were imaged using a microscope to visualize growing microbial colonies. From this visualization, by using wells containing blank droplets as a negative control for growth, we reached three conclusions: (i) microbes from a microbial suspension obtained from a mucosal biopsy from the colon of a healthy human volunteer grow on a replica-SlipChip, and the addition of agarose did not inhibit microbial growth, as shown in Fig. 5(b)); (ii) rapidly and slowly growing microbes in a clinical sample can be successfully confined and cultivated on a replica-SlipChip (Fig. 5(b); and (iii) slipping successfully generates two daughter colonies if, after growth, the original single cell gives rise to a colony consisting of more than 10 cells (see Fig. S6 (ESI†) for distribution of cell numbers from this experiment).

Retrieval of target microbes from a replica-SlipChip demonstrated by isolating *B. vulgatus* from a clinical biopsy

In order to scale up microcolonies on the preserved half of the device for cultivation on an agar plate, we developed a simple technology for rapid retrieval of droplets on the replica-SlipChip (Fig. 6(a) and Fig. S8, ESI†). While a SlipChip enables simple on-chip manipulation of nanoliter-sized droplets, interfacing between the microfluidic device and an agar plate can be challenging. Transferring microbes from a chip to a plate requires careful indexing of droplet positions and avoiding contamination. The splitting capability developed here provides a direct way to access reagents on the device. Further, colonies are spatially indexed at a fixed position; tracking of individual droplets in droplet-based microfluidics[24] can be more challenging because indicators such as air bubbles or fluorescent dyes can be unreliable and may interfere with bacterial growth. We prepared a glass slide with indices for 1000 droplets (fabrication described in the ESI†) and used the same holder for splitting to align the index slide and the chip. 1 μL of buffer was aspirated using an Eppendorf pipettor; this volume merged spontaneously with the ~2 nL droplet on the chip when brought into contact. The combined droplet can then be used for spreading on plates for further growth, or testing with PCR and subsequent sequencing. This method is complementary to those used in other systems,[24,25,27,29,34,48–50] by providing a convenient way to address individual microcolonies on the device without complicated control systems of imaging and fluid manipulation.

We validated the replica-SlipChip's capacity for targeted retrieval and scale-up culture of microcolonies from a complex community by retrieving *B. vulgatus* from a microbial suspension

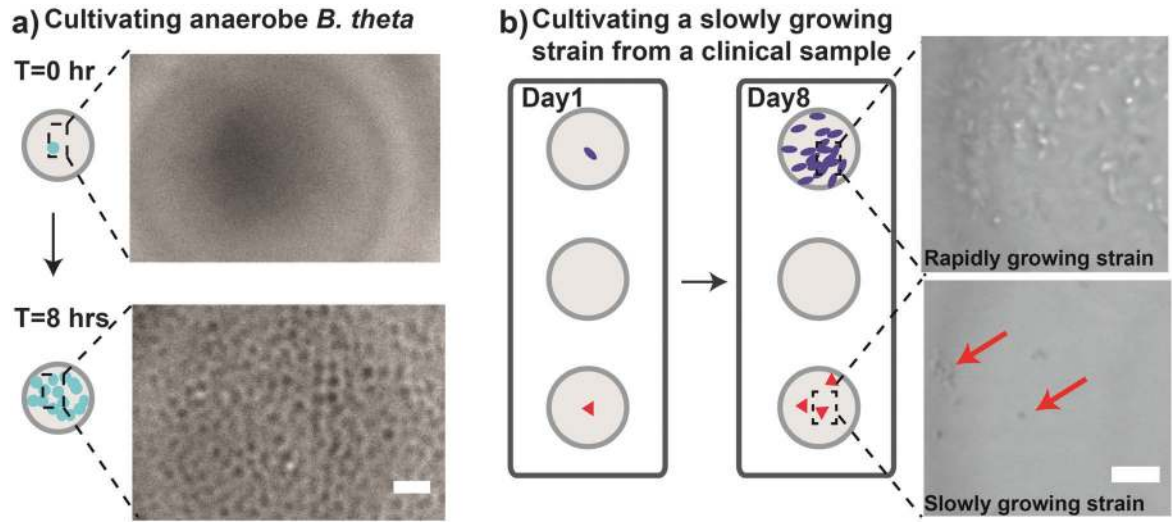

Fig. 5 Illustrations and photographs of anaerobic microbial cultivation on a replica-SlipChip. (a) Growth of anaerobic *B. theta* after 8 h of incubation. (b) Cultivation of a slowly growing strain from a diverse community using stochastic confinement after 8 days of incubation. Photographs in (a) show the same well at two time points, and photographs in (b) were taken at an endpoint after cultivation. Scale bar is 20 μm.

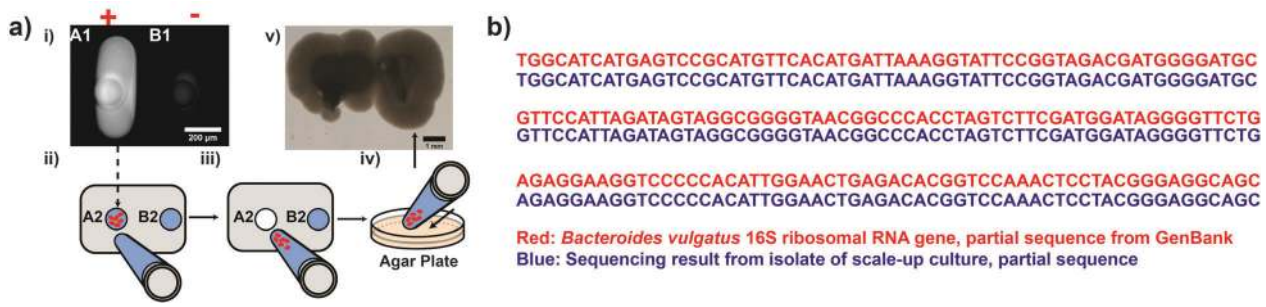

Fig. 6 (a) Retrieval of droplets from the replica-SlipChip and scale-up culture of *B. vulgatus*. (i) Photograph showing positive results for PCR with primers targeting *B. vulgatus*. In this case, well A1 was identified to have *B. vulgatus*. (ii) Schematic of droplet retrieval, showing a pipettor loaded with buffer that is used to retrieve live bacterial cells from duplicate droplets on the sample preservation chip (A1 and A2 were duplicate copies of the original droplet A). (iii) Schematic of ~2 nL droplet merging spontaneously with 1 μL buffer. Microbes (red) are drawn into the pipette tip. (iv) The combined solution is spotted onto an agar plate for scale-up culture. (v) A photograph of a scale-up culture of *B. vulgatus* isolated from the chip. (b) Alignment of partial sequences of the 16S rRNA gene sequence of *B. vulgatus* from GenBank (accession number EU728705.1) (red), and sequencing results from the isolate of the scale-up culture (blue) showed 100% identity at this particular region. Full sequencing results are 99% identical and are provided in the ESI.†

obtained from a mucosal biopsy from the colon of a healthy human volunteer. *B. vulgatus* is abundant in metagenomic data obtained from samples from the human gut.[11,51,52] Using a pair of primers reported[53] to be specific to *B. vulgatus*, the cultivation condition of *B. vulgatus* was identified by using Plate Wash PCR[40] (sequencing results provided in the ESI†). We then loaded the replica-SlipChip with the appropriate dilution of the biopsy with Wilkins-Chalgren Anaerobe (WCA) medium with 0.5% ultra-low gelling temperature agarose. After overnight (8 hours) incubation on the replica-SlipChip, we performed PCR on one half of the device (see ESI† and the accompanying paper[22] for details). In that experiment we obtained 104 PCR-positive wells. We then used the second half of the device to identify the corresponding wells putatively containing microcolonies. When we picked five of those microcolonies and transferred them to an agar plate, we obtained three colonies. We presume that the two false positive results may have come from lysed or non-growing cells, as this experiment was performed with a frozen sample and the viability of microbes is compromised during the freeze–thaw cycle. Sequencing of the 16S rRNA gene of the three isolates was used to confirm that the isolates were indeed *B. vulgatus* (Fig. 6(b); full sequencing results in the ESI†). We concluded that the microfluidic device described in this paper can be used to create individually addressable arrays of replica microbial cultures, and microbial microcolonies could be selected for scaled-up culture as demonstrated by retrieval of anaerobic microbes such as *B. vulgatus* from a clinical sample.

Experimental

Chemicals and materials, microfabrication of a SlipChip, fabrication of the holder, handling of microbial samples, bright field and fluorescence imaging, and sequencing results are reported in the ESI.†

Loading and incubating SlipChips

Empty chips for cultivating microorganisms on the replica-SlipChip were aligned so that wells and channels overlapped. We used tetradecane as lubricating oil. The oil in the channel was removed by applying a vacuum to the inlets and outlets. Once the vacuum was stopped, oil in the gap could flow back to the channel, and therefore the purging process was repeated 3 to 5 times, with 1- to -2 hour intervals between each purging, until no visible oil remained in the channel. A house vacuum was used for purging, and a gas recirculation pump was used as a vacuum source in the anaerobic chamber. If the gas recirculation pump is not available, an Eppendorf pipettor can be used to create a vacuum. Reagents, such as an aqueous solution of red dye or media suspension containing microbial cells, were then loaded. The chip was slipped to overlap the wells in the two plates. Channels were flushed with the gas in the environment (air or anaerobic mix) by purging with a vacuum through the inlets. A piece of Kimwipe was briefly saturated with a 1 : 1 (vol) mixture of water and tetradecane and then placed inside a Petri dish. The replica-SlipChip was then placed into the Petri dish and Parafilm was used to seal the Petri dish. The Petri dish was then incubated at the desired temperature for microbial culture. The Petri dish was placed in a vertical position to prevent uneven distribution of microbial cells in the two plates.

Splitting the replica-SlipChip

A warm aqueous solution containing 0.5–2% (w/v) ultra-low gelling temperature agarose was loaded onto the replica-SlipChip. After incubation, channels were flushed with oil and the chip was slipped back to separate the overlapping wells for splitting. The replica-SlipChip was placed on a 10 °C chilling plate for 1 hour. To place the replica-SlipChip onto the holder, three binder clips were removed from the device and one that fits the indent of the holder was used to hold the device. The through-holes on the device were carefully passed through the pins and the last binder clip was removed. The device, along with the holder, was placed in a Petri dish with enough tetradecane to immerse the chip. After 3 min, if the bottom plate did not separate from the top plate, tweezers were used to tap the device gently to avoid any jamming between the through-holes and pins.

Cell number calculation

The volume of medium in each microcompartment was $\sim$4 nL, and when loading a 1000-microcompartment device at the cell density of 2.5×10^4 CFU mL^{-1}, we expect approximately 100 CFU per chip.

Cultivation of *B. theta*

Stock of *B. theta* was enriched in Cooked Meat Medium overnight at 37 °C in an anaerobic chamber. Cells of *B. theta* were serially diluted to $\sim 10^5$ CFU mL^{-1} with Cooked Meat Medium and loaded onto the replica-SlipChip. The chip was then incubated for 8 hours at 37 °C in the anaerobic chamber.

Cultivating a fresh sample of microbial suspension obtained from a mucosal biopsy from the colon of a healthy human volunteer

A microbial suspension obtained from a mucosal biopsy from the colon of a healthy human volunteer (the sample was archived and de-identified) was prepared by suspending cells from the biopsy sample in GBSS buffer under the microoxic condition.[47] The sample was serially diluted with GBSS buffer and then with AM2 medium with 0.5% ultra-low gelling temperature agarose to 2×10^4 CFU mL^{-1} under the anoxic condition.[47] It was then loaded onto a replica-SlipChip, cultivated for 8 days at 37 °C in an anaerobic chamber, and imaged.

Aliquots of frozen stock were prepared under anoxic conditions[47] using GBSS buffer supplemented with 5% DMSO. Handling of this frozen stock sample is described in the ESI.†

Retrieving droplets on the device

A pipettor was used; when a small pipette tip loaded with 1 μL GBSS buffer was placed near the microcolony of interest on the SlipChip, the $\sim$2 nL droplet merged spontaneously with 1 μL buffer. The combined solution was then aspirated back into the pipettor.

Cultivating and isolating *Bacteroides vulgatus* on the SlipChip

The frozen microbial suspension obtained from a mucosal biopsy from the colon of a healthy human volunteer (the sample was archived and de-identified) was serially diluted with WCA media containing 0.5% of ultra-low gelling temperature agarose to $\sim 10^5$ CFU mL^{-1} and loaded onto the replica-SlipChip. The chip was then incubated at 37 °C overnight in an anaerobic chamber. Cultivar from PCR-positive wells was transferred to a WCA agar plate for scaling up. The identity of isolates was further confirmed by amplifying with universal primers for 16S rRNA gene and sequencing.

Conclusions

In this paper, we validated a microfluidic platform to create individually addressable arrays of replica microbial cultures using a clinical sample. While we validated it here using bacteria, we expect that other microorganisms including archaea and fungi could be cultivated using this platform. We also demonstrated that it can be integrated into a workflow for genetically targeted isolation of bacteria, validated by obtaining a previously unculturable isolate, as exemplified in the accompanying paper.[22] Furthermore, these approaches could be extended to mammalian systems, as shown recently in a microfabricated platform containing two matching arrays of three-dimensional microstructures useful for splitting colonies of adherent (in contrast to bacteria cultivated here) mammalian cells, and performing destructive assays.[54] Enzymatic or mechanical treatment[35] may be employed to retrieve adherent cells or aggregates from the device.

The splitting technology developed in this paper, and the corresponding physical measurements and modeling, may

become useful for applications beyond microbial isolation and cultivation. This technology provides a simple way to generate arrayed droplets on glass slides without the use of a microarrayer, and it is useful for preloading reagents, as demonstrated in Fig. S7 (ESI†). Retrieval of droplets is useful to interface the microfluidic platform with downstream applications. For example, a protein crystal grown on a SlipChip[38] can be retrieved using the splitting technology developed here and used as a seed in bulk experiments to obtain high-quality crystals for X-ray diffraction. It also provides a simple way to validate results from on-chip assays by retrieving the product for further study and characterization in a range of other applications, *e.g.*, when PCR products need to be removed for analysis by sequencing.[49]

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