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Patterning of Microorganisms and Microparticles through Sequential Capillarity-assisted Assembly

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Abstract

Controlled patterning of microorganisms into defined spatial arrangements offers unique possibilities for a broad range of biological applications, including studies of microbial physiology and interactions. At the simplest level, accurate spatial patterning of microorganisms would enable reliable, long-term imaging of large numbers of individual cells and transform the ability to quantitatively study distance-dependent microbe-microbe interactions. More uniquely, coupling accurate spatial patterning and full control over environmental conditions, as offered by microfluidic technology, would provide a powerful and versatile platform for single-cell studies in microbial ecology.

This paper presents a microfluidic platform to produce versatile and user-defined patterns of microorganisms within a microfluidic channel, allowing complete optical access for long-term, high-throughput monitoring. This new microfluidic technology is based on capillarity-assisted particle assembly and exploits the capillary forces arising from the controlled motion of an evaporating suspension inside a microfluidic channel to deposit individual microsized objects in an array of traps microfabricated onto a polydimethylsiloxane (PDMS) substrate. Sequential depositions generate the desired spatial layout of single or multiple types of micro-sized objects, dictated solely by the geometry of the traps and the filling sequence.

The platform has been calibrated using colloidal particles of different dimensions and materials: it has proven to be a powerful tool to generate diverse colloidal patterns and perform surface functionalization of trapped particles. Furthermore, the platform was tested on microbial cells, using *Escherichia coli* cells as a model bacterium. Thousands of individual cells were patterned on the surface, and their growth was monitored over time. In this platform, the coupling of single-cell deposition and microfluidic technology allows both geometric patterning of microorganisms and precise control of environmental conditions. It thus opens a window into the physiology of single

microbes and the ecology of microbe-microbe interactions, as shown by preliminary experiments.

Introduction

Spatial patterning of single microorganisms, particularly within experimental arenas that enable full control over environmental conditions, such as microfluidic devices, is highly desirable in a broad range of contexts. For example, arranging microorganisms into regular arrays would permit the accurate imaging of large numbers of individual cells and the study of their growth, physiology, gene expression in response to environmental stimuli, and drug susceptibility. It would also allow studying cell-cell interactions of particular interest in research into cellular communication (e.g., quorum sensing), cross-feeding (e.g., algal-bacterial symbiosis), or antagonism (e.g., allelopathy), with full control over the spatial localization of cells relative to each other. Cell physiology and evolution studies[1], cell-cell interaction studies[2], phenotypic differentiation screening[3], environmental monitoring[4], and drug screening[5] are among the fields that can greatly benefit from a technology able to achieve such quantitative single-cell analysis.

Several strategies to isolate and handle single cells have been proposed in recent years, from holographic optical traps[6] and heterogeneous surface functionalization methods[7,8,9,10] to single-cell chemostats[11] and droplet microfluidics[12]. These methods are either technically very demanding or affect cell physiology and fail to provide a high-throughput platform to pattern microbes that can be studied over long periods, ensuring single-cell resolution, full optical access, and control over environmental conditions. The goal of this paper is to describe a platform to pattern bacteria with micrometric precision into prescribed spatial arrangements

on a PDMS surface through capillarity-assisted assembly. This platform allows precise and flexible spatial patterning of microbes and enables full optical access and control over environmental conditions, thanks to its microfluidic nature.

The technology behind this platform is an assembly technology developed in recent years, named sCAPA[13,14,15] (sequential capillarity-assisted particle assembly) that was integrated into a microfluidic platform[16]. The meniscus of an evaporating liquid droplet, while receding over a patterned polydimethylsiloxane (PDMS) substrate inside a microfluidic channel, exerts capillary forces that trap the individual colloidal particles suspended in the liquid into micrometric wells microfabricated on the substrate (**Figure 1A**). Suspended particles are first transported to the air-liquid interface by convective currents and then placed into the traps by capillarity. Capillary forces exerted by the moving meniscus act on a larger scale compared to forces involved in particle interactions.

Thus, the assembly mechanism is not influenced by the material, dimensions, and surface properties of the particles. Parameters such as particle concentration, the speed of the meniscus, temperature, and surface tension of the suspension are the only parameters that influence the yield of the patterning process. The reader can find a detailed description of the influence of the aforementioned parameters on the patterning process in[13,14,15]. In the original sCAPA technology[13,14,15], the colloidal patterning process was carried out in an open system and required a high-precision piezoelectric stage to drive the suspension across the

template. This platform exploits a different strategy and allows the patterning to be carried out with standard equipment generally used in microfluidics in a controlled environment, thus minimizing the risks of contaminating the samples.

This microfluidic platform was first optimized on colloidal particles to create regular arrays of inert particles and then successfully applied to bacteria. Both microfluidic platforms are described in this paper (**Figure 1B,C**). Most of the preparatory steps and the experimental equipment described in the protocol are common for the two applications (**Figure 2**). We report colloidal patterning to demonstrate that the technique can be used to perform multiple sequential depositions on the same surface to create complex, multimaterial patterns. In particular, one single particle was deposited per trap for each step to form colloidal arrays with a specific geometry and composition, solely dictated by the traps' geometry and filling sequence. As for bacterial patterning, single depositions are described, resulting in one bacterium being deposited per trap. Once cells are patterned on the surface, the microfluidic channel is flushed with medium to promote bacterial growth, the preliminary step of any single-cell study.

Protocol

1. Silicon master preparation

NOTE: The PDMS templates bearing the microfabricated traps that form the template for colloidal and microbial patterning were fabricated according to the method introduced by Geissler et al.[17]. The silicon master was prepared by conventional lithography in a cleanroom. See the following steps for the procedure and the **Table of Materials** for the equipment.

1. Design the features using computer-aided design (CAD) software.

2. Prepare the chrome-glass mask with a layer of positive photoresist by exposing the designed features with a UV direct laser writer.

   1. Develop the chrome-glass mask with a spin developer using a developer at 1:4 photoresist to water ratio for 15 s.

   2. Immerse the mask in chromium etchant for 50 s.

   3. Plasma-treat a 10 cm silicon wafer for 3 min at 600 W.

   4. Vapor-deposit a layer of hexamethyldisilizane and bake at 110 °C to improve the adhesion toward the photoresist.

   5. Deposit a layer of photoresist at 4,500 × g for 2 min.

   6. Expose the feature to UV through the chrome-glass mask with a mask aligner for 2 s and develop with a developer as for the mask.

3. To complete the fabrication of the silicon master, etch the wafer via deep reactive ion exchange, adjusting the etching time (<2 min) to achieve the desired depth (measured with a profilometer).

2. Microchannel mold preparation

1. Design the channel with CAD software and slice it with a slicer software to convert the designed model into instructions for the 3D printer, setting the slicing distance at 0.05 mm.

2. Print the channel with a 3D printer for ~1 h.

3. Wash and postcure the mold in a dedicated curing and washing machine.

1. Put the mold in a container filled with pure isopropyl alcohol (IPA) and vortex the liquid (wash for 20 min). Take the container filled with IPA out of the machine.

2. Once the washed mold is removed from the IPA container, put it back into the washing and curing machine and postcure it for 15 min at 35 °C.

   NOTE: The **Table of Materials** reports the 3D printer and the curing and washing machine used in the mold preparation protocol. The 3D model was sliced with the 3D printer's proprietary slicer software.

4. Place the print in a UV oven for 12 h and place it in an oven at 80 °C for 12 h.

   NOTE: This step ensures that all the polymer is cured and all uncured polymer is removed from the print as it would prevent PDMS from curing.

5. Silanize the mold through vapor deposition of Trichloro (1H, 1H, 2H, 2H-perfluorooctyl) silane.

   1. Place the 3D mold in a vacuum desiccator along with 20 µL of 100% concentrated Trichloro (1H, 1H, 2H, 2H-perfluorooctyl) silane pipetted on an aluminum foil placed close to the mold.

   2. Create a vacuum in the desiccator to generate vapor and leave for 40 min.

   3. Remove the mold from the desiccator and rinse it with pure ethanol before pouring PDMS on it.

3. Fabrication of the microfluidic chip

1. Prepare a PDMS mixture by mixing the elastomer with its crosslinking agent (**Table of Materials**). Stir the mixture vigorously to blend the two components uniformly until air bubbles are formed, and the PDMS mixture looks opaque.

NOTE: The amount of cross-linker should be 10% by weight of the amount of elastomer.

2. Degas the mixture of elastomer and crosslinking agent in a vacuum desiccator to remove the trapped air bubbles. Transfer the mixture in the desiccator to the container used for mixing (step 3.1). Continue the degassing process until all the bubbles have been removed and the mixture looks transparent again.

   NOTE: The time typically required for desiccation is 30 min.

3. Pour 3 g of the mixture on the silicon master to produce the template, which will serve as the "floor" of the microfluidic chip.

   NOTE: The thickness of the PDMS layer can be tuned by changing the amount of mixture poured on the silicon master.

   1. To obtain a 400 µm-thick template, pour 3 g of PDMS on the silicon master, place the silicon master on a spin coater, and spin coat at 21 × g for 5 s and 54 × g for 10 s, and then degas again as described in step 3.2 to remove trapped air bubbles.

4. Pour 20 g of the PDMS mixture on the 3D-printed mold to make the microchannel, which will serve as the "roof" of the microfluidic chip, and degas it as described in step 3.2 for 30 min.

5. Bake both the silicon wafer and the 3D printed mold at 70 °C for at least 2 h.

6. Peel the PDMS layer off the 3D printed mold, cut the PDMS with a blade around the microchannels, and punch the holes that will serve as inlet and outlet of the microfluidic channel.

7. Peel the PDMS off the silicon master and cut the PDMS layer into smaller pieces with the same dimensions of the

microfluidic channels that will be bonded on top of the templates.

8. Gently rub the templates and microchannels using a 1% detergent solution (see the **Table of Materials**) for 5 min and then rinse with deionized water. Next, rinse the templates and microchannels with isopropanol and rinse them with deionized water. Dry the templates and microchannels at room temperature for 1 min with compressed air at 1 bar.

9. Place the templates and the microchannels in a plasma cleaner with the surfaces to be bonded facing up. Turn on the plasma cleaner, and plasma treat the templates and the microchannels for 40 s. Take them out from the plasma cleaner and immediately bond the microchannels on top of the templates by putting them in contact with one another.

10. Store the microfluidic chips in an oven at 70 °C for five days to ensure PDMS hydrophobic recovery and have a receding contact angle within the optimal range, between 30 and 60°[10,11].

4. Bacterial patterning

1. One day prior to the experiment, grow a population of *Escherichia coli* (strain MG1655 prpsM-GFP). Inoculate the culture directly from the frozen stock and grow overnight for 20 h in lysogeny broth (LB) medium in a shaker incubator at 37 °C. Add 50 µg/mL of kanamycin for the cells to retain the prpsM-GFP plasmid.

2. On the day of the experiment, set the box incubator (**Figure 2A**) at 37 °C several hours before the experiment to have a uniform and stable temperature before starting the experiment. Set up the syringe pumpand the heated glass plate on the microscope stage (**Figure 2B,C**),

setting the temperature at the same temperature as the box incubator.

NOTE: The box incubator in which the entire system, including the microscope (**Figure 2E**), is enclosed ensures that a uniform and constant temperature is maintained throughout the entire experiment when the channel is flushed with medium.

3. Ninety minutes before the experiment, put the microfluidic chip in a vessel filled with 100% ethanol (EtOH) and flush the channel with 100% EtOH for at least 10 min. Place the microfluidic chip in a vacuum desiccator and degas for at least 30 min. Exchange the EtOH with distilled water and vacuum-treat the chip for at least 30 min. Put the microfluidic chip in the oven at 70 °C for 10 min to remove any traces of liquid left in the channel.

NOTE: This step is necessary to prevent bubble formation in the channel when flushed with culture medium. This bubble prevention protocol was adapted from Wang et al.[18].

4. Pipette 1 mL of 3-(*N*-morpholino)propanesulfonic acid (MOPS) medium (1x) into a centrifuge vial and add 10 µL of 0.132 M potassium phosphate (K_2HPO_4). Take the overnight culture out of the 37 °C incubator and aliquot 100 µL into the centrifuge vial and centrifuge the culture at 2,300 × *g* for 2 min. Gently pipette the supernatant out of the centrifuge vials, and resuspend the pellet in 1 mL of fresh MOPS medium with 0.015% v/v of Tween 20 and 0.01% v/v of potassium phosphate.

NOTE: The typical concentration of the bacterial suspension is in the range of 0.015-0.15 vol%, which ensures the formation of an extended and mobile accumulation zone and prevents the accumulation of particles on the substrate. Replacing the overnight

medium with fresh medium minimizes the risks of releasing films of bacteria on the template. The lack of carbon source in the fresh medium prevents cells from growing in the suspension during template patterning. A concentration of 0.015% v/v of Tween 20 in the suspension is necessary for the contact angle of the receding liquid on the PDMS template to fall within the optimal range, between 30 and 60°.

5. Load the bacterial suspension in a 1 mL syringe and connect the syringe to the chip through microfluidic tubing.

   1. To secure the connection between the syringe and the tubing, directly insert a needle with an outer diameter of 0.6 mm into the tubing. To avoid scattering any suspension remaining in the inlet vicinity across the channel, flush fresh medium through the hole used as an outlet during the patterning process (**Figure 3A**-III), rather than the hole used as the inlet.

   2. Mount the syringe on the syringe pump and inject the suspension into the microfluidic chip through the inlet located at the upstream part of the channel until the suspension covers the template region with traps.
   NOTE: During the liquid injection process, air can escape through an outlet located at the downstream end of the channel.

   3. Set the syringe pump to withdraw the bacterial suspension (**Figure 3A**-I) at a flow rate of 0.07-0.2 µL/min, which in the described geometry corresponds to a meniscus receding speed of 80-100 µm/min.

4. Monitor the patterning process via microscope software.
   NOTE: Here, a 10x magnification was used to monitor the receding meniscus on the template, and a 20x magnification was used to monitor the deposition of individual bacteria into the microfabricated traps.

6. Once the template has been patterned with cells (**Figure 3A**-II), increase the withdrawal flow rate to quickly empty the microfluidic channel and flush it with fresh LB that was previously degassed for at least 30 min and prewarmed at 30 °C.

7. Set the syringe pump at a flow rate of 2 µL/min to gently flush the channel. Once the channel has been filled, increase the flow rate (15 µL/min) according to the specific experimental needs.

8. Acquire images of growing bacteria at the desired magnification and time interval.

5. Colloidal patterning

1. Pipette 900 µL of a 0.015% v/v Tween 20 aqueous solution into a centrifuge vial and pipette 100 µL of the original colloidal suspension into it. Centrifuge the suspension at 13,500 × g for 1 min. Gently remove the supernatant and replace it with the aqueous Tween 20 (0.015% v/v) solution. Repeat this process three times to ensure complete replacement of the supplier's solvent from the stock suspension.

2. Load the colloidal suspension in a 1 mL syringe and connect the syringe to the chip through microfluidic tubing. Inject the suspension into the microfluidic chip through the inlet located within the central section, at the

upstream part of the channel, and gradually push the suspension until the template is covered.

3. Withdraw the colloidal suspension at a flow rate of 0.07-0.2 µL/min, which corresponds to a meniscus receding speed of 1-2 µm/min, and image the patterning process via microscope software. Use 10x magnification to monitor the receding meniscus on the template and 20x magnification to observe the deposition of individual particles into the microfabricated traps. Increase the flow rate once the meniscus reaches the end of the template to empty the channel quickly.

NOTE: Colloidal arrays composed of several particles can be produced by running the patterning process from steps 5.1 to 5.3 in series. The channel is loaded with a new colloidal suspension at each iteration according to the desired colloidal arrays' composition. Each patterning step adds one particle to the colloidal array and can be run sequentially until the traps have been filled with the desired particles' sequence. The composition of the resulting colloidal arrays is solely given by the sequence of colloidal suspensions used to fill the traps (**Figure 3B**).

Representative Results

A microfluidic platform that exploits capillarity-assisted assembly to pattern colloidal particles and bacteria into traps microfabricated on a PDMS template was developed. Two different channel geometries have been designed to optimize the patterning of colloids and bacteria through the capillarity-assisted assembly. The first channel geometry (**Figure 1B**) consists of three 23 mm long parallel sections with no physical barrier between them. The two sections on the sides are 5 mm wide and 1 mm high, while the central section is 7 mm wide and 500 µm high. This design helps maintain a well-defined moving droplet with a receding convex-shaped meniscus.

The working principle of this platform is described in detail by Pioli et al.[11]. If the experiment requires filling the lateral channels, as in the case of bacteria culturing, air pockets are usually formed. For this reason, we designed and tested a second geometry that simplifies the filling process when the channel is flushed with medium. In this case, the platform (**Figure 1C**) consists of a single 20 mm long, 3 mm wide, and 500 µm high straight channel.

The temperature in the microfluidic channel is an important parameter to ensure an efficient deposition process. It must be maintained 15 °C above the dew point of water to avoid condensation on the template. The heated glass plate underneath the channel (**Figure 2D**) ensures a uniform temperature across the template to prevent condensation during the patterning process in the region close to the liquid-air interface, characterized by a high vapor concentration in the air. The temperature of the heated glass plate must be the same as that of the box incubator to avoid condensation on the template.

The straight channel geometry was exploited to pattern stationary-phased cells (**Figure 4A**) of a fluorescent *E. coli* strain (MG1655 prpsM-GFP). Bacteria were deposited in 83% of the 5,000 analyzed traps. The cells were first placed in the traps according to the presented protocol and subsequently grown for 4 h at 37 °C in LB flushed at 10 mm/min. Patterned bacteria resumed growth at different times within 1.5 h from when the channel was filled with fresh LB (**Figure 4B**-I), with the median at 44 min. Once growth is resumed, single bacterial cells start forming individual colonies, which expand (**Figure 4B**-II) until a surface layer is formed (3.5 h) and single-cell resolution is lost (**Figure 4B**-III).

This proof-of-concept experiment shows that capillarity-assisted assembly in a microfluidic channel can be used to

pattern a surface with thousands of viable single-bacteria cells. Eleven replicates show that patterned cells grew in 45.5% of cases within a 7 h window. Four tests conducted adding propidium iodide (PI), a live-dead stain[19], to the fresh LB flushed into the channel after the deposition proved that non-growing, patterned bacteria did not get stained. As PI binds to DNA but cannot penetrate cells with an intact membrane, the PI staining experiment shows that neither the patterning process nor the desiccation and rehydration processes damaged the cells' membrane.

The three-section channel geometry was used to produce linear colloidal arrays with different compositions by performing sequential patterning of colloidal particles. **Figure 5** shows the different colloidal arrays formed through sequential patterning, including dimers (**Figure 5A,B**) and trimers (**Figure 5C**), containing two and three sequentially trapped particles, respectively. Green and red fluorescent polystyrene particles were used to assemble both dimers and trimers. Analysis conducted over 55,000 traps show that green-red (G-R) dimers (**Figure 5A,B**) made by particles

with 2 µm and 1 µm in diameter were formed in 93% and 89% of analyzed traps, respectively. Green-red-green (G-R-G) trimers (**Figure 5C**) were formed in 52% of the 55,000 analyzed traps[11]. Dimers and trimers were assembled by running sequential depositions, with the colloidal suspensions moving in the same direction across all sequential depositions (**Figure 3B**). As a result, particles trapped in each deposition step are in direct contact with those trapped in the previous one.

Precise positioning of particles does not require direct contact between deposited particles. The distance between patterned particles can be precisely controlled by performing two depositions in opposite directions, thus trapping such particles at the opposite ends of each trap (**Figure 5D**). The distance between the trapped particles can be tuned by designing traps with the desired length. An additional possibility offered by the platform is chemical patterning of the surface with micrometric precision. This result can be achieved by patterning the template with chemically functionalized particles[11].
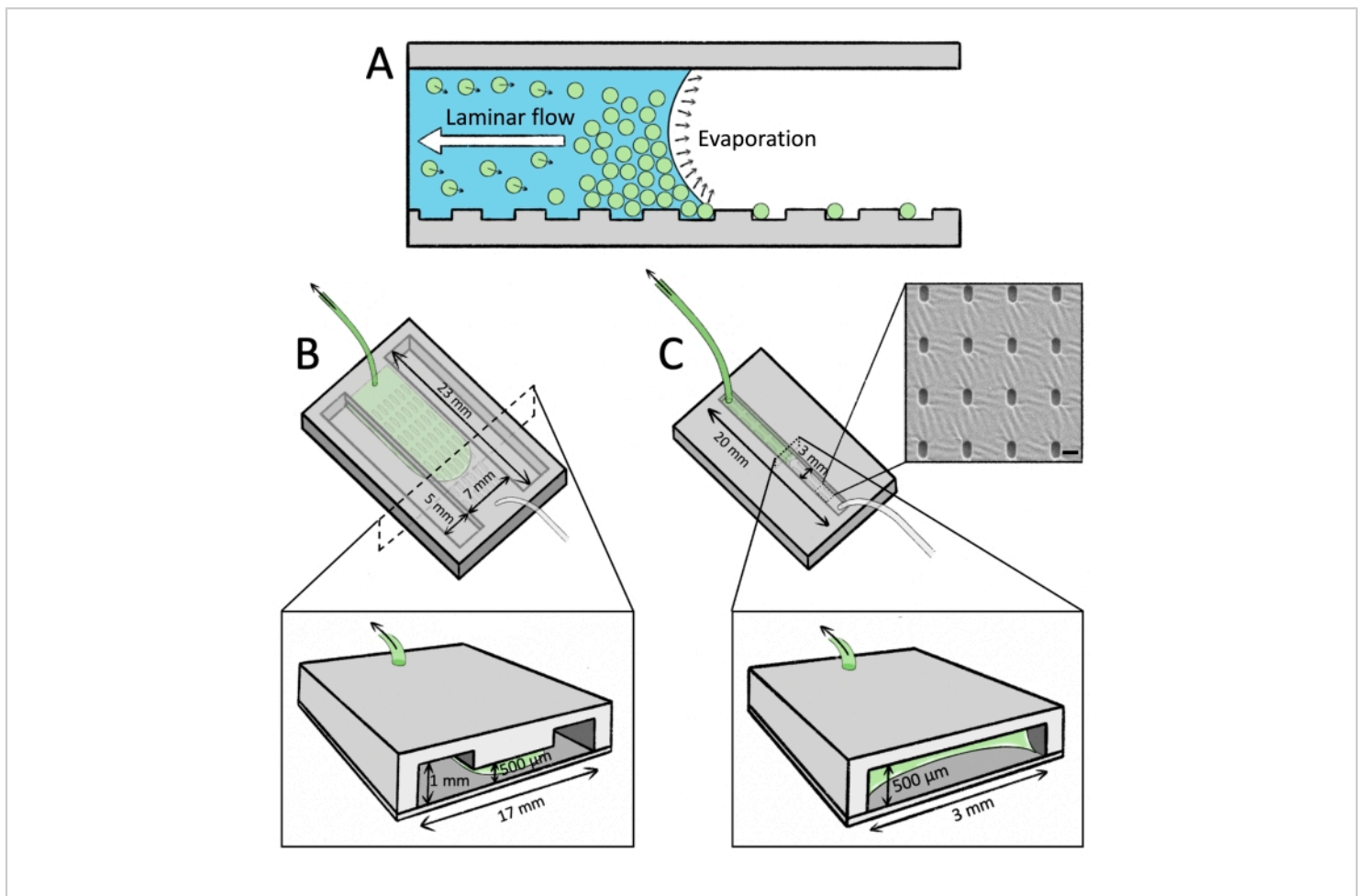
Figure 1: Microfluidic channels' geometries and patterning process. (A) Schematic of a side-view section of the microfluidic channel during patterning of colloidal particles. The liquid suspension evaporates inside the microfluidic channel, and convective currents transport suspended particles toward the air-liquid interface. Particles are thus accumulated and form the accumulation zone. The syringe pump pulls the liquid suspension, prompting it to recede, and individual particles get trapped during the liquid recession on the template. The arrow represents the direction in which the suspension is moving. **(B)** Schematic of the channel geometry used to pattern colloidal particles on the PDMS template. The channel is 23 mm long and 17 mm wide and consists of three sections: a central one that is 7 mm wide and two lateral ones that are 5 mm wide. The template is on the channel's floor, within the central section. The cross-section shows that the central section of the microfluidic channel, where the liquid suspension is confined throughout the patterning process, is the shallowest part of the channel and is 500 µm high, while the two lateral sections are both 1 mm high. **(C)** Schematic of the straight channel geometry used to pattern bacteria. The microfluidic device consists of a 20 mm-long, 3 mm-wide, and 500 µm-high straight channel. The rectangular section of this microfluidic device, shown in the cross-section, simplifies the filling process of the channel with medium. The SEM image shows a small portion of the PDMS template with 2 µm-long, 1 µm-wide, and 500 nm-

deep traps microfabricated on it. Scale bar = 2 µm. Abbreviations: PDMS = polydimethylsiloxane; SEM = scanning electron microscopy. Please click here to view a larger version of this figure.
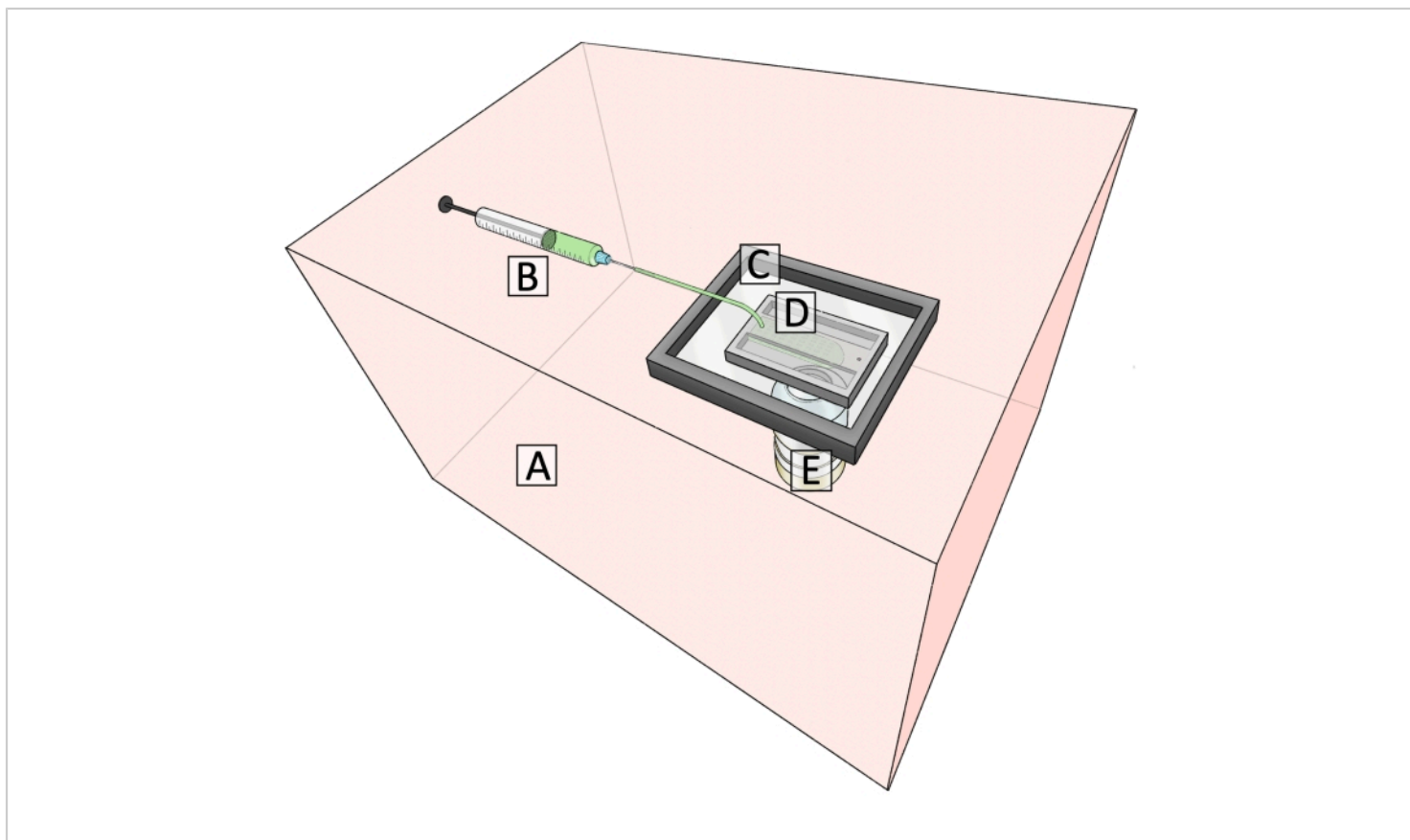

Figure 2: Schematic of the platform for sequential capillarity-assisted assembly in a microfluidic channel. (**A**) Box incubator. The box incubator maintains a uniform and constant temperature (here, 30 °C) inside the microfluidic channel. (**B**) Syringe loaded with liquid suspension. The syringe is controlled by a syringe pump (not shown) and is used to control the motion of the liquid during the patterning process. (**C**) Heated glass plate. The heated glass plate is placed underneath the microfluidic channel and ensures a uniform temperature (here, 30 °C) across the template, which is critical to avoid condensation in the vicinity of the air-liquid interface. (**D**) Microfluidic chip loaded with a liquid suspension. The microfluidic chip's floor bears the traps in which bacteria and colloids get patterned during the patterning process. (**E**) Microscope. The heated glass plate and the microfluidic chip are placed on a microscope stage, granting full optical access during the patterning process and all the following steps. Please click here to view a larger version of this figure.
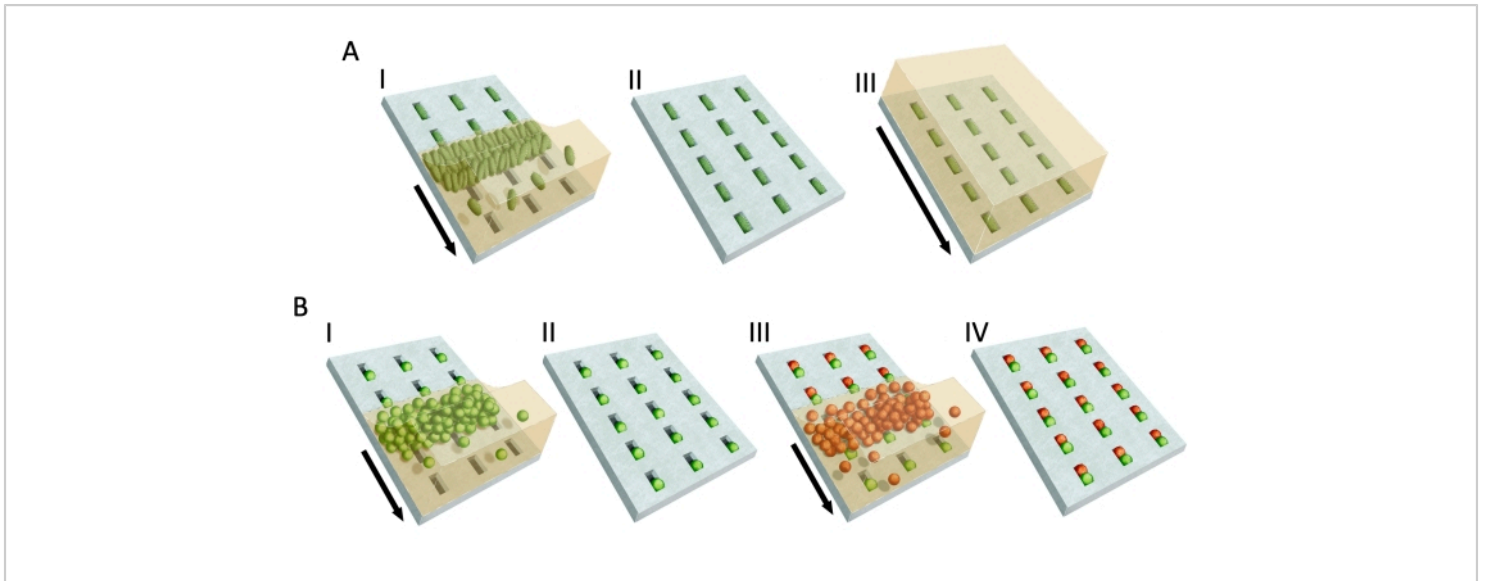
Figure 3: Schematic of the steps involved in bacterial and colloidal patterning. Each panel shows an inner view of the microfluidic channel and only includes a small portion of the PDMS template. (**A**) Patterning of bacteria through capillarity-assisted assembly. (I) The bacterial suspension evaporates inside the microfluidic channel, causing convective currents to transport suspended bacteria to the air-liquid interface, thus forming the accumulation zone. Meanwhile, the suspension is pulled by the syringe pump and recedes on the template. The arrow represents the direction in which the bacterial suspension is moving. The receding suspension sweeps across the template, and individual cells get deposited into the microfabricated traps. (II) Deposited bacteria are exposed to air until the channel is flushed with fresh medium. The deposition process is over when the liquid suspension reaches the end of the template. (III) The microfluidic channel is flushed with fresh medium (i.e., LB). The arrow represents the direction in which the fresh medium is flushed. (**B**) Patterning of colloidal particles through sequential capillarity-assisted assembly. (I) The colloidal suspension recedes on the template while evaporating, and particles accumulate at the air-liquid interface, forming the accumulation zone. Individual particles are deposited into the traps microfabricated on the template. The arrow represents the direction in which the colloidal suspension is moving. (II) The deposition process is complete when the liquid suspension reaches the end of the template. (III) A second deposition is run in the same direction as the first deposition to place a second particle into each trap on the template. (IV) Once the second deposition is over, the template is patterned with dimers of one green and one red particle. Abbreviations: PDMS = polydimethylsiloxane; LB = Lysogeny broth. Please click here to view a larger version of this figure.
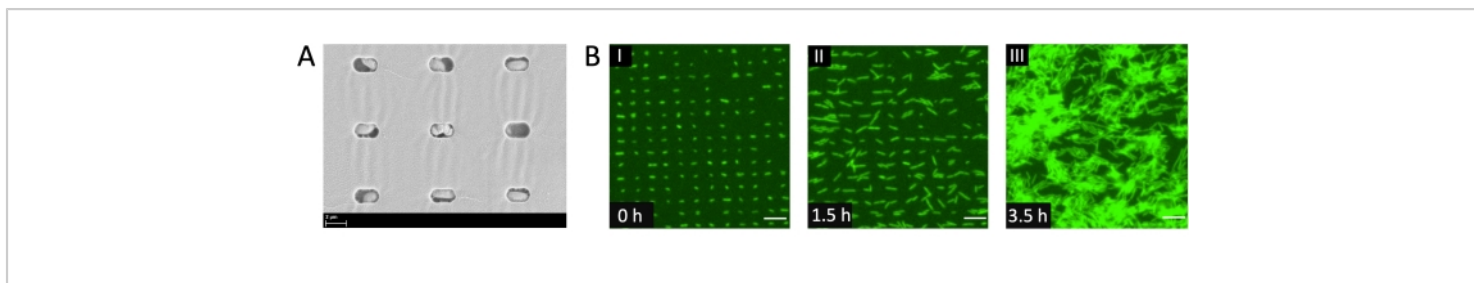
Figure 4: PDMS template patterned with *E. coli* (strain MG1655 prpsM-GFP) cells. (**A**) SEM image of individual *E. coli* cells trapped in 2 µm-long, 1 µm-wide, and 500 nm-deep traps. Cells were trapped after one single deposition. Scale bar = 2 µm. (**B**) Epifluorescence images of a small portion of the PDMS template (approximately 80 µm x 80 µm) with trapped cells of *E. coli* after the microfluidic channel is filled with culture medium (Lysogeny broth) at an initial speed of 1.3 mm/min, which is then increased to 10 mm/min. Trapped cells grow and divide multiple times for 4 h, eventually merging with cells from neighboring traps and covering the surface. Scale bars = 10 µm. Abbreviations: PDMS = polydimethylsiloxane; GFP = green fluorescent protein. Please click here to view a larger version of this figure.
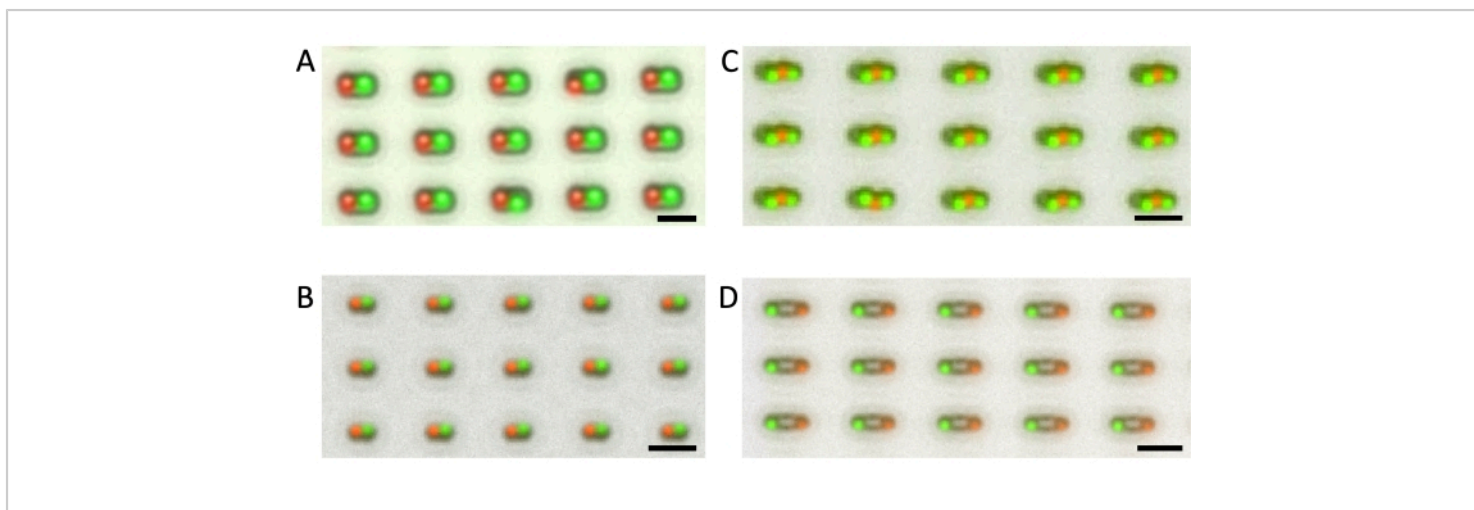
Figure 5: Colloidal clusters assembled through sequential capillarity-assisted particle assembly in the microfluidic platform (first channel geometry). Epifluorescence microscopy image of (**A**) 15 dimers assembled from polystyrene particles with 2 µm in diameter with two sequential depositions. (**B**) Dimers (n = 15) assembled from polystyrene particles with 1 µm in diameter with two sequential depositions. (**C**) Trimers (n = 15) assembled from polystyrene particles with 1 µm in diameter with three sequential depositions. (**D**) Traps (n = 15) with particles patterned at the extremities of each trap by running two sequential depositions in opposite directions. The distance between particles in a trap is 2 µm. Scale bars = 4 µm. Please click here to view a larger version of this figure.

Discussion

The microfluidic platform described here allows the patterning of micro-sized objects, such as colloids and bacteria, into prescribed spatial arrangements on a PDMS substrate. The full control over environmental conditions offered by microfluidics and the ability to pattern cells with micrometric precision granted by sCAPA technology makes it a very promising platform for future physiology and ecology studies.

In the experiments presented in this work, the silicon master was realized using the photoresist reported in the **Table of Materials**. However, any photoresist suited to produce features in the size range described and usable with PDMS molding could be used. The same applies to the 3D printing resin used to prepare the microchannel mold. Any resin capable of producing features of the described dimension and compatible with PDMS molding could be used.

In this protocol, storing the microfluidic chips at 70 °C for 5 days optimizes the surface hydrophilicity (contact angle falling between 30 and 60°) when Tween 20 is added to the bacterial or colloidal suspension. This procedure ensures the best reproducibility for the receding contact angle under these experimental conditions; however, other storing times and temperatures may work as well.

Two applications of this microfluidic technique have been presented: 1) bacterial patterning involving individual depositions of bacterial cells into the traps on the PDMS substrate, and 2) colloidal patterning involving sequential depositions of colloids, obtaining colloidal arrays of different compositions. This platform has been fully optimized to ensure high yields of bacterial deposition, with thousands of cells patterned on the PDMS template. The combination of capillarity-assisted assembly and microfluidics ensures single-cell resolution and stable flow of nutrients for several hours, with full optical access throughout the process. The patterned cells are capable of resuming growth once the microfluidic channel is flushed with fresh medium, although the viability of the patterned cells still needs to be optimized.

Currently, the major limitation of this technique is the lack of growth of patterned cells in 54.5% of experiments within a 7 h window. This aspect may be related to the specific bacterial species and needs to be further investigated to determine the root cause of the stress that prevents cells from regrowing. Air exposure and the force exerted by the meniscus during the patterning process may be among the major contributors to bacterial stress. Several possible solutions can be implemented to reduce such sources of stress, including the use of deeper traps to reduce the force applied on cells by the meniscus during the patterning process. An additional option to reduce the stress associated with air exposure would be to decrease the temperature of the heated glass plate. A temperature of 30 °C ensures a sufficient evaporation rate of the liquid suspension during the patterning process and would reduce the heat bacteria are exposed to after they are patterned on the template before medium is flushed.

Once fully optimized, we envision that this platform will be used in a variety of biological studies involving quantitative single-cell analysis. The high-throughput nature of the technique allows patterning of thousands of cells, providing large statistics within the same experimental arena. Full optical access enables tracking the behavior, growth, and molecular activities of large numbers of single microorganisms over time, thus providing major advantages in the study of phenotypic heterogeneity under homogeneous environmental conditions. The microfluidic nature of this platform ensures full controllability over environmental

conditions such as media composition, flow rate, and temperature, just to name a few.

Disclosures

The authors have no conflicts of interest to disclose.

Acknowledgments

References

1. Choi, C. H. et al. Preparation of bacteria microarray using selective patterning of polyelectrolyte multilayer and poly(ethylene glycol)-poly(lactide) deblock copolymer. *Macromolecular Research.* **18** (3), 254-259 (2010).

2. Smriga, S., Fernandez, V. I., Mitchell, J. G., Stocker, R. Chemotaxis toward phytoplankton drives organic matter partitioning among marine bacteria. *Proceedings of the National Academy of Sciences of the United States of America.* **113** (6), 1576-1581 (2016).

3. Thomas, C. M, Nielsen, K. M. Mechanisms of, and barriers to, horizontal gene transfer between bacteria. *Nature Reviews Microbiology.* **3** (9), 711-721 (2005).

4. Suo, Z., Avci, R., Yang, X., Pascual, D. W. Efficient Immobilization and patterning of live bacterial cells. *Langmuir.* **24** (8), 4161-4167 (2008).

5. Kane, B. J., Zinner, M. J., Yarmush, M. L., Toner, M. Liver-specific functional studies in a microfluidic array of primary mammalian hepatocytes. *Analytical Chemistry.* **78** (13), 4291-4298 (2006).

6. Akselrod, G. M. et al. Laser-guided assembly of heterotypic three-dimensional living cell microarrays. *Biophysical Journal.* **91** (9), 3465-3473 (2006).

7. Cerf, A., Cau, J. C., Vieu, C., Dague, E. Nanomechanical properties of dead or alive single-patterned bacteria. *Langmuir.* **25** (10), 5731-5736 (2009).

8. Cerf, A., Cau, J. C., Vieu, C. Controlled assembly of bacteria on chemical patterns using soft lithography. *Colloids and Surfaces: B Biointerfaces.* **65** (2), 285-291 (2008).

9. Rozhok, S. et al. Attachment of motile bacterial cells to prealigned holed microarrays. *Langmuir.* **22** (26), 11251-11254 (2006).

10. Xu, L. et al. Microcontact printing of living bacteria arrays with cellular resolution. *Nano Letters.* **7** (7), 2068-2072 (2007).

11. Ingham, C. J. et al. The micro-Petri dish, a million-well growth chip for the culture and high-throughput screening of microorganisms. *Proceedings of the National Academy of Sciences of the United States of America.* **104** (46), 18217-18222 (2007).

12. Moffitt, J. R., Lee, J. B., Cluzel, P. The single-cell chemostat: an agarose-based, microfluidic device for high-throughput, single-cell studies of bacteria and

bacterial communities. *Lab on a Chip.* **12** (8), 1487-1494 (2012).

13. Ni, S., Isa, L., Wolf, H. Capillary assembly as a tool for the heterogeneous integration of micro- and nanoscale objects. *Soft Matter.* **14** (16), 2978-2995 (2018).

14. Ni, S., Leemann, J., Buttinoni, I., Isa, L., Wolf, H. Programmable colloidal molecules from sequential capillarity-assisted particle assembly. *Science Advances.* **2** (4), e1501779-e1501779 (2016).

15. Ni, S., Leemann, J., Wolf, H., Isa, L. Nanoparticle Insights into mechanisms of capillary assembly. *Faraday Discussions.* **181**, 225-242 (2014).

16. Pioli, R. et al. Sequential capillarity-assisted particle assembly in a microfluidic channel. *Lab on a Chip.* **21** (5), 888-895 (2021).

17. Geissler, M. et al. Fabrication of metal nanowires using microcontact printing. *Langmuir.* **19** (15), 6301-6311 (2003).

18. Wang, Y. et al. Systematic prevention of bubble formation and accumulation for long-term culture of pancreatic islet cells in microfluidic device. *Biomedical Microdevices.* **14** (2), 419-426 (2012).

19. Crowley, L. C. et al. Measuring cell death by propidium iodide uptake and flow cytometry. *Cold Spring Harbor Protocols.* **7**, 647-651 (2016).