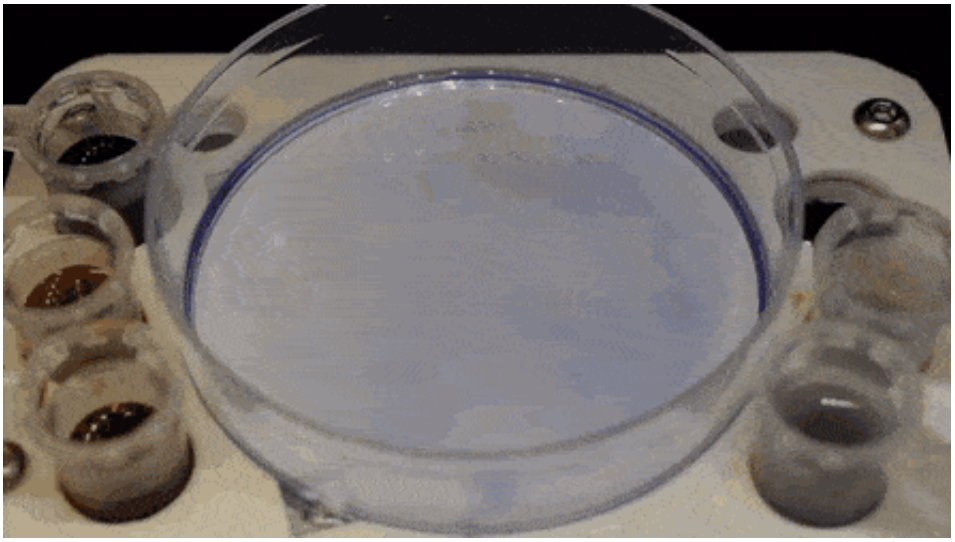
Raising fluid walls around living cells

June 14 2019, by Thamarasee Jeewandara



Reconfiguring patterns of the fluid walls. View full movie on, Credit: Science Advances, doi: 10.1126/sciadv.aav8002

Cell culture plates that are in everyday use in biology can be effectively transformed into microfluidic devices, opening paths for biologists to miniaturize cell-based workflows. In a recent report, Ph.D. researcher Cristian Soitu and co-workers in the departments of Engineering Science and Pathology at the University of Oxford, Oxford, U.K., described a simple method to create microfluidic arrangements around cells. In the study, the cells were already growing on standard Petri dish surfaces, when the scientists used the interface between the immiscible fluid media in the container as a building material.

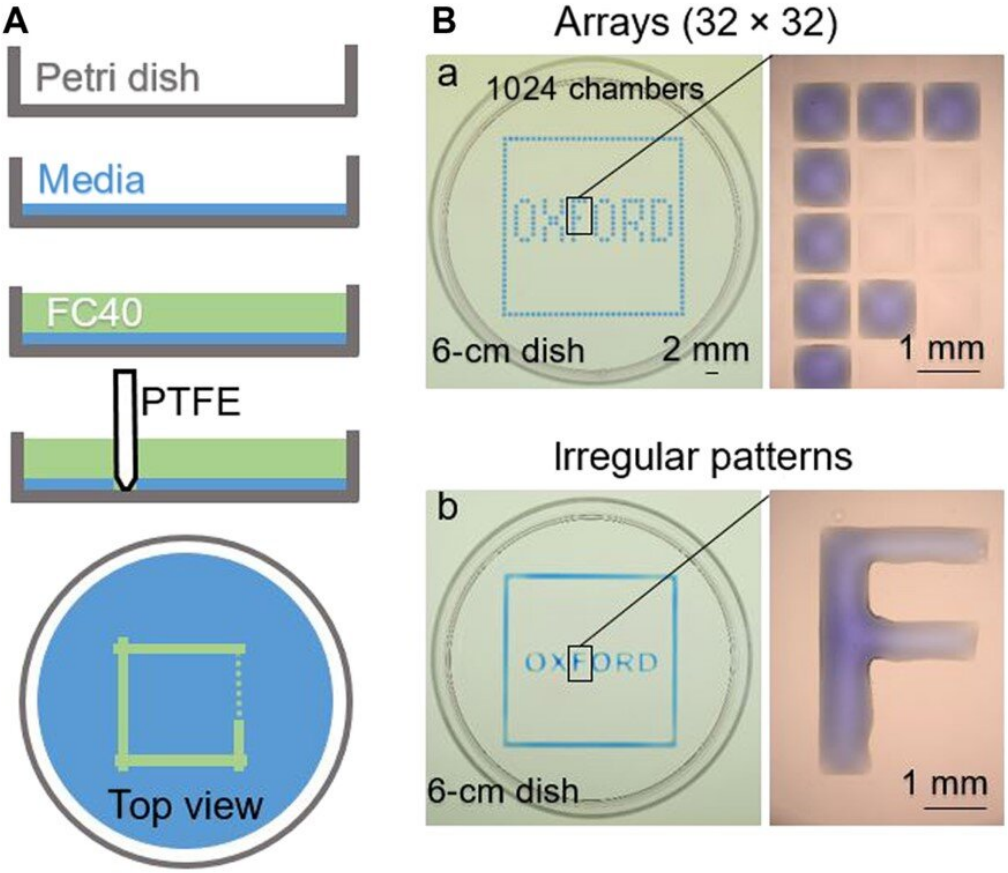
They re-purposed the conventional cell culture dishes into sophisticated microfluidic devices on demand by reshaping fluid structures around living cells. Soitu describes the new fluid-shaping technique built by his research team as "fluid structures for those cells with fear of commitment when choosing a home—they can be easily removed and new ones (with a different geometry) built in place." The research is now published on *Science Advances*

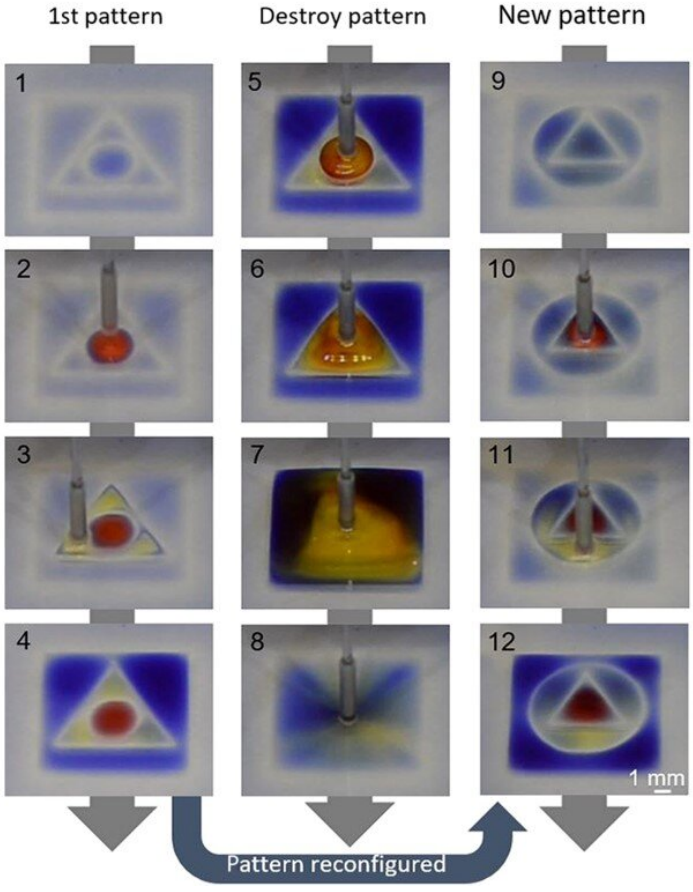
The researchers demonstrated the method using workflows involving cell cloning; selective cloning of a specific clone from among others in a dish; drug treatments; and wound healing. The research work demonstrated a versatile approach, coupled to biologically friendly features to promote the microfluidics technology among biologists. Microfluidics-based approaches have gained popularity in many workflows, although their uptake in mainstream biology remains slow due to a variety of contributing reasons, including:

1. Material incompatibility for cell growth
2. Microfluidic architectures that are enclosed and inaccessible
3. Predetermined geometries that cannot be reconfigured during experiments—causing cost of manufacture and operation
4. Workflows designed by engineers that do not align with pre-existing techniques developed by biologists.

In the past, scientists created 3-D constructs with fluid walls at the nanoscale, although their biocompatibility remains to be assessed. In the present work, therefore, Soitu et al. developed a method to make arrays of isolated microfluidic chambers on virgin petri dishes to accommodate major workflows in cell biology. Possible examples include cell feeding and transfer, cloning, cryopreservation, fixation and immunolabeling, cell lysis and reverse transcription polymerase chain reaction (RT-PCR) and CRISPR-Cas9 gene editing. In previous experiments of such workflows scientists added the cells after microfluidics fabrication.

In the present work, the researchers created a variety of microfluidic arrangements on standard petri dishes containing adherent cells and reconfigured them in real-time. They isolated and retrieved cell clones to perform proof-of-concept drug tests and wound-healing assays and introduced the new technique to create and reconfigure microfluidic circuits on petri dishes while cells grew and divided, with many potential applications in mainstream biology.


A
Petri dish
Media
FC40
PTFE
Top view
B
Arrays (32 × 32)
a
1024 chambers
6-cm dish
2 mm
1 mm
Irregular patterns
b
6-cm dish
1 mm


1st pattern
Destroy pattern
New pattern
1
2
3
4
5
6
7
8
9
10
11
12
1 mm
Pattern reconfigured

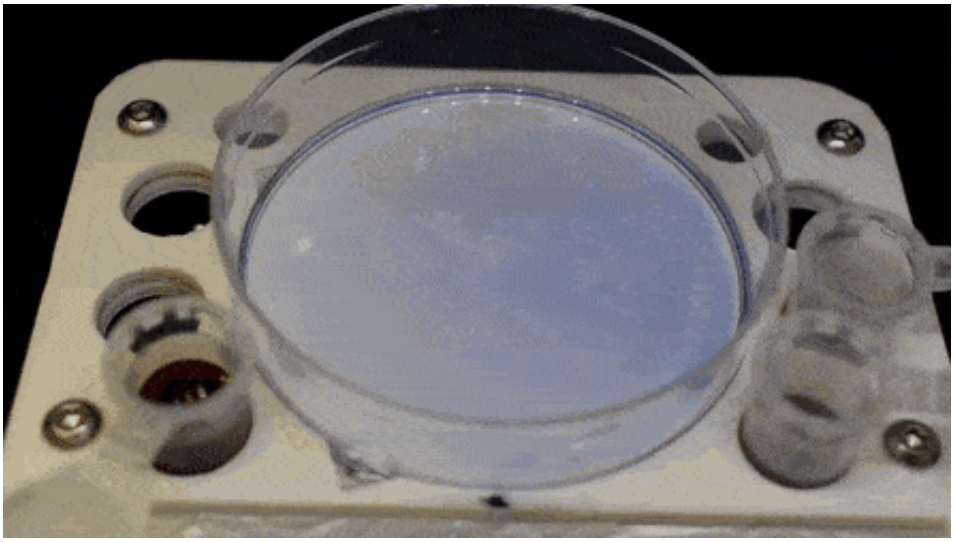
TOP: Chamber construction. (A) Principle. Dulbecco's modified Eagle's medium (DMEM) + 10% fetal bovine serum (FBS) is added to a virgin petri dish, and most of the medium is removed to leave a thin film covering the bottom, which is overlaid with FC40. The stylus is moved across the bottom to create a microfluidic arrangement. When complete, the initial volume of DMEM + 10% FBS will be divided into two parts separated by a continuous liquid wall of FC40 pinned to the substrate. (B) Different patterns. (a) Forming equally spaced vertical and horizontal lines creates an array (32 × 32; 1-mm spacing). Next, 60 nl of blue dye is added by the printer to selected chambers; peripheral chambers receive blue dye to give the blue square, and internal ones give the word "OXFORD." The magnification (right) shows individual chambers without and with dye. (b) A similar pattern is created by forming two squares (one slightly larger than the other) with the stylus and then adding dye manually to the space in between; each letter is made by forming its sides and again manually filling the interior. The magnification shows that the letter "F" is one continuous body of liquid. Photo credit: Cristian Soitu, University of Oxford. BOTTOM: Reconfiguring microfluidic arrangements. Images show frames from the movie. (1) An initial pattern is printed: a circle (radius, 1.5 mm) inside a triangle (side, 7 mm) inside a square (side, 9 mm). (2 to 4) Different dyes are added to each compartment (1.5 μl of red dye, 1.5 μl of yellow dye, and 5 μl of blue dye); dyes are confined within FC40 walls. (5) More yellow dye is added to the circle. (6) After adding 3 μl of yellow dye, the circular pinning line ruptures and contents spill into the triangle. (7) After adding 24 μl, the triangular pinning line ruptures and contents spill into the square. (8) Sixty microliters is withdrawn from the square. (9) A new pattern is printed—a triangle (side, 4.5 mm) in a circle (radius, 3.3 mm)—in the initial square. (10 to 12) Colored dyes are added to the three different compartments as before. Photo credit: Cristian Soitu, University of Oxford. Credit: Science Advances, doi: 10.1126/sciadv.aav8002

The new technique and the proof-of-concept experiments

In the experiments that followed, the researchers first covered the

bottom of a petri dish with tissue culture medium and removed most of the medium to form a thin film covering the polystyrene substrate. They overlaid the thin film with an immiscible fluorocarbon (FC40) to prevent evaporation and as a barrier against external contaminants to maintain sterility of the medium. Then using a Teflon tip, the researchers contacted the bottom of the dish, displacing the aqueous phase to form microfluidic arrangements in the shape of interest—in this instance, a square. Using the technique, the researchers brought the open microfluidic platform's advantages to standard cell culture-ware.

Soitu et al. shaped the aqueous phase to create a grid with low volumes of liquid as previously demonstrated by the same team, and viewed them with selective dyes in selective chambers. For instance, the peripheral chambers received a blue dye (forming a blue square) and those in the interior formed the word "OXFORD."



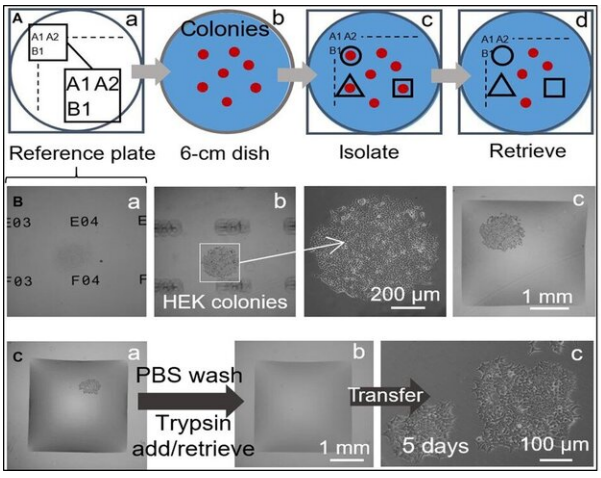
Creating chambers for the isolation of cell clones. View full movie on, Credit: Science Advances, doi: 10.1126/sciadv.aav8002

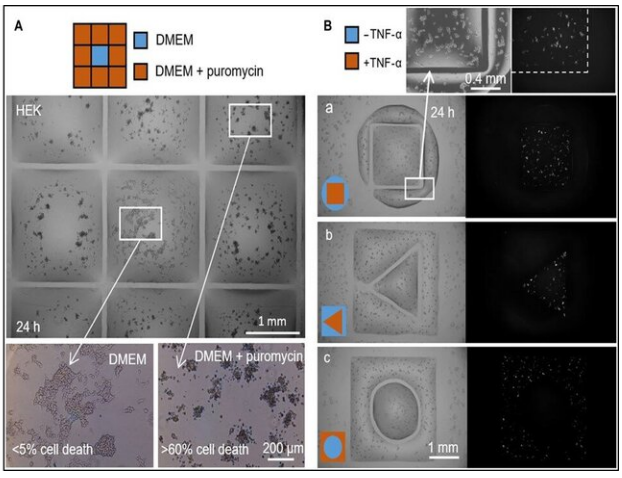
The researchers "printed" a circle within a triangle within a square and used microliters of three dyes to separately view the three shapes; where FC40 prevented the dyes from mixing. The results showed capability to build and destroy FC40 walls to effectively confine the liquids in any desired 2-D shape.

After the preliminary proof-of-concept results, Soitu et al. generated arrays of chambers to recapitulate the cloning of mouse mammary tumor cells (NM18), for which they initially created grids, followed by cell addition thereafter. The researchers first allowed the cells to grow freely surrounded by the FC40 wall permeable to both O_2 and CO_2, and then by growing single cells into clones before surrounding them with fluid walls of different shapes.

They showed that fluid walls with different 2-D footprints could be built easily around living cells, as long as the colonies remained isolated from each other during subsequent treatment or retrieval. Previous studies that grew cells within confined, pre-patterned surfaces required surface treatment prior to cell adhesion—contributing to the notable exception in the present technique.

Applications in clone picking and drug testing





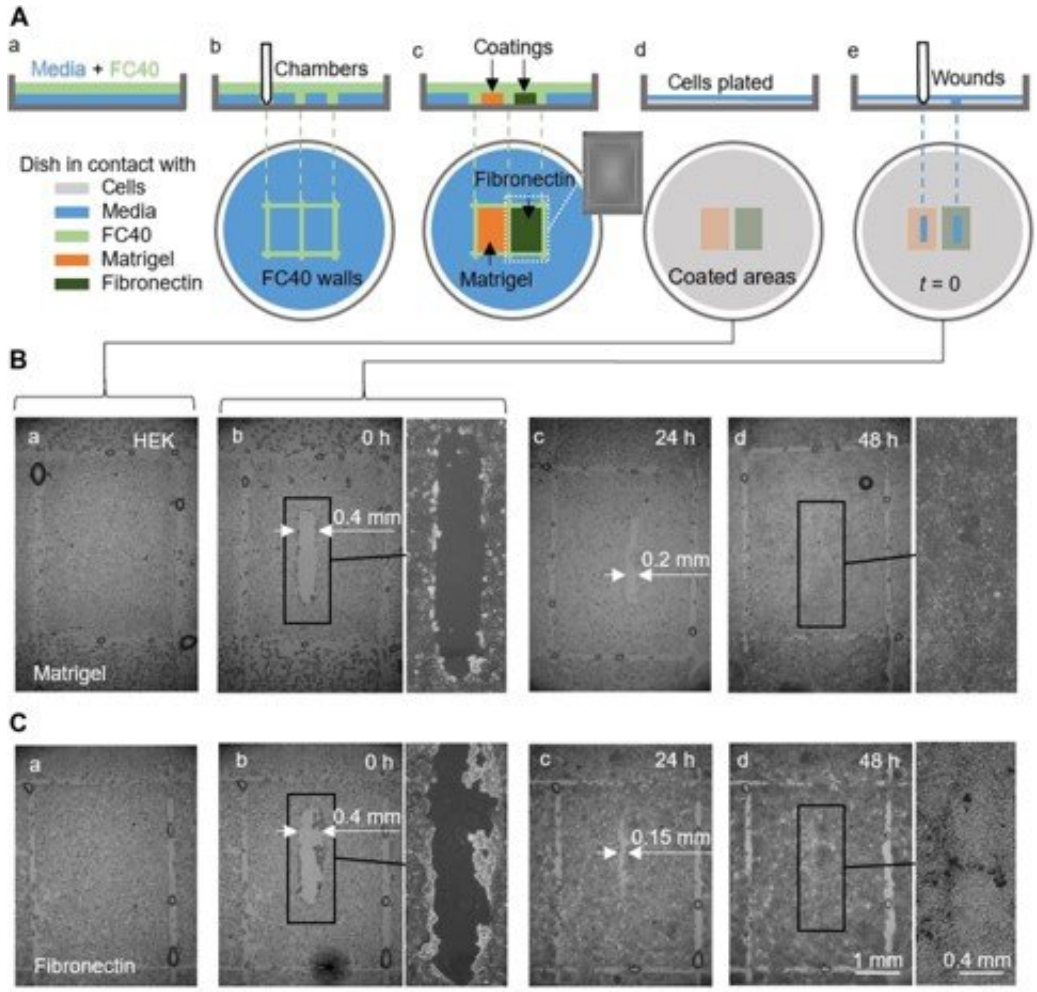
LEFT: Semi-automating selective clone picking (HEK cells). The printer adds/removes a microliter to/from chambers at different stages. (A) Approach. (a) Locations on a glass "reference plate" are marked by unique identifiers (i.e., A1, A2 …, B1…). (b) A 6-cm dish with colonies (red) is placed on the reference plate. (c) After recording colony locations and inputting them into a script, fluid walls are printed around selected clones (black lines). (d) Clones are retrieved from these chambers. (B) Isolating a clone. HEK cells were plated at low density (~1 cell/cm2) and grown (8 days) into clones, the dish was placed on a reference plate, and walls were built around selected clones. Three different z-axis views of one clone are shown. (a) Reference plate with unique identifiers in focus. (b) Colony in focus (identifiers out of focus) with magnification. (c) Colony after building surrounding walls. (C) Clone picking. (a) Square wall built around one living colony. The printer washes cells by adding/retrieving 1 μl of PBS; it then adds 1 μl of trypsin. (b) The dish is incubated (37°C; 5 min) to detach cells from the surface, and the printer retrieves 1 μl containing the cell-rich suspension (and transfers it to a microcentrifuge tube) to leave the now-empty chamber. (c) Retrieved cells are plated manually in a 12-well microtiter plate and grown conventionally for 5 days; cells attach and grow. RIGHT: Two drug treatments side by side with untreated cells. Fluid walls were built around HEK cells (300,000 cells; 6-cm dish) grown for 24 hours. (A) Puromycin (3 × 3 grid; 2 mm × 2 mm chambers). The printer adds 1 μl of medium to the central chamber and 1 μl of medium + puromycin to peripheral ones (final concentration, 10 μg/ml), as indicated in the cartoon. Cell viability is assessed after incubation (37°; 24 hours) using a trypan blue exclusion assay. Cells in outer chambers are dead (more than 60% in each one), whereas those in the central one remain alive (less than 5% cell death). This assay has been replicated three times. (B) TNF-α. Pairs of chambers with distinct shapes are printed, one surrounding the other. The printer adds 0.5 μl of medium ± TNF-α (final concentration, 10 ng/ml) to one or other volume (as in cartoons). As cells encode a GFP-reporter gene controlled by a promoter switched on by TNF-α, they fluoresce green on exposure to the cytokine. Fluorescence images show that only cells in the treated volume fluoresce green. Volume pairs had the following dimensions: (a) square (side, 1.8 mm) in circle (radius, 1.75 mm); (b) triangle (side, 1 mm) in square (side, 3.5 mm); (c) circle (radius, 1 mm) in square (side, 3.5 mm). Credit: Science

Advances, doi: 10.1126/sciadv.aav8002

In the next step, the researchers created a reference plate on which they placed a dish containing live cell colonies of interest to isolate cell clones of interest from others by printing fluid walls around them. On isolation, they could pick the colonies, recover the cells and grow them conventionally to multiply as expected. Since the fluid walls could effectively confine the liquids, Soitu et al. tested their efficiency by adding puromycin – a small molecule translator inhibitor that kills mammalian cells.

In the drug screening experimental setup, they allowed the central chamber to receive growth medium alone, while the drug was delivered to the surrounding chambers in a high lethal dose, to show the efficacy of FC40 separation when only the cell lines in the central chamber survived. In a second example, Soitu et al. exploited the property of a human embryonic kidney cell line genetically modified to encode a green fluorescent promoter gene. Which switched on in the presence of tumor necrosis factor-α to fluoresce green. The fluid walls formed effective barriers to drug exposure, verifying the technique's drug screening potential.

Applications in wound healing



A proof-of-concept wound-healing assay using one dish precoated with Matrigel and fibronectin in different regions. (A) Cartoon illustrating workflow. (a) A thin layer of medium is overlaid with FC40. (b) Two chambers (3 mm × 4 mm each) are printed side by side. (c) Surfaces in chambers are coated with Matrigel or fibronectin (2 μl; final concentration of 1 μg/cm2; 1 hour); the inset shows an image of one chamber. Fluid walls are now destroyed, and the dish is washed with 3 ml of medium to remove unattached coatings. (d) HEK cells (600,000) are plated in the dish. (e) After 24 hours, cells have formed a monolayer, and a wound (0.4 mm × 2 mm) is created by scraping the stylus over the surface to remove cells in its path. Healing of the wound is now monitored microscopically. (B and C) Images of wounds in monolayers grown on Matrigel or fibronectin. (a and b) Immediately before and after wounding (some droplets of FC40 remain where walls originally stood). (c) After 24 hours, cell growth reduces wound widths to