Microfluidics on Standard Petri Dishes for Bioscientists

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Few microfluidic devices are used in biomedical labs, despite the obvious potential; reasons given include the devices are rarely made with cell-friendly materials, and liquids are inaccessible buried behind solid confining walls. An open microfluidic approach is reviewed in which aqueous circuits with almost any imaginable 2D shape are fabricated in minutes on standard polystyrene Petri dishes by reshaping two liquids (cell-culture media plus an immiscible and bioinert fluorocarbon, FC40). Then, the aqueous phase becomes confined by fluid FC40 walls firmly pinned to the dish by interfacial forces. Such walls can be pierced at any point with pipets and liquids added or removed through them, while flows can be driven actively using external pumps or passively by exploiting local differences in Laplace pressure. As walls are robust, permeable to O2 plus CO2, and transparent, cells are grown in incubators and monitored microscopically as usual. It is hoped that this simple, accessible, and affordable fluid-shaping technology provides bioscientists with an easy entrée into microfluidics.

1. Introduction

Microfluidic approaches aim to increase throughput while minimizing cost and waste. Many microfluidic devices have been made for bioscientists (often out of polydimethylsiloxane, PDMS) using techniques applied successfully to shrink integrated circuits in computer chips, and some devices are incorporated into the specialized equipment they use (e.g., the tape sequencers made by Illumina and Oxford Nanopore). However, a recent review states “...most... biotechnologists are still not used to integrate microfluidic systems into their typical experimental procedures in a regular manner.” Many reasons are given for this, with probably the most important being that devices are complex and rarely made with materials familiar to biologists. Moreover, the aqueous phase is usually inaccessible buried behind solid walls, although open microfluidics improves this (Figure 1A). Clearly, an optimal solution is to construct devices out of the culture media and polystyrene dishes that biologists use daily to grow their cells. But how might one do so? Adaptations of existing approaches are possible, including confining cells in one phase in an aqueous two-phase system and by modifying dish surfaces. An approach that uses fluid (not solid) walls—an interface between two immiscible liquids—to confine media on untreated dishes provides a simpler answer.

The approach is based on three principles. First, at the microscale, gravity is weak. Consider raindrops stuck to windows; they defy gravity pinned to glass by strong interfacial forces. Therefore, a three-way traverse holding a “pen” can print “letters” of culture medium on a standard 6 cm Petri dish, and air–water interfaces hold the letters in place (Figure 1B). Second, nanoliter volumes in such letters (which have maximum widths and heights of ~500 and ~150 µm) soon evaporate, but this can be prevented by overlaying an immiscible and lighter-than-water oil. But if gravity—and so buoyancy—is irrelevant, denser oils become alternatives. For reasons we will see, the fluorocarbon, FC40, is particularly attractive as <7 ppm water dissolve in it, compared to <200 ppm for the silicone oil often used by biologists. (It was selected after screening only some of the many fluorocarbons currently available, so others may prove suitable.) Third, medium can be added/removed to/from such letters without change in aqueous footprint. Consider a 500 mL water drop sitting on a dish (Figure 1C). On adding (or removing) water, drop footprint increases (or decreases) only when the advancing contact angle, θA (or receding contact angle, θR) is reached. Between θA and θR, such contact-angle hysteresis ensures that drop footprint remains unchanged despite volume changes. Incredibly, for media used to culture human cells, θA is >70° and θR is <3°, so drops can hold a wide range of volumes as fluid walls/ceilings morph above unaltered footprints (Figure 1D).

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2. “Printing” Almost Any 2D Pattern

Fluid walls are built by reshaping media and FC40 with different tools in ways that artists recognize (Figure 2A). Often, they paint a pattern with a brush; in “positive printing”, a “pen” prints the circuit. They also cover a canvas in paint, and then remove some to develop the pattern (as in sgraffito); in “negative printing”, the bottom of a dish is covered with a thin layer of medium overlaid with FC40, and a “stylus” or “microjet” removes medium locally to replace it with FC40. Thus, a stylus (made of fluorophilic polytetrafluoroethylene, PTFE) or a submerged jet bring FC40 down through the medium into contact with the dish; as FC40 wets polystyrene better than medium, it remains stuck to the dish. In all cases, circuit fabrication depends on wetting sequence and interfacial forces acting on medium, FC40, polystyrene, and a tool.

Building straight fluid walls can yield “grids” with many chambers (Figure 2B) that are used like wells in microplates: liquids are simply added/removed by pipetting through FC40 instead of air. Surprisingly, these chambers accept a wider range of working volumes than those in 96-well microplates with the same interwell spacing (i.e., ~30-fold compared to ~8-fold for a standard well). Even more surprisingly, fluid walls are remarkably robust (Figure 2C), and dishes supporting them can be carried around labs like any filled dish. Complex circuits are built in much the same time it takes to draw freehand the plan on a piece of paper (e.g., a “human circulatory system” in ~90 min; Figure 2D).

Each printing method has advantages and disadvantages. For example, precision is reduced by changes in tool shape (e.g., as serum proteins in media aggregate on a pen, or a stylus wears when dragged over a dish). Additionally, a pen’s outer diameter, and jetting nozzle’s inner diameter, are major determinants of the minimum widths of aqueous features and FC40 walls, respectively. Consequently, jetting is often the method of choice, as it is contactless and forgiving. Then, we routinely use a portable “printer” small enough to fit in a biosafety cabinet (Figure 2E) that jets FC40 through a nozzle (internal diameter 70 µm) at 8 µL s⁻¹ to build walls =120 µm wide—and so sterile grids and circuits.
3. Benefits of Liquid FC40 Walls

At the microscale, liquid FC40 walls have many advantages over solid ones. [14–16] Their fabrication in minutes allows rapid prototyping (contrast the days taken to make PDMS devices in specialized clean rooms). Moreover, circuit layouts can even be reconfigured around living cells on the fly during experiments (e.g., flow through conduits can be started/stopped repeatedly by building/removing blocking walls, and new conduits/reservoirs can be added). [16–17] Significantly, FC40 is as transparent as water with a refractive index close it (i.e., 1.29 vs 1.33), so one can see through these fluid walls with little diffractive distortion. [18] Sampling pipets can also be inserted anywhere in circuits, and walls self-heal automatically when they are withdrawn. Additionally, air bubbles causing catastrophic failure in conventional devices [19] are lost spontaneously to the atmosphere through fluid walls.

FC40 is arguably one of the most bioinert immiscible liquids known. [12] The CF bond is not found in nature, and it is the strongest in organic chemistry ($\approx 485 \text{ kJ mol}^{-1}$, compared to $\approx 413 \text{ kJ mol}^{-1}$ for CH). Consequently, fluorocarbons are both bioinert and unreactive. Biologists also think of chemicals as hydrophilic or hydrophobic, but fluorocarbons constitute...
another group that is strikingly illustrated by the three-way emulsions formed when water, hydrocarbons, and fluorocarbons are shaken together. As a result, it is likely that most molecules found in cells will be insoluble in FC40. Moreover, FC40 carries approximately tenfold more O₂ than water (and approximately twofold more CO₂), as well as binding neither gas, so its close relatives have been used as human blood substitutes and for liquid ventilation of neonatal babies.[20] In combination, these properties mean that cells under FC40 can be grown in standard CO₂ incubators, and viewed on standard microscopes. FC40 walls even provide additional sterility barriers. For example, if every second chamber in a grid is seeded with bacteria, growth occurs only in inoculated chambers as others remain sterile. If now more medium is fed to each chamber, aqueous drops jump from tip to chamber, as uninoculated chambers remain sterile.[15] Consequently, our printers carry another tool—a dispensing needle that is used repeatedly, often without washing between repeats (as in Figure 2E, inset).

4. Flow with and without External Pumps

Flow through circuits can be driven by external pumps; steel needles connected to pumps are inserted through fluid walls to yield leak-free joints.[14,16] Flow rates can vary widely (in Figure 3A, two inputs merge to flow as laminar streams down one conduit to a sink that automatically self-empties). Note, however, progressively increasing the flow into any fluid-walled conduit inevitably increases θ, and fluid walls move outward once θ is reached and burst when θ rises above 90°.

Flow can also be driven without external pumps. Drops with small radii of curvature harbor higher Laplace pressures than those with larger radii (Figure 3B-i; Laplace pressure \( = \frac{2\gamma}{R} \), where γ is interfacial tension and R is radius of curvature).\[21\]

In Figure 3B-ii, all six dumbbell-shaped circuits are initially identical, but when decreasing dye volumes are pipetted successively into drops 6–2, consequential changes in Laplace pressure drive flows rightward at different rates (e.g., 30 s after the last addition, dye in circuit 2 reaches its sink before that added earlier to circuit 6). The circuit in Figure 3B-iii serially dilutes and mixes dyes; pipetting dye into input drops increases local pressures so chamber a fills only with red dye, f only with blue, and b–e with dilute mixtures. Flow rates in circuits without external pumps depend on many factors (e.g., circuit geometry, density, viscosity, and interfacial tension), but can be predicted,[22] and—for a maximal when the contact angle of the source drop is 90° and the sink drop is completely flat. Geometrical changes can have a large impact: increasing conduit width from 590 to 880 µm in a dumbbell-shaped circuit can increase flow rate sixfold.[14]

5. Examples: Cell Cloning and Feeding

Grids and circuits have now been used for all core cell-culture methods (e.g., feeding,[15] replating,[15] cloning,[15,23] and cryopreservation)\[15\] with organisms ranging from bacteria\[15\] through yeasts\[24] and worms\[15] to man,[14–15,23] as well as for drug screening,[14–15] chemotaxis[14] plus cell-wounding assays,[25] lysis plus RT-PCR,[14] transfection plus genome editing,[15] and fixation plus immunolabeling.[15] Note that although most of these applications are cell-based, the general technology can nevertheless be applied wherever small volumes require manipulation. We now give two example cell-based applications.

Mammalian cells are often cloned by splitting a dilute cell suspension among wells in microplates before clones are picked. However, Poisson statistics ensure most wells get no cells, and a few only one (usually \( < 10\% \)). Anyone who has cloned this way worries that picked colonies may be derived from \( > 1 \) founder, so they perform another cloning round to increase the chances of achieving monoclonality. Moreover, one can never be sure a colony is truly derived from one progenitor, as edge effects obscure views of the periphery of wells.[18] Jetting fluid walls (shaped like Voronoi polygons) enables analogs of cloning rings to be built around almost all single cells in a dish (Figure 4A); then, after growing clones, pipetting trypsin into polygons, and picking colonies, cloning efficiencies as high as those obtained conventionally can be obtained. This enables...
the Poisson limit to be beaten in the sense that >90% polygons contain a single cell. Additionally, the excellent optical clarity afforded by fluid walls gives users confidence which polygons contain only one founder, and so to pick clones after 1 week (instead of ~2) and forego second cloning rounds.\[16,23\]

In another example, fresh medium is fed continuously to 48 sets of mouse myoblasts as they differentiate into myotubes over 7 days (Figure 4B-i,ii).\[16\] Cells are deposited in 48 drops on a virgin dish and allowed to attach (Figure 4B-iii), a circuit jetted around them (Figure 4B-iv), and fresh medium perfused (1 µL day\(^{-1}\)) through each of 48 chambers; cells fuse to give syncytial myotubes expressing a component of the neuromuscular junction (i.e., DOK7 tagged with EGFP; Figure 4B-v). This circuit can easily be adapted to screen for drugs affecting this developmental pathway (e.g., as syncytia form, each chamber is isolated from others by building new FC40 walls across inputs and outputs, drugs added, and fluorescence monitored).

6. Conclusion

We describe a methodology for constructing and operating microfluidic devices using just a trio of cell-friendly materials (cell culture media, polystyrene Petri dishes, FC40) plus a three-way traverse, three simple tools, syringe pumps, and pipettors. All parts of these circuits are accessible from above, so cells in them can easily be sampled. What are the major shortcomings? First, our devices will never be as robust as solid-walled ones (e.g., they survive careful carriage by bike or car, but not over speed bumps). Second, they cannot support high flow rates that increase contact angles beyond \(\theta_A \leq 90^\circ\), as then fluid walls shift or rupture; therefore, they are unsuited for applications like high-throughput droplet-based microfluidics.\[26\] Third, despite the limited solubility of water in FC40, some static media in features <20 µm wide evaporate, and this limits further miniaturization. Fourth, circuits are currently limited to two dimensions. Fifth, while circuit operation may be simple, circuit design often requires specialized know-how. Nevertheless, we hope the simplicity and robustness of this affordable approach will increase adoption of microfluidics in biolabs.

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Conflict of Interest

Oxford University Innovation—the technology transfer company of the University of Oxford—has filed patent applications for fluid-walled technologies on behalf of P.R.C. and E.J.W. iotaSciences Ltd. is exploiting this technology. P.R.C. and E.J.W. each hold equity in the company and receive salaries or fees from it; the company also provides scholarships for C.D. and F.N., printers, and FC40.

Keywords

cell cloning, fluid walls, fluorocarbon FC40, Laplace pressure, microfluidics, open microfluidics

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