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Perspective

Synthetic life on a chip

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In this article, we argue that on-chip microfluidic systems provide an attractive technology when it comes to designing synthetic cells. We emphasize the importance of the surrounding environment for both living systems in nature and for developing artificial self-sustaining entities. On-chip microfluidic devices provide a high degree of control over the production of cell-like synthetic entities as well as over the local microenvironment that these soft-matter-based synthetic cells experience. Rapid progress in microfluidic fabrication technology has led to a variety of production and manipulation tools that establish on-chip environments as a versatile platform and arguably the best route forward for realizing synthetic life.

The curious thing called life

Some of the most intriguing questions that scientists — and laymen for that matter — ask, revolve around the theme of 'What is life?'. For example, 'What distinguishes living systems from lifeless matter', 'By what criteria can an entity be called alive?', 'Can we build life from scratch?', 'What minimal elements are needed to constitute a living cell?'. Scientists have been attempting to tackle these questions from experimental and theoretical points of view, while also ruminating over the usefulness of questions related to defining life [1–3]. While satisfactory answers have remained lacking for over at least a century, the emerging field of synthetic biology may now provide new avenues to shed some light on this. Indeed, efforts are springing up to attempt to assemble a cell-like object from lifeless molecular components in such a way that it will exhibit many of the attributes of living cells — in other words, to manufacture a synthetic cell that is alive [4,5]. This is expected to lead to vast new insights into cell biology and create new opportunities in biotechnology, while such research may also be highly relevant for astrobiology and how life began in the first place on our primitive Earth. The field aimed at resolving the origin of life is facing enigmatic problems as it is very hard to get a hang of how millions of biomolecules self-organize to form autonomous self-sustaining systems. Systematically working on simplified minimal systems may help to disentangle some of the enormous complexity.

In this perspective, we reflect on a possible route towards making synthetic cells, which we provisionally define as functional, autonomous, and self-propagating entities that thrive within a specific environment. While embarking on the monumental task of revealing the basic principles of life with a synthetic-cell approach, it is useful to ask whether there are general lessons to consider from observing the current life forms in nature, and what specific technologies are available that might facilitate the way towards building synthetic cells in a bottom-up fashion.

The role of the external environment

Upon looking at the remarkable variety of life forms that exist on Earth, one thing is immediately noticeable: the local environment plays a critical role for the survival of a particular life form. To survive and proliferate, we humans, for example, require quite specific conditions, such as a certain range of external temperature, oxygen content of the air, water and food supplies, a symbiosis with our microbiota. Changes in these conditions can be tolerated to some extent, but drastic changes, say a temperature shift to ±100°C, would make human life unsustainable. The defined nature of the

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environment is thus crucial to our existence. This general point becomes even clearer when you consider the life of an obligatory parasite, an organism that cannot sustain itself without another host organism that provides it with indispensable nutrients. For example, *Nerocila* ectoparasites attach themselves to a host fish and entirely depends on the host for survival [6]. While without doubt *Nerocila* is alive, it would not be able to survive without the extremely specific environment of its host which provides it with essential components. An example at the unicellular level is *Mycoplasma genitalium*, a pathogenic bacterium that lives within the urinary tracts of humans (Figure 1a). The environment of the post-kidney urinary tracts is well defined, and rich in essential metabolites that have allowed *M. genitalium* to evolve to a rather minimal cellular organism with one of the smallest known genomes, where many of the cellular networks are missing that other bacterial systems possess to robustly survive in more demanding conditions [7,8]. Indeed, it is no surprise that the *Mycoplasma* species were selected by Venter and co-workers as the organisms of choice in The Minimal Genome Project, a study to find the smallest set of genetic material necessary to sustain life [9]. Notably, mycoplasmas are just one of the many obligatory host-associated bacteria with reduced genomes [8], emphasizing the crucial role the external environment plays in the life of an organism.

Life, thus, benefits from a specific external environment, a fact that generally is taken rather for granted. If the environment is stable, i.e. well defined over a long time, and rich in essential metabolites and other bio-organic residues that derive from other life forms (as opposed to mere inorganic components in the environment), the living form can be simplified in terms of the functions that it needs to perform. If, on the other hand, the environment is poor and strongly fluctuating, organisms need a robust array of functionalities to survive. Defining life for an object thus involves a subtle balance between the control and complexity that is provided by the environment against the built-in functions of the organism itself (Figure 1b). It is interesting to consider whether we can extend this concept and take advantage of it in our pursuit of creating synthetic cells where we aim to mimic basic life-like characteristics, say a growth-replication-division cycle, in a population of microcontainers. For example, we may try to provide a stable, very specific, and rich environment in an attempt to make a synthetic cell that is as minimal possible with regard to its internal complexity and functionality. But how can one spatiotemporally regulate and control the external environment while observing the microscopic synthetic cells? On-chip microfluidic technology provides an exquisite solution for this.

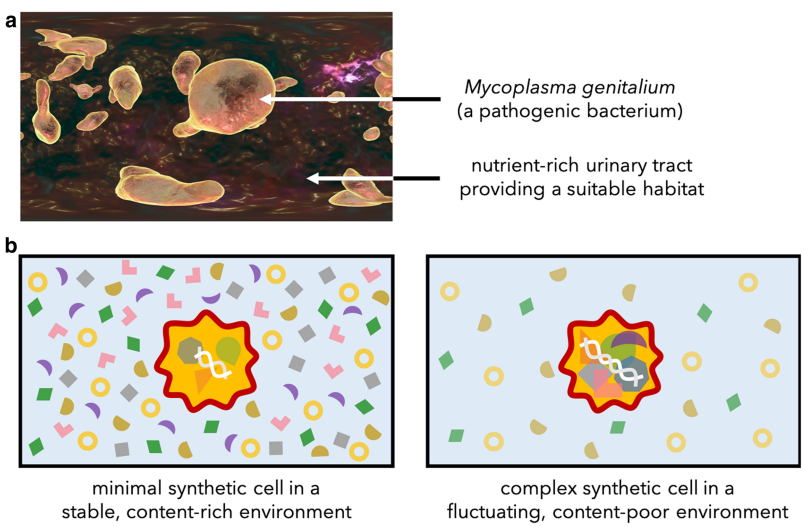

Figure 1. The importance of a suitable environment to sustain life.

(**a**) Artist impression of the pathogenic bacterium *Mycoplasma genitalium* that inhabits the urinary tract of humans, which provides it with a nourishing environment. (**b**) The complexity of a living form, or of a synthetic cell for that matter, depends on the surrounding environment. If the environment is stable and provides the cell with a large variety of nutrients and other essential molecules, the synthetic cell may be relatively minimalistic (left). Making the environment more fluctuating and less specific does necessitate the increase in the complexity of the internal machineries of the synthetic cell (right).

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Microfluidics: an optimal way to control the environment

Microfluidic systems constitute a technology developed to handling small fluid volumes (in the μl range or less) flowing at the micrometer scale (typically with velocities of μl/min or less). A microfluidic architecture typically comprises of a network of microchannels, ranging from sub-100 nm to hundreds of μm in diameter, that are mutually connected in a user-defined manner, through which fluids of specified composition can be flown with a high degree of control (Figure 2a). Such a set-up ensures a low Reynold's number where viscous forces dominate over inertial forces. As a result, the highly unpredictable nonlinear behavior described by the Navier–Stokes equation is reduced to a linear Stokes equation that describes a regime of predictable laminar flow without any turbulence. The unique environment of microfluidic systems thus enables superb control of molecules in space and time. A wide range of valves, splitters, mixers, gates, traps, and other local manipulation

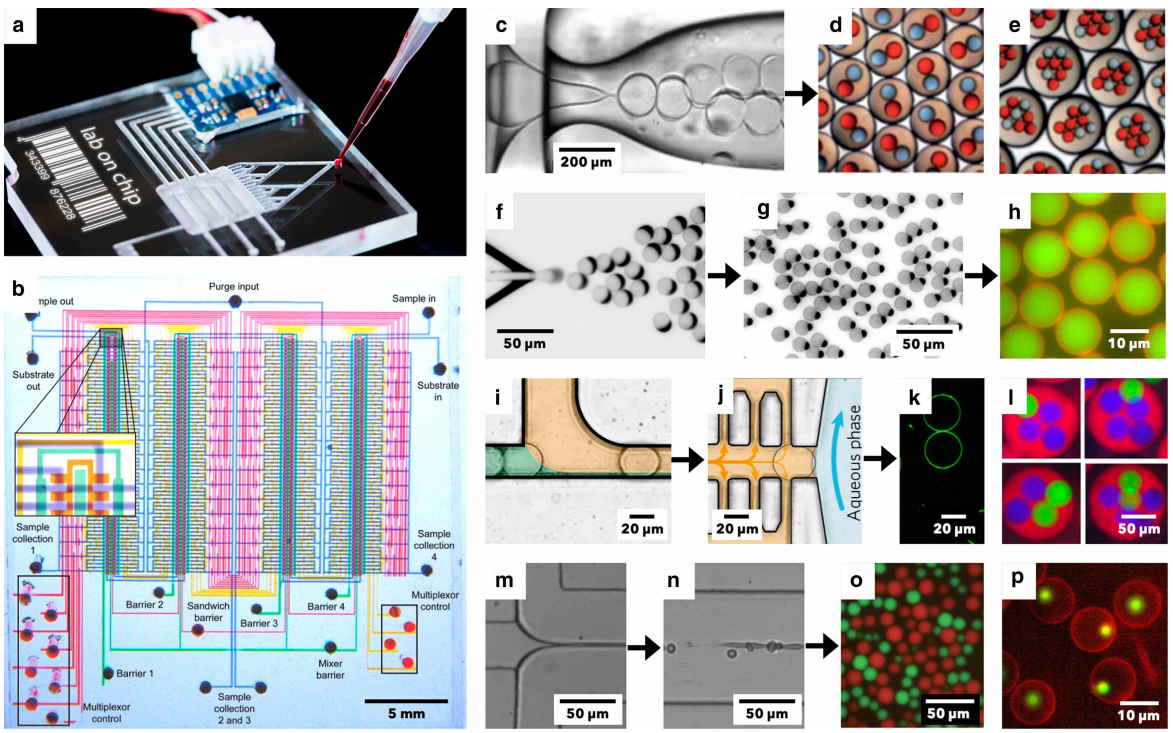

Figure 2. The large variety of on-chip technologies available to produce synthetic cells.

(**a**) Example of a lab-on-a-chip device that shows its miniature size, microchannel architecture capable of handling minute fluid samples, and its ability to couple with diverse modules such as electronics. (**b**) A large-scale integrated microfluidic circuit containing an intricated network of thousands of channels, valves, and chambers, demonstrating the capability of a microfluidic chip to create and control a complex environmental set-up. (**c**–**e**) High-throughput production of monodisperse double-emulsion droplets using glass-capillary devices (**c**). These glass-capillary devices can be further designed to allow sub-compartmentalization (red and blue inner drops), exemplified by two (**d**) and eight (**e**) inner drops inside the main droplet. (**f**–**h**) OLA showing the initial formation of double-emulsion droplets at the production junction (**f**), partially dewetted liposomes with protruding octanol pockets (**g**), and completely dewetted monodisperse liposomes (**h**). (**i**–**k**) Droplet-stabilized liposome formation, where the polymer-coating on the droplets is first destabilized at the T-junction by the oil phase (in yellow) containing surfactants (**i**) and droplets are eventually released into the aqueous phase (**j**), forming unilamellar liposomes (**k**). (**l**) Vesosomes (liposomes-in-liposome structures) with different numbers and ratios of interior liposomes (green and blue circles), formed using glass-capillary devices. (**m**–**o**) Microfluidic formation of coacervates, where the bulk coacervate phase is hydrodynamically focused (**m**) and pinched off to form irregular segments (**n**), which eventually form stable coacervate droplets (**o**). (**p**) Coacervate-in-liposome structures showing freely diffusing polylysine/ATP coacervates (green blobs) formed inside OLA-based liposomes. Panels are adapted from references as follows: (**b**) [10]; (**c**) [14]; (**d**,**e**) [15]; (**f**,**g**) [16]; (**h**) Cees Dekker lab; (**i**–**k**) [17]; (**l**) [18]; (**m**–**o**) [19]; (**p**) [20].

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tools have been developed to manipulate the flow of fluids, molecules, particles, and cells on chip. Highly sophisticated and intricate microfluidic circuits can be designed to create user-specific microenvironments in a high-throughput fashion [10] (Figure 2b). Continuous development in microfluidics, coupled with advances in materials and fabrication, has opened up a tremendous potential to form complex multifunctional microfluidic systems that can handle thousands of cells in parallel, which can be particularly useful in the field of biomedicine and bioengineering [11]. In the light of the ideas sketched above regarding organism–environment relations, it is clear that microfluidics can provide unprecedented control over the environment and the synthetic cells, especially in comparison with traditional bulk experiments. For example, pipetting fluids in Eppendorf tubes or microtiter plates containing delicate synthetic cells involves a high risk of turbulence-induced damage and slow mixing of contents, both of which can be easily avoided in microfluidic systems. Indeed, over the years, microfluidic schemes have been developed for a variety of on-chip production methods to make cell-like microcontainers that can potentially act as a scaffold to create synthetic cells, as well as manipulation tools to position, control, and maneuver such soft-matter-based micron-sized objects [12,13]. Below, we briefly discuss these two points.

Producing synthetic cells on a chip

Pioneering work by the Weitz lab, which produced water-in-oil-in-water double-emulsion droplets using glass capillary devices in a process akin to bubble-blowing (Figure 2c), set the tone of using microfluidics to create cell-like containers [14]. These micron-sized droplets with ideal encapsulation properties can be formed in a highly controlled and a high-throughput fashion, with follow-up work enabling the formation of compartmentalized multi-component droplets [15] (Figure 2d,e). Recently, this droplet-based approach was redesigned to form liposomes (bilayer-bounded aqueous compartments in an aqueous external environment). Having a lipid bilayer as the boundary, such liposomes are close mimics of natural cells and thus more relevant for functional synthetic cell containers. The two prominent methods that use this approach are PDMS (polydimethylsiloxane)-based Octanol-assisted Liposome Assembly (OLA) [21] (Figure 2f–h) and glass-capillary-based double emulsion-dewetting [22]. In both cases, the organic solvent phase physically separates within a few minutes to form monodisperse unilamellar liposomes with a high encapsulation efficiency. These methods provide great advantages as compared with the popular bulk production methods such as hydration [23], extrusion [24], and electroformation [25], which suffer from polydispersed samples, variability in the encapsulated content, and a much lower encapsulation efficiency [26,27]. Several other methods have emerged to form liposomes on chip [26], and new techniques are being developed continuously (Figure 2i–k), such as recently developed droplet-stabilized liposome production [17]. In parallel, these on-chip techniques are being used to make other promising scaffolds for building a synthetic cell [28], such as polymersomes [29,30] (using amphiphilic block copolymers) and proteinosomes [31] (using protein–polymer conjugates). The on-chip approach further allows the formation of sophisticated and biologically relevant nested assemblies, such as liposome-in-liposome structures [18], also known as vesosomes, resembling eukaryotic cells with membrane-bound organelles such as the nucleus (Figure 2l).

The microfluidic approach is also being extended into the domain of biomolecular condensates, membraneless organelles that are crucial to maintaining cellular biochemistry [32]. Usually formed through the process of complex coacervation, the bulk coacervate phase can be broken down by hydrodynamic focusing into relatively monodisperse droplets [19] (Figure 2m–o). This could be a very useful technique to study the emerging role of condensates in the compartmentalization of reactions such as RNA catalysis [33], which is normally performed via bulk-produced polydisperse condensates. Combining the two containers, coacervates-in-liposome structures have been recently designed, either by encapsulating both the necessary components and modulating the phase transition parameter such as temperature [34] or by administering a coacervate component across the membrane through membrane-embedded protein pores [20] (Figure 2p). The rapid development of various on-chip techniques is thus allowing a wide choice of methods to create cell-like containers as per the experimental need. The impressive level of control and sophistication provided by microfluidics is very difficult to achieve in bulk experimentation. Apart from the ability to synthesize objects in a highly controlled and a high-throughput fashion, the on-chip approach simultaneously gives the opportunity to store and to protect these soft-matter-based objects by providing a suitable microenvironment for storage, manipulation, and readout.

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Manipulating synthetic cells on a chip

A major advantage of microfluidics is that synthetic cells, once produced on chip, can be further manipulated in a variety of useful ways, via numerous specific modules [35]. The past years have seen the development of many features that could be useful for functionalizing minimal cells:

i. Trapping: Keeping the synthetic cells in a fixed position is beneficial if one wants to modulate the external buffer conditions and study the cellular response. As a simple solution, objects can be immobilized in physical traps and monitored for hours [36] (Figure 3a). This enables long-term observation and facilitates changing the external conditions without disturbance. Interestingly, the trap geometries can also be

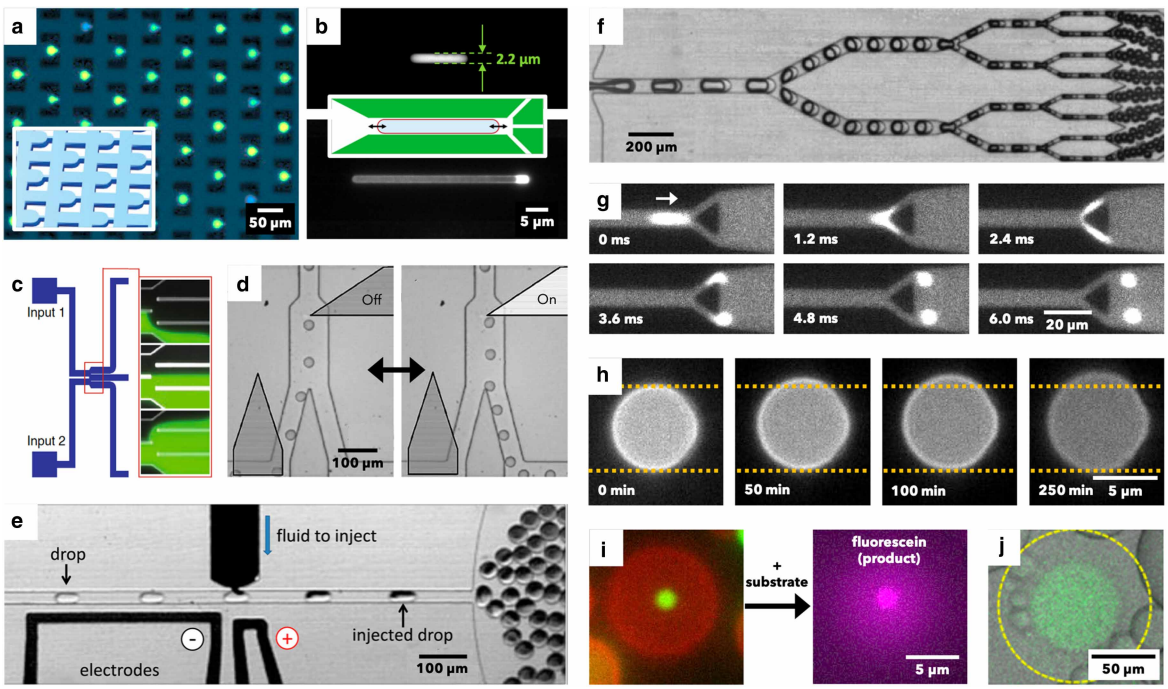

Figure 3. Variety of on-chip modules available to manipulate synthetic cells.

(**a**–**b**) Trapping: An array of physical traps (design shown in the inset) showing highly efficient immobilization of liposomes on chip (**a**). Tubular trap design (inset in **b**) to deform synthetic cells into a specific shape, for example into rod-shaped geometry of different dimensions. The trapped objects are droplets (upper image in **b**) and double-emulsion droplets (lower image in **b**). (**c**) Controlling the external environment: Dial-a-wave junction with three distinct switching states (100% from input1, 50% each from input 1 and 2, 100% from input 2). This module can be combined with, for example, physical traps to change the local environment of the synthetic cells in a user-defined manner. (**d**) Sorting: a dielectrophoresis-based high-speed sorting of droplets. In the absence of an electric field, the droplets flow into the low-resistance channel (left panel), while, upon applying the electric field, they are attracted towards the energized electrode and enter the other channel. (**e**) Local injection: An on-chip picoinjector injects a well-defined amount of fluid into pre-formed droplets. The process is triggered by the electric field, which is applied by the in-built electrodes. (**f**,**g**) Multiplication and division: Double-emulsion droplets splitting three times in series, resulting in eight-fold amplification (**f**). Highly symmetric and leakage-free division of a cell-sized liposome across a mechanical splitter. The arrow indicates the direction of motion (**g**). (**h**) Growth: Time-lapse images showing membrane tension-mediated growth of a trapped liposome. A solution containing small feeder liposomes, which fuse with the trapped liposome, is continuously flushed resulting in the observed growth. The dashed horizontal lines are drawn in order to guide the eye. (**i**,**j**) Compartmentalized reactions: A coacervate-in-liposome structure (left panel in **i**) showing an enzymatic reaction predominantly getting carried out in the coacervate phase as judged by the fluorescence intensity of the resulting product (right panel in **i**). *In vitro* transcription being carried out specifically in the nucleus-mimicking liposome of a vesosome, as judged from the fluorescence (**j**). The yellow circle indicates the outer liposome boundary. Panels are adapted from references as follows:- (**a**) [36]; (**b**) [37]; (**c**) [39]; (**d**) [40]; (**e**) [42]; (**f**) [43]; (**g**) [44]; (**h**) [45], (**i**) [20]; (**j**) [18].

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chosen such that they deform the containers into desired shapes [37], for example, a rod shape resembling an *E. coli* bacterium (Figure 3b).

ii. Controlling the external environment: Once immobilized or confined within a region, monitoring the environment becomes an easy task, for example, through feeding channels and switchable valves [38]. One particularly useful feature is a dial-a-wave junction, where fluids from two input channels can be mixed in the desired ratio over a wide range (continuously from 100% of input 1 (and 0% of input 2) to 0% of input 1 (and 100% of input 2), Figure 3c) [39]. Combining this module with trap arrays facilitates to easily change the local environment in a user-defined manner.

iii. Sorting: For large populations of vesicles, sorting modules are useful when it comes to selecting a specific fraction of vesicles, for example in experiments on directed evolution. Such sorting of droplets has been successfully demonstrated using dielectrophoresis, where in-built electrodes are able to sort water-in-oil droplets in a high-throughput manner [40] (Figure 3d). Importantly, such sorting can be coupled with fluorescence-detection [41], similar to the fluorescence-activated cell sorting.

iv. Local injection: Adding components in a defined manner to an existing object is very handy, especially when one wants to activate reactions in a particular sequence or achieve a step-by-step bottom-up assembly [17]. Electromicrofluidics has been shown to be capable of sequentially injecting picolitre fluid volumes into pre-formed droplets [42] (Figure 3e).

v. Multiplication and division: Division is a fundamental requisite for life, needed for achieving perpetuation. Using straightforward triangular PDMS-based splitter posts, double-emulsion droplets and even liposomes can be divided efficiently (Figure 3f,g) [43,44]. Putting such splitters in series can lead to a substantial amplification of the number of droplets [43] (Figure 3f).

vi. Growth: Growth is another fundamental characteristic of living systems that also will be a mandatory feature of synthetic cells. Recent work has demonstrated membrane tension-mediated growth of liposomes by trapping the liposomes and inducing membrane fusion with feeder liposomes present in the external bath [45] (Figure 3h).

vii. Compartmentalization of reactions: Microfluidic fabrication schemes enable the formation of sophisticated structures, which also provides a direct solution for segregating various biochemical reactions, something living cells have developed over the course of evolution. For example, coacervate-in-liposome structures can be used to carry out enzymatic reactions specifically within the condensates [20] (Figure 3i). Vesosomes can be used to mimic the eukaryotic cell structure and limit *in vitro* transcription to the nucleus-mimicking liposome [18] (Figure 3j).

Outlook: towards synthetic life on a chip

We started this perspective by emphasizing the importance of the external environment when it comes to the emergence and sustenance of living systems. One should rather not think of defining a living entity as an individual system in isolation, because its maintenance is inevitably connected to its habitat. Depending on the complexity and richness of the habitat, a living form can be a relatively simple system, as is exemplified with the case of parasitic pathogens. We argue that the same logic can beneficially be applied to synthetic life forms: one should be able to design minimal artificial cells by incubating them in a highly sophisticated microenvironment that will be responsible for their nourishment. An outstanding technology that provides such a well-defined dynamic environment is microfluidics. From the brief expose given above, it may be clear that microfluidics has truly changed the experimental approach, providing ample options for novel designs when it comes to creating and functionalizing synthetic cells. The high degree of control achieved with on-chip systems in creating, sustaining, and manipulating synthetic cells is next to none, and can be expected to play a vital role in the future of synthetic biology. At the same time, it should be noted that depending on the level of sophistication required, using on-chip systems to one's advantage does require considerable infrastructure (e.g. clean room facilities) and expertise on chip design, best fabrication routines, and the like. Furthermore, combining different modules (for example production, growth, and division) on a single chip is not straightforward and further efforts are needed to seamlessly integrate multiple modules into a single device.

To conclude, although the spatial constraints may be different, as a chip is a more specific environment than the natural one, we expect that future life-on-a-chip will essentially not be too much different from the natural life as we know it: functional, autonomous, and self-propagating units that are able to work within a particular set of external conditions.

Summary

- The external environment is essential to a living system and determines how complex a natural/synthetic cell needs to be in order to sustain and perpetuate itself.
- When it comes to creating synthetic cells, it is beneficial to start with designing a content-rich and stable environment, in order to minimize the complexity of the synthetic cells.
- On-chip microfluidic techniques provide a versatile toolbox to produce synthetic cells in a controlled manner and to manipulate the cells as well as the external environment, and hence can be expected to significantly impact the future creation of synthetic life.

Abbreviations

OLA, Octanol-assisted Liposome Assembly; PDMS, polydimethylsiloxane.

Author Contribution

S.D. and C.D. wrote the manuscript.

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Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

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