3D-printed synthetic tissues

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‘Bottom-up’ approaches in synthetic biology have been used to construct synthetic cells from simple biological components. By contrast, relatively little work has been done on synthetic tissues in which collections of cells cooperate to achieve functionality that cannot be generated by individual compartments. We have developed a 3D printer, which can create structures containing hundreds or thousands of communicating aqueous droplets arranged in programmed patterns. These tissue-like materials can adopt properties such as the ability to fold or conduct electrical signals. Furthermore, the properties of the materials can be extended, so that they become true synthetic tissues through the performance of sophisticated functions such as protein synthesis. In addition, we have shown that 3D-printed synthetic tissues can be controlled and energized externally, for example by light. Printed synthetic tissues might find a variety of uses in medicine and could even be interfaceditly with living tissues. As they contain no genome and cannot replicate, synthetic tissues are comparatively safe for medical applications.

3D-printed aqueous droplets

We have developed a 3D printer to create structures containing hundreds or thousands of aqueous droplets arranged according to programmed patterns. Recently, we used the approach to produce synthetic tissues, which ultimately may have applications in medicine, for example in the repair of damaged organs. By tissues, we mean biological materials with emergent functional properties that surpass the sum of the capabilities of the individual compartments. While bottom-up synthetic biology proceeds apace, including the construction of synthetic cells, there has been little work on synthetic tissues in which these cells behave in a cooperative manner.

Curiously, our approach to synthetic tissues evolved from an attempt to miniaturize single-channel current recording through biological channels and pores in lipid bilayers. We formed two aqueous droplets (~200 nL) in an oil that contained lipid molecules. A monolayer of lipid formed around each droplet, and when the droplets were brought together a bilayer formed at the interface, which we named a droplet interface bilayer (DIB) (Figure 1a). With a membrane protein of interest inserted into the bilayer and an electrode in each droplet, current recordings were made. DIBs have been widely adopted for biophysical studies.

If two droplets can be joined together, why not three, four or 10? We proceeded to build multi-droplet systems, first in two and then in three dimensions, in which each droplet was connected to one or more of its neighbours through a DIB. For example, a 3-layer, 10-droplet pyramid was made by the micromanipulation of droplets containing paramagnetic beads (Figure 1b). These networks can be endowed with useful functional properties. For example, a 4-droplet network, containing a diode-like mutant of the α-hemolysin (αHL) transmembrane protein pore arranged vectorially in each DIB, carries out full-wave rectification of an electrical signal (Figure 1c).

The methods we had devised to build small droplet networks were not practicable for larger structures. For this, we constructed a piezo-based 3D printer capable of printing thousands of aqueous droplets (~50 pL) in patterned 3D assemblies (Figure 2a). These printed structures can be viewed as mechanically resilient systems, resembling soft balls held together by springs. The printed droplet networks can be formed inside oil drops in an aqueous environment, in which case the final structure is encased in a lipid bilayer through which communication with the external environment can be mediated by membrane proteins. Again, these structures can afford functional properties. For example, a 4-petal structure was printed onto a flat surface from layers of droplets with different internal osmolarities. After printing, water flow across the bilayers caused the structure to fold into a hollow sphere (Figure 2b). With their multiple communicating compartments and properties analogous to living organisms (here shape change), we consider these printed structures to be ‘tissue-like materials’.

Controlling synthetic tissues with light

Our early work used only simple reagents inside 3D-printed droplets to produce electrical communication and shape change. Furthermore, the properties of the materials could not be altered after printing. To produce synthetic tissues, namely materials closer to biological tissues, we needed to enhance the properties of the constituent compartments so that they more closely resembled living cells. Therefore, we elaborated the droplets by adding the ability to synthesize protein from encapsulated DNA with an in vitro expression system.
Beyond the Cell

Such synthetic cells had previously been made from individual vesicles bounded by lipid bilayers\(^1\), but these entities had not been elaborated into multi-compartment systems. By printing networks of hundreds of synthetic cells, we sought to produce the emergent properties associated with tissues by allowing communication between the compartments, which had been a principal feature of our simple tissue-like materials. Additionally, we aimed to control the synthetic tissues externally, by using an in vitro expression system turned on by light.

We quickly found that our original aqueous droplet networks were only stable when they contained simple salt solutions with, at most, very low concentrations of protein. The lipid bilayers between each compartment broke when a high concentration of protein was present, such as that in an expression system, which contains ribosomes and many different enzymes. We overcame this by altering the lipid composition of the DIBs to include lipids that contained head-groups derivatized with polyethylene glycol (PEG), which projected into the aqueous interiors of the droplets\(^2\). PEG-head-groups have previously been shown to stabilize bilayers, by shielding them from the adjacent solution\(^1\). Aided by the PEG lipids, we printed synthetic tissues comprising hundreds of synthetic cells patterned in defined geometries. Protein could then be expressed inside each of the synthetic cells.

We next sought to externally control the activity of these synthetic tissues. A common means by which to control biological systems is with light\(^1\), which can penetrate living cells without disrupting membrane-bound compartments or upsetting cellular pathways. Two previously published methods have demonstrated control of in vitro protein expression with light\(^1,2\). However, we developed our own way of controlling expression (Figure 3a). In particular, we required an off-state that was not leaky. This was accomplished by covalently attaching the small molecule biotin at several sites on the promoter region of the gene of interest. Biotin binds strongly to the large protein streptavidin, which was used as a steric blocker of the promoter sequence thereby preventing transcription of the DNA by RNA polymerase. Therefore, the first step in protein expression, the formation of messenger RNA did not occur. We also inserted photocleavable linkers between the promoter and the biotins, which allowed removal of the block with low energy ultraviolet light, permitting transcription followed by translation of the messenger RNA into protein.

By combining the stabilized aqueous droplet networks with the LA-DNA, we produced light-activated synthetic tissues\(^1\). In one example, the αHL pore was produced within the synthetic cells by using the expression system under the control of the LA-DNA promoter (Figure 3b). Following 3D printing, the synthetic tissues showed no functional activity, as the LA-DNA was not transcribed and no protein was produced. After irradiation, αHL was synthesized, forming pores in the DIBs through which an electrical current could pass. By patterning the synthetic cells that contained LA-DNA during printing, or by using guided irradiation, extended 3D pathways of cells producing αHL were generated within synthetic tissues. This resulted in light-
activated directional electrical communication realized with external electrodes (Figure 3c). Rapid, directional electrical signalling is precisely the role of neurons, although they work through a different mechanism. Therefore, our approach demonstrates the power of external light-control of expression and has parallels with the field of optogenetics, where neural activity is controlled by light in living organisms\textsuperscript{16}.

We are presently expanding the repertoire of proteins that can be expressed inside the synthetic tissues. For example, light-activated channels and pumps generate currents across bilayers in the absence of electrodes. Synthetic tissues containing them could be used to transduce light signals or as micrometre-sized power generators. Patterning by 3D printing and by light will add valuable levels of complexity to these systems.

**Future directions**

A long-term goal is to use 3D-printed synthetic tissues in medicine. Potential uses include drug delivery and tissue replacement in surgery. In drug delivery, the multi-compartment nature of the materials will allow the release of binary or ternary agents in which components (e.g. an enzyme and a substrate) are combined at a desired site to generate potent effectors\textsuperscript{17}. In surgery, synthetic tissues might be used to replace damaged tissue: for example, to provide a provisional electrical connection after nerve damage. Synthetic tissues are likely to be less problematic than therapies based on living cells, for which questions of immunogenicity and uncontrolled proliferation arise.

While the rapid progress in this new area suggests that these ideas are not far-fetched, there remains a long way to go towards these and other applications. In the meantime, numerous issues of significant technical interest must be addressed. First, improvements in construction techniques and materials are required. Safety considerations suggest that synthetic tissues would preferably be robust rather than self-renewing. Hydrogel blocks, which are not restricted to a spherical shape, have potential uses...
been used as components for network construction in place of aqueous droplets\(^8\) (Figure 4a). Droplet networks might also be gelled or encapsulated after printing to provide robustness. 4D printing (printing followed by folding) might also be a useful construction approach, as our shape change experiments already suggest\(^7\). Larger objects could be made more rapidly through the use of 3D printers with multiple printing heads and printed objects themselves might be used as building blocks for extended structures.

Second, the synthetic tissues must encompass a far wider variety of functional properties. We have already explored shape change, electrical signalling and drug release\(^1,10\). Additional possibilities include the in situ synthesis of short-lived biological effectors that can’t be packaged into pills and drips (e.g. S-nitroso compounds or hydroperoxides), the ability to sense the environment and respond, the means to take up, modify and release molecules, the capacity to interact directly with neighbouring biological tissues and the ability to be energized from external sources (e.g. light). We have previously engineered derivatives of the αHL pore that mimic gap junctions\(^18\) (Figure 4e). We have previously engineered derivatives and the ability to be energized from external sources (e.g. light). We have previously engineered derivatives of the αHL pore that mimic gap junctions\(^18\) (Figure 4e). We have previously engineered derivatives and the ability to be energized from external sources (e.g. light). We have previously engineered derivatives of the αHL pore that mimic gap junctions\(^18\) (Figure 4e).

Additional possibilities include magnetic\(^17\) and mechanical stimulation\(^19\). While the ability to turn systems ‘on’ can be extremely useful, ‘on-off’ switches are obviously more desirable.

Still further in the future, we foresee a myriad of potential technologies, many of which are likely to be controversial, at least initially. We envisage autonomous soft robots\(^20\) based on 3D-printed synthetic tissues. We see printers capable of climbing into and printing directly in surgical sites and implants that allow humans to detect previously imperceptible molecules, sights and sounds.

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**References**


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