cardiovascular calcification, this work also epitomizes the scientific approach of forming an interdisciplinary team to address difficult biological questions, and stands out as an example of how methods originating from materials science can profoundly impact other fields. Prospectively, finding advances resulting from the convergence of biology and materials science should come as no surprise, as perhaps foretold by biologist E. O. Wilson: "The love of complexity without reductionism makes art; the love of complexity with reductionism makes science"[8]. ❐

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BIOPRINTING

Functional droplet networks

Tissue-mimicking printed networks of droplets separated by lipid bilayers that can be functionalized with membrane proteins are able to spontaneously fold and transmit electrical currents along predefined paths.

Naside Gozde Durmus, Savas Tasoglu and Utkan Demirci

To make artificial constructs that mimic the structural complexity of native tissues, scientists typically use photolithography, soft lithography, stamping or microfluidic approaches. Recent advances in such micro- and nanoscale technologies, emerged at the convergence of engineering, biology, chemistry and materials science, have also enabled great progress in the understanding of living systems[1]. Yet the microenvironment of native tissues — which consists of multiple cell types precisely organized in three dimensions — is still complex in comparison to what can be achieved today with fabrication techniques. In particular, the spatiotemporal manipulation of cells remains a challenge. Moreover, fabricated scaffolds are typically rigid and thus lack the potential to mimic contractile tissues, such as cardiac muscle or vascular structures[2]. Bioprinting — the rapid, layer-by-layer deposition of cells and extracellular matrix — has also been used to make tissue mimics at the microscale, but imparting them with functionality has remained elusive. Now, Hagan Bayley and colleagues report in *Science* the fabrication, by means of a bioprinting approach, of three-dimensional (3D) tissue-like materials that fold in ways similar to muscles and, like neural tissues, transmit electrical signals[3] (Fig. 1).

Bayley and co-workers used an automated 3D printer to eject aqueous droplets (each about 65 pl) into a lipid-containing oil bath, and assembled the droplets into networks by programming

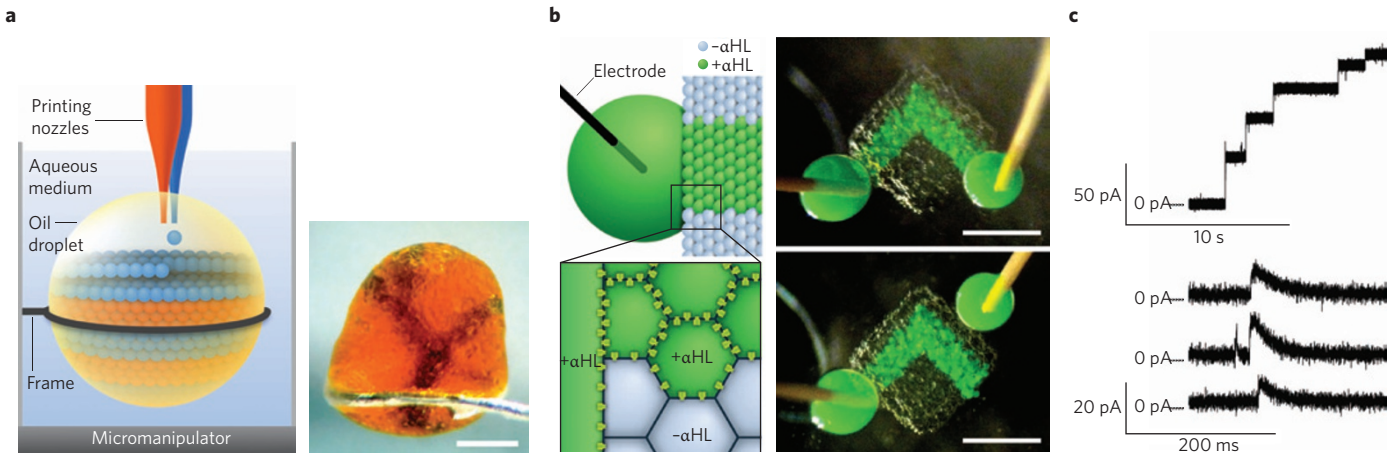

Figure 1 | Printed droplet networks with electrically conductive paths. **a**, Left: schematic of aqueous droplets (red and blue) being printed into a lipid-containing oil droplet placed on a movable frame and suspended in aqueous medium. Right: micrograph of a network of black-dyed droplets within a matrix of orange droplets in aqueous solution. Scale bar, 400 μm. **b**, Schematics (left) and photographs (right) of an ionically conductive pathway (green) in a droplet network (embedded in a solution at pH 8) containing the membrane-pore-forming α-hemolysin (αHL) protein (green triangles). The two large droplets, positioned at either the ends of the ionically conductive trail (top), or one large droplet at one end and the other in contact with the non-conductive region (bottom), are impaled with Ag/AgCl electrodes. Scale bars, 500 μm. **c**, Stepwise (top) and transient (bottom) increases in the measured ionic current under an applied potential of 50 mV for the configurations shown in **b**. Figure reproduced with permission from ref. 3, © 2013 AAAS.

the movement of the tray supporting the bath so as to accurately establish the position of each ejected droplet (Fig. 1a). The lipids gathered at the droplets' oil/water interfaces and formed cell membrane-like bilayers. The bilayer networks were self-supporting and stable for several weeks, and had elastic moduli similar to brain, fat and other soft tissues. The researchers then explored the possibility of using the bioprinting method to impart the material with tissue-like functionality. They printed a network of bilayers containing the membrane-pore protein α-hemolysin (αHL) in a way that creates an ionically conductive pathway across an insulating network (Fig. 1b). A stepwise increase in ionic current was observed in the presence of electrical stimulation (Fig. 1c), thus indicating that, similarly to neural networks, printed networks can also transmit signals. Moreover, computer simulations showed that electrical signals were transmitted within the network of αHL-containing droplets through the αHL pores in the bilayers. Furthermore, the researchers showed that networks of droplets with regions of high and low osmolarity swelled or shrank in response to the flow of water resulting from osmotic-pressure differences (Fig. 2a), and that droplet networks can be programmed to fold into a variety of shapes (for example, a flower-shaped network is shown to fold into a hollow sphere; Fig. 2b).

Bayley and colleagues' method holds great potential for applications in bioengineering and medicine. For instance, self-folding printed networks could be programmed to respond and adapt to changes in the surrounding physiological environment. Indeed, smart delivery systems have been designed to respond to changes in pH, temperature, specific ions or chemical groups, or light[4]. Moreover, self-actuating or externally stimulated soft, inexpensive hydrogel robots could be engineered for detecting toxic substances, biosensing, biofilm cleaning, energy harvesting or environmental remediation. Self-folding, stimuli-responsive droplets might be further integrated with microfluidics to locally capture, isolate and release rare cells for diagnostic applications (for example, circulating tumour cells for cancer detection[5] or CD4 cells for HIV monitoring[6]).

Bioprinting technologies could potentially revolutionize the field of tissue engineering and regenerative medicine if cells, chemicals and biological samples can all be deposited with sufficient spatiotemporal accuracy and high throughput. Inkjet, valve-based and laser-jet printing technologies have successfully printed a variety of cell types — including neurons, endothelial cells and human mesenchymal stem cells — with over 90% viability[7]. Moreover, acoustic technologies have achieved the encapsulation of single (or a few) cells into picolitre-sized droplets, enabling droplet-based cryopreservation of cells[8] and the printing of uniform, size-controlled embryonic bodies for stem cell differentiation[1,7,9]. However, significant challenges remain. For example, encapsulation of single cells is a highly probabilistic phenomenon. Also, printing cells at high densities often leads to clogging problems. And the varying cytocompatibility and functionality post-printing between printing technologies limits their integration and applicability.

Still, it may be possible to integrate bioprinting with single-cell encapsulation for scaffold-free tissue-engineering applications. For example, cells could be deposited, layer by layer, at high packing densities (a sort of '3D cellular epitaxy'[10]). This type of approach may enable new directions in single-cell studies to explore latency in HIV, or cell heterogeneity in 3D tumour microenvironments, for example. In the long term, advances in bioprinting may lead to applications in clinical settings, where functional tissue-patches and 3D tissue-like constructs control the microenvironment of native tissues or mimic cancer tumour microenvironments[11,12]. Although creating artificial tissue-like structures that possess the functionality, specificity and complexity of native tissues and organs remains a far-fetched challenge, in the short term we expect to be able to engineer biologically active soft constructs for use as cancer models, or in stem cell patterning and differentiation, soft robotics, drug delivery and diagnostics. ❐

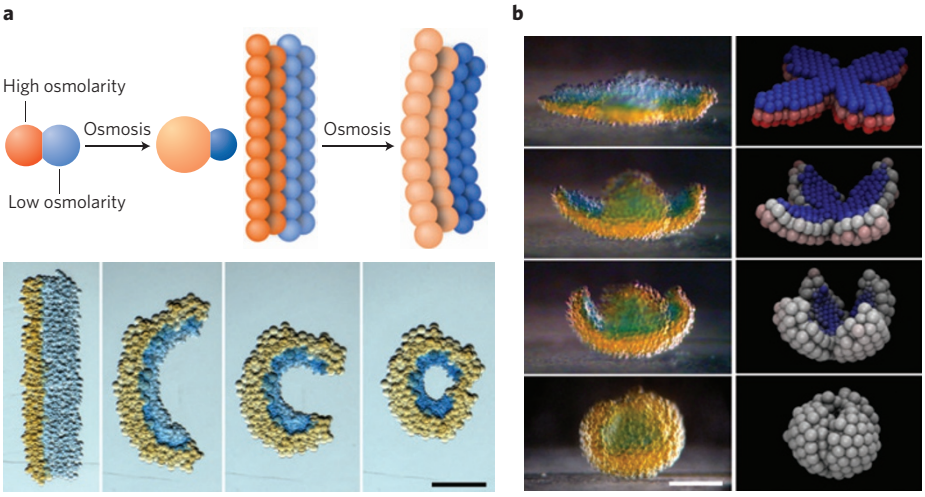

Figure 2 | Self-folding networks of printed droplets. **a**, Top left: schematic of two droplets of different osmolarities sitting next to each other with a lipid bilayer at the interface. Top right: schematic of strips of droplets with regions of high and low osmolarity. Bottom: water transport across the bilayer resulting from osmolarity differences causes strips of droplets to swell or shrink. Scale bar, 250 μm. **b**, Self-folding of flower-shaped droplet networks (left, photographs; right, simulations). Scale bar, 200 μm. Figure reproduced with permission from ref. 3, © 2013 AAAS.

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