MATERIALS SCIENCE

Bioprinting in space and time

A goal of tissue engineers is to use 3D printers to assemble living filaments into tissues that then transform into more-mature forms through predictable shape changes. A study of single living filaments provides a basis for this approach.

ZEV GARTNER & ALEX HUGHES

Rapid advances in 3D-printing technologies have raised the prospect of printing organ-like, cell-dense tissues directly using living inks — combinations of cells and polymeric materials. When living inks are placed under physiological conditions, the cells exert mechanical forces on the polymer matrix and dynamically change the shape and mechanical properties of the ink. To aid the development of 3D printing for tissue engineering, a quantitative understanding of the properties of living inks is needed, so that the evolution of their shapes can be predicted, and perhaps controlled, once placed in culture[1,2].

Writing in *Nature Communications*, Morley *et al.*[3] provide one of the most complete quantitative descriptions so far of a living ink and its mechanical properties. Their findings lay the foundations for 4D bioprinting, a process in which printed biomaterials could be guided through a series of morphogenetic steps (biological processes that alter the shape of the printed object) that converge on a functionally and structurally advanced final form.

The most widely used 3D printers are extrusion-based devices, in which the ink is pushed through a nozzle to form a filament that has a particular diameter and geometry[4,5]. Tissue engineers have developed slurries of microparticles into which soft materials, such as mixtures of cells and components of the extracellular matrix (ECM; the 'mortar' that binds cells into tissues), can be 3D-printed. The slurry prevents the collapse of the resulting structural elements under gravity[6,7]. In their experiments, Morley *et al.* used a free-form printing technique to extrude filaments of a living ink into a slurry formed from polymeric microparticles that turns into a fluid as the printhead moves through the medium.

The living ink consisted of live fibroblasts (the cells most commonly found in connective tissue in animals) and the ubiquitous ECM protein collagen-1, which provided a matrix material that the fibroblasts could attach to and cause to contract. The printed filaments had a range of geometries and different compositions of fibroblasts and collagen-1. The authors used the filaments as models of the simplest building block of a printed tissue — akin to a single beam in the supportive framework (truss) of a building.

Morley *et al.* measured the time-dependent changes in filament geometry that occurred after printing, as the cells applied traction to the collagen-1 and remodelled the structure of the matrix. By systematically varying the filaments' thickness and length, as well as their collagen-1 and cell composition, the authors obtained a comprehensive understanding of the mechanical behaviour of filaments of living material. Although the study was restricted to simple filament geometries, the resulting data could, in principle, be fitted to mechanical models that describe the deformation of tissues that have more-complex filament geometries and patterns.

In one key series of experiments, the authors observed four types of filament behaviour under cell traction that can be explained quantitatively in terms of the material properties of the filaments and the stiffness of the microparticle slurry (Fig. 1). In microparticle slurries that have low stiffness, the filaments buckled into wave-like shapes that relieve internal stresses applied by the cells. If the slurry material was made stiffer, however, it prevented the buckling. At medium slurry stiffnesses, the filaments either broke into smaller segments or shortened, depending on the concentration of collagen-1 in the filament. The authors present a theoretical framework that predicts how controllable parameters of a 3D printer will determine which of these behaviours will occur.

Morley *et al.* propose that their theoretical framework provides quantitative engineering guidelines for 4D bioprinting[8]. For example, one could imagine printing arrangements of cells and ECM components that spontaneously change shape to create synthetic representations of tissues and organs, such as kidneys, lungs or blood vessels, that are more true to life than can currently be achieved.

However, challenges remain before this vision can become a reality. The engineering of functional tissues using 4D bioprinting will require the integration of an extensive list of living cells and ECM components into the material extruded by the printer, all of which will interact with one another biochemically and mechanically. How will multiple coupled filaments behave in a composite, interconnected set of trusses? How will they push and pull one another? And will the cell dynamics themselves change as a structure curves or becomes more compact[9]? It is also not clear how more-complex objects might be engineered to achieve one stable outcome using morphogenetic processes, or whether such processes will be robust to cell behaviours not analysed by Morley and colleagues, such as proliferation, differentiation and motility.

Finally, it should be noted that all extrusion-based printing techniques inherently suffer

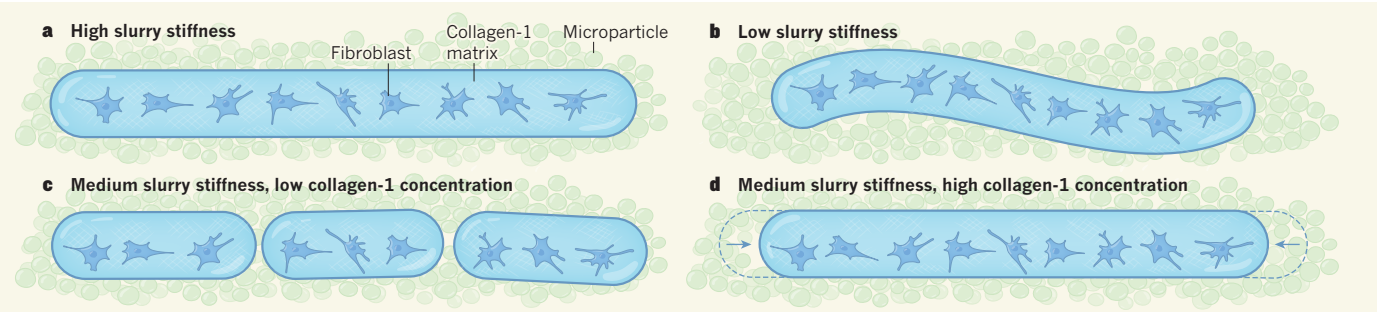

Figure 1 | Predictable changes to living filaments. Morley *et al.*[3] used a 3D printer to print filaments of a 'living ink' in a culture material consisting of a slurry of microscopic polymeric particles suspended in a growth medium. The living ink was a mixture of cells called fibroblasts and collagen-1 (a structural protein). The authors observed that the filaments underwent one of four types of behaviour depending on the stiffness of the slurry and the concentration of collagen-1 in the ink. **a**, When the stiffness of the microparticle slurry is high, the filaments do not change shape. **b**, When the stiffness of the slurry is low, the filaments buckle. **c**, **d**, At medium slurry stiffnesses, the filaments break into shorter sections when the collagen-1 concentration is low (**c**), or shorten when the collagen-1 concentration is high (**d**). The authors' quantitative analysis of filament behaviour provides a basis for using 3D printing for tissue engineering.

from issues of spatial resolution. Intriguingly, Morley and co-workers' observations suggest a possible workaround for tissue engineering: they find that filaments contract within a certain parameter regime. In this regime, tissues would behave like Shrinky Dinks — toys that shrink when heated, but retain their initial shape[10]. The effective spatial resolution of printed tissue constructs might therefore be much better than the diameter of the printer's nozzle would typically allow, because of the compacting effect of cell tractions throughout the printed object. The challenges of 4D bioprinting thus provide exciting opportunities for engineers to grapple with tissue-developmental processes, and to treat them as controllable design motifs. ■

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INFECTIOUS DISEASES

A trial to tackle tiger mosquitoes

A fresh approach to suppressing the Asian tiger mosquito, a highly invasive species that transmits disease-causing viruses, has been used to nearly eradicate these insects from two test sites in China. SEE ARTICLE P.56

PETER A. ARMBRUSTER

Mosquito-borne viruses, including dengue, chikungunya and Zika, are major public-health threats[1]. Because neither vaccines nor effective drug treatments are available for most mosquito-borne viruses, vector control — that is, suppression of the mosquito populations that transmit viruses — remains the primary means of reducing disease incidence. The Asian tiger mosquito (*Aedes albopictus*) has spread rapidly in recent years, is increasingly prevalent in densely populated urban environments and is resistant to conventional vector-control practices[2]. On page 56, Zheng *et al.*[3] describe a new control strategy that almost completely eliminated *Ae. albopictus* from two experimental field sites, providing encouragement for future approaches to control *Ae. albopictus* and other vector mosquitoes.

Over the past two decades, various innovative strategies to reduce the transmission of disease-causing viruses and microbes by mosquitoes have been developed[4]. These strategies aim either to reduce mosquito populations (known as population suppression) or to make wild mosquitoes unable to transmit infectious diseases by spreading genetic modifications or bacterial infections through natural populations (known as population replacement).

Bacteria from the genus *Wolbachia* live in the cells of insect hosts, are maternally inherited and affect the reproduction of their host in such a way that they can be leveraged for both population suppression and population replacement. For example, when male mosquitoes infected with certain *Wolbachia* strains are released and mate with wild females that are not infected with the same *Wolbachia* strain, the females are unable to produce viable eggs (Fig. 1a). Alternatively, releasing males and females that are all infected with a strain of *Wolbachia* that makes mosquitoes less able to transmit viruses can lead to the spread of this strain through the wild population (Fig. 1b). Indeed, field trials of *Wolbachia*-based population replacement of the closely related *Aedes aegypti* are currently being conducted in five countries[4]. In addition, previous attempts at *Wolbachia*-based suppression of populations of several different mosquito species have shown some success[4].

Ae. albopictus is highly invasive and has spread rapidly from its native Asia to all continents except Antarctica over the past 40 years[5]. This mosquito is difficult to control, in part because larvae develop in a wide variety of artificial containers that are challenging to treat thoroughly with insecticides, and its desiccation-resistant eggs can survive in a dormant state for long periods.

Zheng and colleagues aimed to release male *Ae. albopictus* infected with a selected *Wolbachia* strain to suppress established populations in residential areas of two islands in a river in Guangzhou, China. Wild populations of tiger mosquitoes are infected with two strains of *Wolbachia* that do not block virus transmission[6]. The authors therefore infected *Ae. albopictus* with a third strain of *Wolbachia*, called *w*Pip, from the mosquito *Culex pipiens*, to produce a laboratory colony of mosquitoes that they called the HC population.

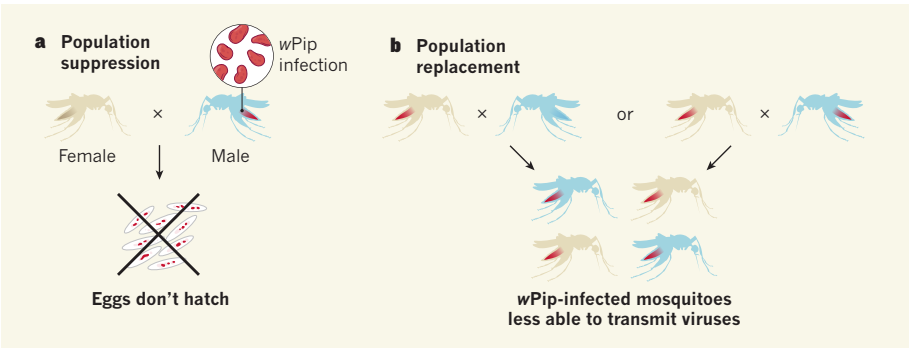

Figure 1 | Controlling populations of disease-transmitting mosquitoes using *Wolbachia* bacteria. **a**, One method of suppressing a mosquito population, termed population suppression, is to release male mosquitoes that are infected with a strain of *Wolbachia* bacteria that makes them unable to produce viable offspring with females that are not infected with the same *Wolbachia* strain. Zheng *et al.*[3] aimed to suppress tiger mosquito (*Aedes albopictus*) populations in this way by releasing male tiger mosquitoes carrying a *Wolbachia* strain called *w*Pip from another species of mosquito (*Culex pipiens*). **b**, Females infected with a given strain of *Wolbachia* can produce viable offspring with males regardless of whether the males are infected with the same strain. Thus, if *w*Pip-infected females were accidentally released, wild females would produce fewer offspring than the *w*Pip-infected females, and the *w*Pip infection would spread rapidly, leading to population replacement. However, Zheng *et al.* showed that *w*Pip-infected mosquitoes were less susceptible to infection by disease-causing viruses than were wild mosquitoes. Therefore, population replacement would still have resulted in reduced virus transmission.