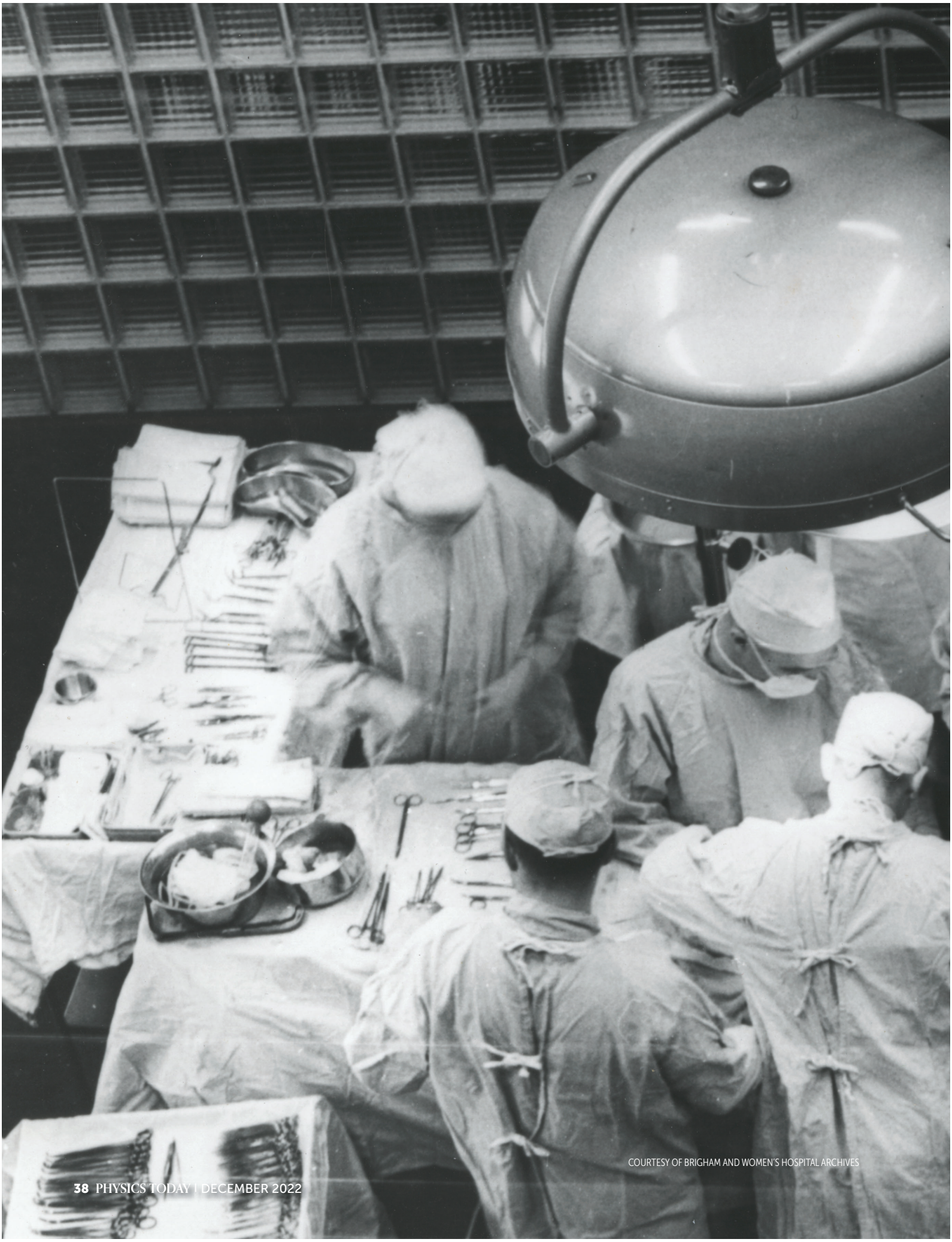

COURTESY OF BRIGHAM AND WOMEN'S HOSPITAL ARCHIVES

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FABRICATING HUMAN TISSUES: HOW PHYSICS CAN HELP

Ashkan Shafiee, Elham Ghadiri, and Robert Langer

By understanding and applying the physics of cellular self-assembly, scientists aim to predict tissue behaviors and accelerate the regeneration of human tissues and organs.

On 23 December 1954, the first successful organ transplantation was accomplished by a team of scientists and clinicians, including Joseph Murray, who was awarded the 1990 Nobel Prize in Physiology or Medicine for that breakthrough procedure.[1] It was performed at the Peter Bent Brigham Hospital in Boston. The surgery, captured in the photograph on the opposite page, involved transferring a kidney from Ronald Herrick to his identical twin, Richard. Having both donor and recipient genetically identical reduced the risk of adverse immune reactions and eliminated the chance of organ rejection.

In subsequent years, immune suppressive medicines enabled organ transplantation from genetically unrelated donors. In 1968 a Harvard University ad hoc committee on brain death (a neurological criterion for organ donation) recommended that individuals who have irreversible loss of brain function can be considered deceased.[2] The same year, the Uniform Law Commission drafted the Uniform Anatomical Gift Act, a regulatory framework for organ donation. Ever since, numerous types of organs—including heart, lung, liver, and pancreas—have been transplanted.

Despite remarkable advances in organ transplantation, the shortage of organ donors and the large number of patients who need a replacement have produced long waiting lists, which puts

patients' lives at risk. The shortage inspired one of us (Langer) and Joseph Vacanti to introduce tissue engineering in the 1980s and early 1990s, wherein physical sciences, cell biology, and chemical engineering would be used to regenerate human tissues and eventually organs.[3] To that end, new hope has dawned for patients with end-stage organ failure.

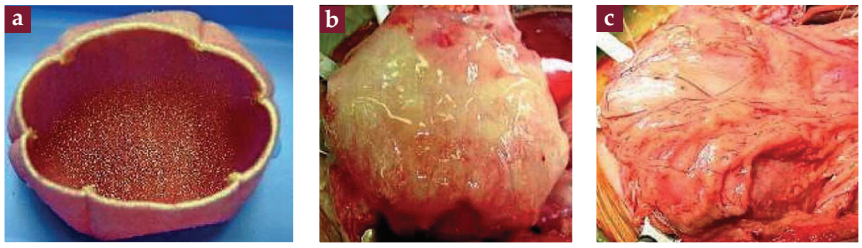

FIGURE 1. TISSUE-ENGINEERED human organs are transplanted into a patient. **(a)** A scaffold is shaped like a bladder and seeded with the patient's own cells. **(b)** The engineered bladder is then sutured to the patient's bladder. **(c)** A surgical material known as fibrin glue covers the scaffold's exterior. (Adapted with permission from ref. 4.)

Biofabrication

Engineered tissues can be made from cells from a patient's own body, another individual's body, or another species—referred to as autografts, allografts, and xenografts, respectively. Among them, the most promising are autografts because they largely eliminate the chance of rejection and require little, if any, immunosuppressive medication.

In 2006 Anthony Atala (Wake Forest University School of Medicine) and coworkers reported the biofabrication and transplantation of human bladders created from the patients' own cells[4] (see figure 1). The same group later reported the fabrication and transplantation of other organs, such as urethras and vaginas.

Autologous organ and tissue fabrication involves several steps, including cell biopsy and culturing, biofabrication of three-dimensional biological constructs, tissue maturation, and transplantation. The time needed to create a new organ, however, may be much longer than a patient can afford to wait, particularly when they urgently require transplantation.

From the point of view of a physicist, human organ fabrication is a 4D project because time is a tremendously important factor when considering the big picture of tissue and organ regeneration. Physicists are trying to understand the behavior of multicellular systems and the dynamics of cellular self-assembly in an effort to improve their ability to accelerate tissue and organ fabrication. Although cells are the building blocks of tissues and organs and are investigated largely in terms of their genetic and biological attributes, it is physical laws that they must ultimately follow, irrespective of the underlying biological processes.[5] Therefore, understanding and applying the physical principles of the dynamics of multicellular systems may help control the tissue regeneration procedure. Consequently, the physics of tissue engineering has gained more attention recently.

One can envision four types of organs: flat, such as skin; hollow and tubular, such as the urethra and vagina; hollow and nontubular, such as the bladder; and solid, such as the heart, liver, and kidneys. The challenge when fabricating solid organs is to vascularize the tissues to make thicker structures while maintaining their viability and function. Although scientists have fabricated some organs, the procedure is still not widely available. The goal is to eventually manufacture all the organs that patients need.

Biofabrication can be scaffold based or scaffold free. In scaffold-based tissue engineering, different natural or synthesized materials—polymers, mostly—can be used to mold the cells into appropriate structures during fabrication and after transplantation. The scaffolds are chiefly made of materials that degrade over time, thereby allowing tissue and organ integration into the body. As such, familiarity with the physics of how the cells interact with the scaffolds may help improve the biofabrication process.

Scaffold-free tissue engineering, on the other hand, uses cellular structures; no template biomaterials are required. Therefore, cellular self-assembly and the dynamics of multicellular systems play an important role in helping the fabricated tissue reach the maturation phase and to be ready for the next steps.

Bioprinting

There are a number of biofabrication techniques used for tissue engineering. Bioprinting is one promising technology for tissue and organ fabrication—and, ultimately, organ manufacturing—given that it is a reliable and precise technique for making 3D biological structures. (To learn about different bioprinting approaches, see reference 6.)

Organ and tissue printing is a term used in the tissue engineering, bioengineering, and biomaterials communities. It refers to a series of activities performed to biofabricate human organs using computer-controllable 3D printers. Although bioprinting shares many concepts with the ordinary 3D printing of objects, a key difference is that in normal 3D printing, the object is ready immediately after printing, whereas in bioprinting, the postbioprinting process is essential for achieving a reliable biological structure.

Immediately after the bioprinting, there are only 3D biological structures composed of cells and supporting materials that must undergo cellular self-assembly to become the tissue construct ready for the following steps. Cellular self-assembly—including but not limited to cell migration to physiologically appropriate locations and tissue fusions that provide mechanical integrity for the bioprinted tissue—occurs postbioprinting. Here again, understanding and applying the physics of the multicellular system can help engineers bioprint tissues and organs.

Physics of multicellular systems

Scientists looked toward the developing embryo, which is the quintessential tissue and organ engineering process, to understand the physics behind organogenesis. Their work relied on Malcolm Steinberg's pioneering model of tissue liquidity, a loose analogy that allowed scientists to use the known properties of liquids to assess tissue behavior.[5,7] That analogy was based on the many similarities between tissues and liquids. For example, tissues try to minimize their surface energy and form a sphere out of cylindrical or cubical shapes. Spheroids made from the same type of tissue fuse together to create larger tis-

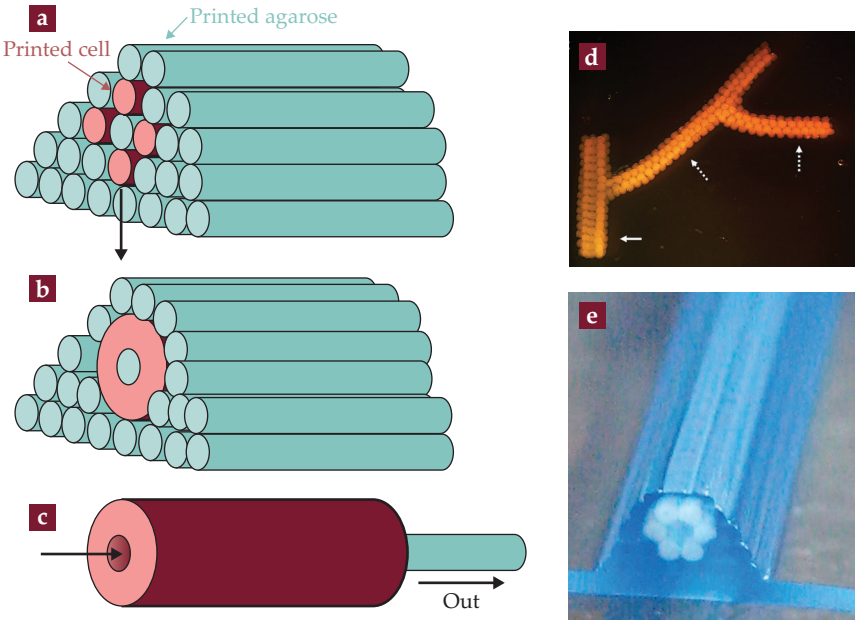

FIGURE 2. BIOPRINTING BLOOD VESSELS, scaffold free. **(a)** In this schematic, red rods depict cellular bioinks, each composed of millions of cells, and green ones depict agarose, a printable hydrogel that serves as a structural support layer. **(b)** After fusion is complete and cellular self-assembly occurs, the bioprinted structure is ready for the next steps. The time that elapses for the construct to change from the structure in panel a to that in panel b is critical for the final regenerated tissue. **(c)** The supportive layers of agarose can then be removed, leaving a hollow tube. (Panels a–c adapted from ref. 10.) **(d)** This bioprinted branched tubular structure has diffe rent inner diameters: 1.2 mm (solid arrow) and 0.9 mm (dashed arrows). **(e)** This image shows a bioprinted blood vessel just after the printing process. Blue rods are agarose cylinders and white rods are bioinks composed of cells. (Panels d–e adapted with permission from ref. 9.)

sue, as would, for example, two droplets of water. Moreover, different tissue types separate, based on their viscoelastic properties, much like droplets of immiscible liquids, such as oil and water.

In what's known as the differential adhesion hypothesis, Steinberg stated in 1963 that cells in different tissues adhere to each other with different strengths.[7] That property was subsequently shown to be a feature of 3D cellular structures, not 2D monolayers. Different tissue-fabrication techniques from the same cell source could provide different strengths.[8]

Gabor Forgacs of the University of Missouri and his collaborators, including Steinberg, quantified the biophysical parameters of many tissue-related properties, such as the apparent tissue surface tension. A measure of how cells adhere in a tissue, surface tension can help scientists predict the interaction pattern of different tissues. For example, tissues with higher surface tensions are engulfed by those with lower surface tensions, similar to how oil engulfs water.

That prediction of the interaction patterns of two tissues is helpful when calculating the self-assembly of a mixture of different cell types postbioprinting, given that bioprinted structures such as blood vessels and nerves undergo cellular self-assembly to become sturdy biological structures with physiologically appropriate cell localization.[9] Cyrille Norotte (then at the University of Missouri) and collaborators used the prediction of tissue interaction to print human blood vessels, in which microtissues comprising smooth muscle cells had a higher surface tension than did those comprising fibroblasts. The postbioprinting cellular self-assembly mimicked the exact pattern observed in human blood vessels.[9]

A bioprinter can deposit bioinks on supportive layers, which are mostly hydrogels, such as agarose. Bioinks are either spherical or cylindrical paste-like materials composed of millions of cells that are loaded into a bioprinter's cartridge to be 3D printed. Figure 2 demonstrates the concept of cellular self-assembly, postbioprinting.

Models for cellular behavior

Most primary works to understand the physics of cellular self-assembly and the dynamics of multicellular systems investigated embryonic morphogenesis. To that end, cell sorting, movement of cell collections, and early morphogenesis have all attracted the attention of researchers.[5]

More recent works have concentrated on tissue-engineering applications and the physics of tissue regeneration, with the ultimate goal of predicting and controlling the time it takes tissue to mature.[10] Most models successfully predicted the behavior of tissue with a limited number of cells, and one of the challenges that remained was addressing tissue behavior for multicellular systems with millions of cells.

Garrett Odell and coworkers from the University of California, Berkeley, performed investigations on multicellular systems more than 40 years ago when they sought to understand cellular behavior in sea urchin embryos.[11] They hypothesized that a decrease in the apical circumference of the epithelial layers of a blastula—a multicellular aggregate—occurs because of the contractile activity of actin filaments. In a developing embryo, the process of cleavage of the single-cell-stage zygote results in the formation of the blastula and is considered an example of early morphogenesis.[5]

Eirikur Palsson of the City University of New York and Hans Othmer of the University of Minnesota introduced a model for cell movement in a slime mold, in which they considered cells to be deformable viscoelastic ellipsoids, and integrated signal transduction and cell–cell signaling into their model.[12] The subcellular-element method, which was able to describe the dynamics of larger numbers of interacting cells, was introduced by Thea Newman, then at Arizona State University.[13] She considered the subcellular elements as fundamental dynamical variables and used the inter- and intracellular potentials to understand the dynamics of multicellular systems.

The ultimate goal of understanding the physics of cellular self-assembly is to predict and control tissue behavior. Controlling self-assembly can be used to either decelerate tissue migration in oncology or accelerate tissue maturation in tissue engineering. Most of the models presented so far, however, failed to describe the behavior of cells beyond a certain number. Therefore, physicists have been working hard to predict the

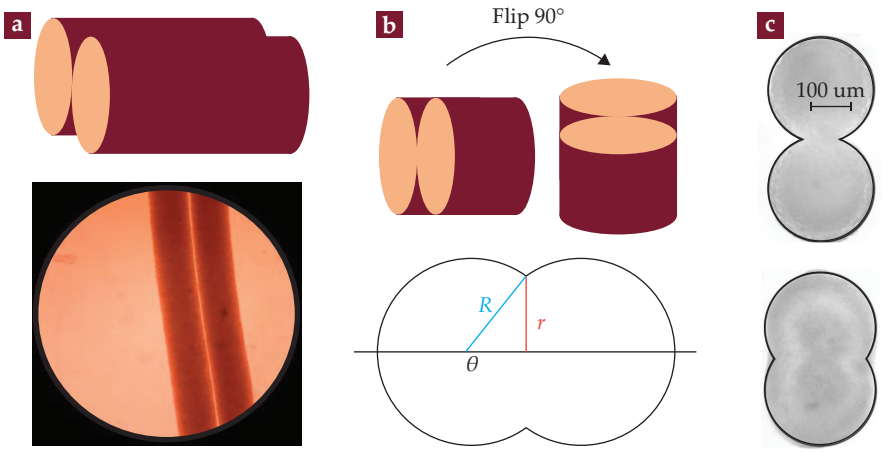

FIGURE 3. BIOINK FUSION. (a) Shown schematically, two cylindrical bioinks sit next to each other, immediately after being bioprinted. **(b)** A few hours later, they have evolved into fused pieces and can be cut into smaller sections. By flipping the fused bioinks by 90° and recording their cross-sectional geometric parameters *r*, *R*, and *θ*, at different times during that evolution, researchers can obtain the characteristic fusion time—a quantitative measure of the cellular self-assembly procedure. **(c)** Twenty hours of evolution separate the top and bottom light-microscopy images of fused cylindrical bioinks. (Adapted from ref. 10.)

time of tissue relocation, postbiofabrication, across the scales to address tissue sizes and greater cell numbers.

That time of tissue relocation is essential in the process of tissue regeneration. In figure 2, for example, if the biological construct is removed from the container in which it was bioprinted before it gains its mechanical integrity during cellular self-assembly, the structure may fall apart. On the other hand, if the structure is given more time than it requires to mature, it may develop some necrotic cores with dead cells that may impact the functionality of the structure after transplantation.

To predict the optimum time, Ioan Kosztin of the University of Missouri and collaborators, including Forgacs, introduced cellular particle dynamics (CPD).[14] It provides a theoretical, computational, and experimental framework to address the tissue dynamics and time of tissue fusion, postbioprinting.[15,16] CPD involves examining the fusion of two spherical and cylindrical bioinks or microtissues that are used to 3D print biological structures. After the bioprinting is complete, tissue fusion allows the biofabricated tissue to adopt the mechanical properties required for its function and is one of the most important steps in tissue maturation. Figure 3 shows the fusion of two cylindrical bioinks and the experimental procedure to measure the fusion time.

The CPD formalism coarse-grains each cell into a finite number of equal regions and considers them cellular particles (CPs). The interactions among those CPs are introduced using short-range contact forces, and their dynamics are explained by an overdamped Langevin equation.[14] The ultimate goal of that formalism is to predict the time evolution of a multicellular system, specifically the change in the biological structure's shape.

In 2014 Matthew McCune, then at the University of Missouri, and collaborators accomplished that goal by computing and recording the simulated trajectories of all CPs. Using the formalism, they were able to predict the fusion of same- and different-sized spherical bioinks as well as cylindrical bioinks. Their framework consists of a comprehensive experimental component to evaluate the theoretical part and calibrate the simulation.

Controlling time with zipper CAMs

Although CPD was able to predict the fusion time of different structures with different cell types and geometries, it was not capable of controlling the time.[15,16] To the best of our knowledge, the only model that provides different biophysical parameters to control and fine-tune the behavior of multicellular systems is the zipper cell adhesion molecules (CAMs) model introduced by two of us (Shafiee and Ghadiri) and our collaborators.[8,10,17]

CAMs are cell surface proteins by which cells bind either to each other or to the extracellular matrix—a network of proteins and molecules essential for tissue integrity. The dynamics of those molecules play an important role in the zipper CAM framework, in which the analysis of experimental data for tissue fusion considers how the cell surface adhesion molecules are involved in tissue dynamics.

Forgacs and colleagues previously showed the relationship between the number and strength of CAMs on the surface of the cells and their respective surface tensions; the researchers found that a higher number of CAMs causes a higher tissue surface tension. On the other hand, using the zipper CAMs model, Shafiee, Norotte, and Ghadiri showed that cellular bioinks with higher surface tension have faster fusion and tissue maturation rates.[8] The finding implies that a higher number of CAMs or stronger ones may help accelerate tissue fusion.

In that framework, cells are imagined to lie on ribbons, or lines, atop spherical and cylindrical bioinks. The fusion of two bioinks starts when they are located in close vicinity to each other. The outermost cells of two close bioinks detect each other on their surfaces via their CAMs and develop new bonds. After the CAMs of all cells positioned on the first imaginary line bond, their proximity will force the next line of cells to approach each other. Based on the adhesion molecules' chemistry, their length when they form bonds is shorter than that of their free state. Figure 4 demonstrates the basics of the zipper CAMs model for fusing cylindrical bioinks.

It is essential to understand that in the model, cells attach to each other one by one, and the imaginary lines are mentioned only to demonstrate the location of a group of cells involved in each step. The cellular attachment resembles a zipper. In a zipper, the slider passes through teeth that engage each other and close one by one. Likewise, in the zipper CAMs model, the cells use their CAMs to attach to each other when they develop their bonds.

With that analogy, therefore, statistical mechanics was used for the zipper CAMs model, with energy equal to zero for the (unbonded) ground state and ε for a (bonded) excited state. The researchers used the appropriate partition function to establish a mathematical technique to identify the energy and force in-

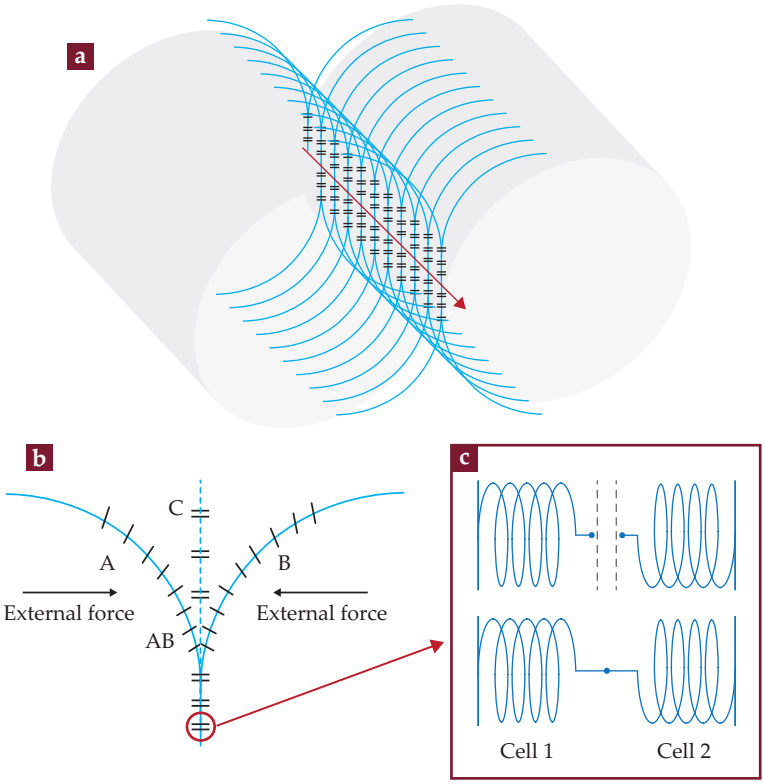

FIGURE 4. ZIPPER CAM. The zipper cell adhesion molecules (CAMs) model describes how to use different biophysical parameters to accelerate tissue formation. **(a)** In the case of cylindrical bioinks, the cellular attachment is considered as two series of closing zippers (above and below the contact line, shown in red). **(b)** The solid curves show the original site of adhesion molecules on the surface of each bioink. The dashed line represents the final location of cells after attachment when fusion is complete. Cell adhesion molecules of outermost cells on each bioink are shown as A and B in the unbonded, ground state; C represents the developed bond, the excited state. **(c)** The adhesion molecules can also be studied as springs, wherein a deviation bond length and applied external force help the bond develop faster. (Adapted from ref. 10.)

volved in tissue fusion—and hence cellular self-assembly. The identification of energy and force in cellular self-assembly was not previously possible.

The framework then predicted that by applying an external force—by pushing the bioinks toward each other—the cellular attachment could be further expedited.[10] Shafiee and collaborators therefore investigated the effect of external force on tissue dynamics and confirmed that tissue fusion was indeed faster. They developed a mechanotransduction-based bioprinter to evaluate the prediction and used an external force to push the bioinks toward each other immediately after bioprinting.

The external force had to be limited, however, to prevent any damage to cells and tissues. The mechanotransduction-based bioprinter deposits bioinks inside the grooves of agarose so that the two bioinks experience the force from the walls of the grooves pushing them toward each other. That configuration could help the fusion procedure by providing cells with a limited external force to facilitate their migration. A normal bioprinter deposits bioinks on a flat surface, and the tissue fusion occurs only by using the energy and force involved in self-assembly, fueled only by the biochemistry of CAMs.

Comparing different bioprinted structures from a mechanotransduction-based bioprinter and normal bioprinters demonstrates that the fusion occurs almost three times as fast for the products of the mechanotransduction bioprinter.[10] The comparison attests to the use of CAMs as a building block of tissue movement and to the versatility of the zipper CAMs model in terms of controlling or accelerating tissue maturation.

Future directions

The physics of tissue engineering in general and of bioprinting in particular is a relatively new field that could provide numerous opportunities for tissue and organ fabrication and regeneration. Increasing the number of physicists who can investigate the different aspects of tissue dynamics could help the field move forward and introduce more complex structures and additional materials.

It appears that the amount of computational and theoretical work aimed at predicting tissue behavior far exceeds the number of experimental studies.[18] On one hand, it is good to have a number of different computational approaches that save money and time, but the fruits of those endeavors have not significantly reached the tissue-engineering community.

It would be helpful to have more physicists working together with biologists and bioengineers to optimize the physics of every single step of tissue fabrication. As stronger experimental approaches to the physics of tissue engineering are developed, computational investigations can help advance our abilities to tackle more complex problems in the field. Physics can identify different biophysical parameters with which to fine-tune tissue behavior and introduce innovative techniques and technologies to adjust those parameters to achieve the desired tissue behavior.

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