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Opinion

# Next-generation engineered microsystems for cell biology: a systems-level roadmap

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**Engineered microsystems for *in vitro* studies of cultured cells are evolving from simple 2D platforms to 3D architectures and organoid cultures. Despite advances in reproducing ever more sophisticated biology in these systems, there remain foundational challenges in re-creating key aspects of tissue composition, architecture, and mechanics that are critical to recapitulating *in vivo* processes. Against the backdrop of current progress in 3D fabrication methods, we evaluate the key requirements for the next generation of cellular platforms. We postulate that these future platforms – apart from building tissue-like structures – will need to have the ability to readily sense and autonomously modulate tissue responses over time, as occurs in natural microenvironments. Such interactive robotic platforms that report and guide cellular events will enable us to probe a previously inaccessible class of questions in cell biology.**

## Highlights

The tissue microenvironment – diverse cells, extracellular matrices (ECMs), soluble factors, physical forces, and their respective organizations – is dynamic and regulates cell fate and function. Engineered microsystems re-create relevant cues *in vitro* to experiment without the complex crosstalk within an organism.

3D fabrication advances are driving these microsystems from 2D to biomimetic 3D geometries. Organoid cultures are achieving organ-like cellular diversity. The combination of these two developments is proving to be powerful.

Additive fabrication allows depositing cells and ECMs into large organotypic constructs with features greater than ~100 μm, whereas subtractive photoablation achieves micrometer-scale resolution but cannot position cells or ECMs. Next-generation engineered microsystems need to achieve both large-scale and fine resolution.

Controlling cues noninvasively midculture using advances such as in optogenetics or magnetic actuation will help simulate rapid microenvironmental changes seen in development, regeneration, and disease.

## Re-creating tissue microenvironments *in vitro*

In the past century, isolating cells from their *in vivo* environment and culturing them *in vitro* has provided a key path to identify critical machinery, signaling, and processes that drive cellular function, establishing the foundations for modern molecular and cellular biology. However, despite these critical advances, it has also become clear that cells *in vitro* often fail to recapitulate behaviors observed *in vivo*. Cells residing in tissues continuously monitor and respond to various dynamic stimuli in their microenvironment that are not present in typical cell culture settings. Components of the microenvironment [i.e., all the distinct cell types, extracellular matrices (ECMs), soluble factors, and physical forces] collectively provide the external cues that regulate cellular function. Furthermore, these cues are neither static nor spatially uniform. Tissues are composed of cells that actively move about; ECM architecture (alignment, composition, pores, and stiffness) is continually remodeled and vastly inhomogeneous across multiple length scales; soluble growth factors appear in a time-varying manner and in coordinated spatial patterns and gradients; and both cell-generated tractions and external forces can be anisotropic and occur in transients.

Although it is conceptually simple to introduce multiple cell types, ECMs, and growth factors into a single construct, re-creating physiologically relevant architectures is challenging and yet critically important to achieving function. The specific organization of cells and ECMs determines how muscle generates and transmits forces [1,2], the efficiency of oxygen transport between lung alveoli and nearby capillaries [3], or whether fluid transport through a tissue is experienced as interstitial flow or flow through cell-lined lumens [4].

Recently, engineered microsystems have emerged to address this gap, with the goal of re-creating and controlling one or a few of these diverse signals and their appropriate spatial organization *de novo* to accurately mimic the tissue microenvironment. Although engineering all these signals synthetically outside an organism may seem like an impossible challenge, the key appeal of

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engineering new microsystems is that they allow investigators to dissect the specific roles of different cues in regulating cell and tissue function – control knobs that cannot be independently tuned *in vivo*. Characterizing each context (or cue) individually and in small groups has offered new insights into how such cues regulate cell behavior, ultimately lending modularity and reusability to our distilled map of intracellular processes.

Recognition of the importance of tissue organization has driven the development of numerous microsystems to investigate processes previously studied only in *in vivo* models. For example, it has been possible to study the function of the alveolar–capillary interface in the lungs using sandwiched cultures of epithelial and endothelial cells separated by ECM-coated stretchable membranes [5], assess the vascular barrier using endothelial cell–lined cavities in collagen [6], or measure the contractile forces produced from microtissues of cardiomyocytes suspended between deformable posts [7].

These engineered models have been buoyed by advances in fabrication technologies such as 3D printing, allowing the assembly of complex, predefined geometries. However, they are typically seeded with homogeneous cell populations and therefore do not truly reflect the diversity of cells *in vivo*, which organoids succeed at. By contrast, unlike top-down fabricated devices, stem cells cultured in ECMs can form self-assembled organoids that better represent the cellular heterogeneity seen in organs (such as work from Clevers and colleagues on the formation of intestinal crypt and villus-like domains from Lgr5 stem cells [8] or recent reports of brain organoids that were shown to develop bilateral eyelike features [9]). However, organoids come in an uncontrolled spectrum of sizes and typically in closed geometries that limit accessibility of the luminal space. It is possible that a combination of these two approaches (i.e., employing top-down fabricated structures to steer organoid morphogenesis) could prove to be a powerful paradigm for the future of engineered microsystems. Recent evidence comes from intestinal stem cells that were cultured on top-down fabricated crypt/villus-like structures that yielded more robust functions than regular organoids, produced rarer cells, and had accessible lumens, enabling the infusion of nutrients and clearance of dead cells (thereby promoting health and prolonging lifespan) [10]. This reinforces the continued role that fabrication approaches will play in all future engineered microsystems.

With the growing sophistication of engineered microsystems, these systems are increasingly viewed as a path toward the translational goals of tissue engineering. However, there are distinct priorities when building tissues to study biological processes versus the ambitious goal of replacing damaged human organs, despite the overlapping importance of re-creating tissue organization and composition. Engineered tissues used to study specific cellular mechanisms or to characterize responses to stimuli are only required to accurately model physiology of those specific aspects. How well they perform other functions is only a secondary consideration, if at all. Augmenting these tissues with sensors (to help in probing function) or actuators (to rapidly apply different stimuli) is critical to gaining fundamental insights into how these biological systems operate. By contrast, for engineered tissues designed to take over from human organs, the primary focus will be on their ability to meaningfully restore all organ functions over long intervals while partaking in interorgan communications. Although tissues that replace organs do not need built-in sensors that report tissue function to the outside world, such sensors would nonetheless help in continually tracking how well engineered organs integrate with the body. Moreover, feedback from tissue-embedded instrumentation will fast-track the iterative process likely needed to achieve functional, organ-scale tissues.

Rather than providing an in-depth review of existing engineering microsystems that have been developed (see [11–13] for such reviews), here we discuss some of the recent engineering

## Glossary

**Magnetogenetics:** a toolkit to control cellular activity of genetically edited cells through magnetic stimulation. Commonly, magnetic stimulation is converted to changes in force or heat that are then sensed by the cells; for example, alternating magnetic fields can heat magnetic nanoparticles that trigger temperature-sensitive channels (such as TRPV1) to regulate gene expression.
**Optogenetics:** a class of tools for the control of cellular activity of genetically predefined cells using light. This is achieved by engineering light-responsive proteins in cells; for example, cell protrusion and movement can be controlled optically by fusing light-sensitive domains to Rac1.
**Photoabsorbers:** an additive used to attenuate light in projection stereolithography resins. To improve the resolution of printed parts in the *z*-axis (layer thickness), light blockers are used to confine photopolymerization within a thin layer and attenuate light quickly outside this layer.
**Photocavitation:** the formation of cavities such as bubbles or voids in a solid or liquid, initiated by light. In the context of photoablation of water-based hydrogels, it is the mechanism by which concentrated energy from laser sources leads to the formation of a bubble that first expands rapidly. This energy is dissipated by rupturing a small volume of the polymer network, after which the bubble collapses.
**Proteome:** the group of all proteins expressed at a given time. This includes subsets such as the secretome, which is the set of proteins expressed and secreted into the extracellular area, of which ECM-related proteins fall under the subcategory of matrisome. Quantifying the proteome offers robust insight into the state and function of a cell or tissue.
**Soft lithography:** a collective set of techniques that use soft elastomeric stamps and molds for fabrication and patterning. Typically, there are two steps in soft lithography: first, fabricating the 'master' mold from which the elastomeric stamp is obtained, and second, using the elastomeric stamp to pattern and fabricate new structures. For instance, polydimethylsiloxane (PDMS) stamps, commonly used in soft lithography, can be used to pattern adhesive proteins to control cell adhesion.

advances that may contribute to the next generations of 3D culture platforms. We also describe the challenges and opportunities in mimicking the extreme dynamics of natural microenvironments *in vitro,* as well as fabricating larger, complex tissue-engineered constructs.

## New fabrication methods are advancing 3D platforms

**Soft lithography** (see Glossary) and layer-by-layer patterning precipitated the shift from planar cell culture to patterned substrates and microfluidic platforms about three decades ago. Currently, two classes of 3D fabrication methods have enabled the development and propagation of 3D culture-based engineered microsystems: 3D printing and photoablation. 3D printing covers a range of additive manufacturing processes where small volumes of materials (e.g., cells, ECMs, sacrificial materials) are deposited sequentially to build up a structure; this is interchangeably called 'bioprinting' when used for building biological structures for cell biology and tissue engineering applications. 'Photoablation' refers to a class of fabrication processes where small volumes of a biomaterial are degraded using light to sculpt the final structure starting from a block of biomaterial (Figure 1A).

Additive assembly processes allow one to position cells, surrounding ECMs, and growth factors into desired structural architectures, including conduits. Therefore, conceptually, entire tissue constructs can be bioprinted. Bioprinting comes in a variety of types based on the technical toolkit, ranging from extrusion of materials or cells through a single nozzle to layer-by-layer crosslinking of polymers (Figure 1B). Most often these processes are evaluated on the basis of three specific metrics: resolution, material flexibility, and speed. The resolution defines the smallest length scales that can be consistently achieved by the process, often set by the smallest volume of material that can be reliably deposited. This determines the size of the smallest 'building blocks' used to assemble the final printed construct. Equally important is whether the printing process can assemble a wide range of inks. Printing living cells and natural ECMs imposes severe limits on the process, print time, and level of environmental control, thereby making it substantially more challenging than printing acellular synthetic parts. Although no one process currently excels across all three metrics, they have all contributed to the emerging generation of highly engineered, organ-specific microsystems. Complex alveoli-like constructs have been printed via **stereolithography** using poly(ethylene glycol) diacrylate hydrogels with food dyes such as tartrazine as **photoabsorbers** [14]. These printed hydrogel structures also function as supports for hepatic tissues and allow host engraftment, showing potential for therapeutic use. In contrast to printing a synthetic hydrogel, it has been possible to create vascular conduits directly in dense cell aggregates by depositing sacrificial materials that subsequently can be cleared out [15]. There has also been substantial progress in printing both ECMs and cells directly. Anatomically faithful sections of the heart can be fabricated by printing collagen scaffolds alongside stem cell–derived cardiomyocytes [16]. Bioprinting has also been used to deposit organoid-forming stem cells into appropriate matrices in controlled macroscale architectures [17]. While this method is typically limited in resolution (~hundreds of μm), this approach allows the morphogenetic program of organoid formation to take over to then produce the smaller-scale features, such as lumens and crypt/villus-like structures when starting from intestinal stem cells. These examples are some recent highlights; different types of 3D printing and their applications to various biological questions are reviewed more comprehensively elsewhere [18,19].

Subtractive processes create structure by removing material from an existing shape, in contrast to additive techniques that add small volumes of new material. Therefore, subtractive methods such as photoablation are limited to creating structure alone and are not used for directly positioning cells and ECMs. During photoablation, both synthetic and natural hydrogels can be degraded using lasers by disrupting the polymer network directly or through **photocavitation** of water.

**Stereolithography:** also known as SLA printing, is an additive manufacturing process where a focused laser beam is used to crosslink a photopolymer resin one layer at a time. The pattern drawn on each layer solidifies the select areas, and this is repeated layer by layer to form the 3D object. The final part is removed from the resin tank and rinsed to clear the unused resin.

**Transcriptome:** a set of all the RNA transcripts present in a cell. mRNA transcripts are an important part of this full readout and are essential to making proteins. RNA transcript counts can indicate which genes are active or transcribed more than others.

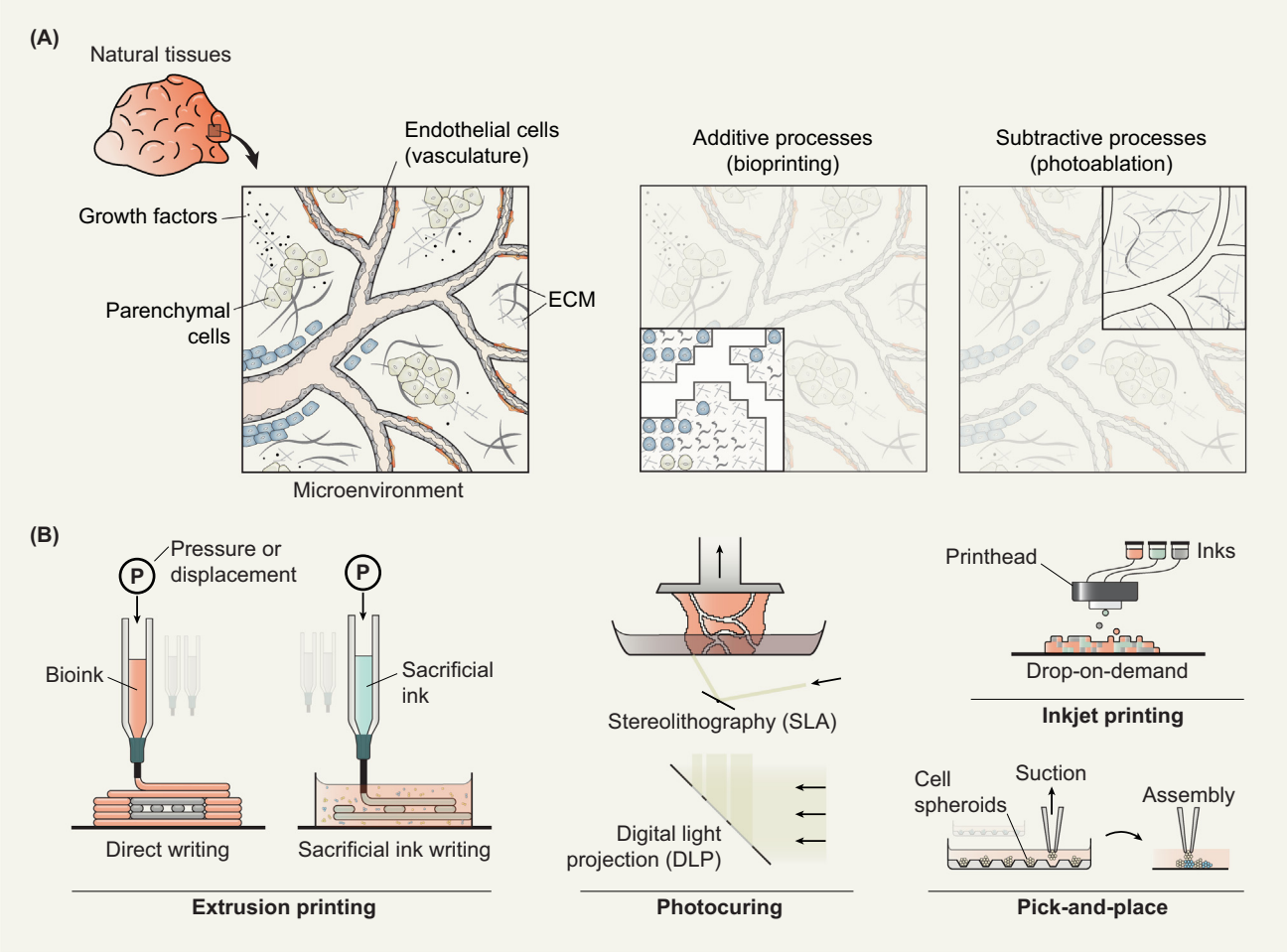


Figure 1. Engineering the tissue microenvironment *in vitro*. (A) Natural tissue microenvironments consist of many diverse components: cells, extracellular matrices (ECMs), and growth factors in intricate arrangements. To re-create more complex arrangements in 3D engineered systems, two classes of fabrication strategies are emerging. Additive manufacturing methods involve assembling cells and ECMs at coarse resolutions (and sacrificial materials to define voids). By contrast, subtractive methods rely on degrading portions of an existing construct. This does not allow one to directly position cells and ECMs, but this approach offers high resolution in defining cavities. (B) Bioprinting is a group of fabrication techniques that involve sequentially adding small volumes of bioinks to create a larger construct. Extrusion printing, a widely used bioprinting technique, involves depositing materials through a nozzle. In stereolithography and digital light projection, light is used to crosslink selective regions of a vat containing a monomer ink layer by layer to create the final structure. Inkjet printing techniques allow the deposition of droplets of inks through a printhead with multiple nozzles. In pick-and-place techniques, separately assembled cell spheroids are rearranged using suction.

These cavities are subsequently covered with cells that migrate from proximal areas or through direct cell seeding. Despite its inability to assemble cells and matrices, photoablation offers extremely high (micrometer-scale) resolution in defining cavities because one can confine energy into very small focal volumes by relying on multiphoton absorption [20]. This precision remarkably enables engineered capillary-scale channels (~10 μm) in collagen that are then covered with endothelial cells to model human microvasculature [21]. Photoablation can also be used to engineer complex scaffold architectures [22]. Subtractive schemes are expeditious when the amount of material to be removed is less than the material required to create the full construct additively (e.g., in the fabrication of branched vasculature and other ductal forms).

Both 3D printing and photoablation are promising because they offer the design freedom required to realize the breadth of designs that reflect different tissues. Although we focus on these two developing technologies, a different strategy has been used for a large fraction of engineered microsystems of the past. These specialized fabrication recipes are based on a sequential combination of processes such as soft lithography, molding, casting, and stamping. For instance, soft lithography can be used to form a supporting structure that allows accurate positioning of a needle mold around which natural ECMs can be cast. Upon cell seeding, this process produces endothelial cell–lined linear vascular conduits with perfect circular cross-sections and high precision in natural ECMs. While such recipes lack general purpose utility, they typically outperform more general methods such as 3D printing in resolution and repeatability within design constraints.

The automation tools and precision gantries that are used in 3D printers readily lend themselves to other uses. They can be used to perform precision pick-and-place tasks with cell spheroids of diverse cell types into complex arrangements [23]. Similarly, they can be used as robots that handle liquids to execute organoid expansion with at least the same efficiency as humans [24]. As it stands, there is little doubt that such automation technologies will play a frontline role in the high throughput use of future engineered microsystems.

## Next-generation challenges in tackling structural organization

Despite the treasure trove of technologies that have emerged in the past 2 decades, there are many basic challenges that confront the next generation of engineered microsystems. Existing fabrication methods still struggle with spatial resolution, achieving structures such as lumens and conduits that are millimeters down to hundreds of micrometers for most bioprinting methods currently [14–16,25] and micrometer scale for subtractive techniques such as photoablation [21]. No existing method can arrange materials such as collagen, laminin, or fibronectin at the length scales of most cellular basement membranes (typically 50–100 nm) in nonplanar architectures. Even though it is well established now that the basement membrane is a critical regulator of cell function both in health and in pathological conditions, such as in cancer [26], our ability to freely define basement membrane architecture and composition in engineered constructs is limited.

Most mature tissues also exhibit distinct cell populations separated at micrometer-length scales that cannot be engineered from scratch into freeform architectures *in vitro*. For instance, gas exchange occurs in alveolar sacs that are arranged in macroscale 3D architectures, but the stratified layers of squamous epithelium, basement membranes, and capillaries are layered at submicrometer-length scales. How to position multiple cells into such precise cellular arrangements in 3D architectures is unclear. Although cell layers stacked on either side of a thin, porous planar membrane can provide representative functional readouts and help in screening drug toxicity, the planar constraints of such membranes will substantially impede the translational aspirations of next-generation engineered microsystems, where it is essential to re-create microscopic cell–cell interactions in freeform, dynamically remodeling 3D constructs.

Engineering tissue constructs that capture cellular arrangements at multiple length scales is a formidable challenge, but this is a key requirement to someday realize highly functional constructs that can support damaged human organs. The need for creating multiscale features and hierarchical spatial arrangements is especially evident in fabricating vascular networks (Box 1). Positioning both biomaterials and cells to simultaneously preserve microscale intercellular relationships and the macroscale 3D tissue architecture is also where existing 3D fabrication technologies are most challenged; they work optimally at a small range of length scales. For example, when a nozzle is used to deposit cells or materials, the deposition rate must be optimized to limit shear damage to cells [27], and nozzles should be sufficiently larger than cells (typically

Box 1. Challenges in engineering vasculature

The vasculature in the body is an intricate network of endothelial cell–lined blood vessels that nourish tissues. Engineering large constructs of dense vascular networks *in vitro* is one of the grand challenges in the field of tissue engineering, a critical step in the path to creating organs, and an excellent use case for illustrating the challenges of 3D fabrication. The hierarchical branching of blood vessels from arteries to arterioles and capillaries is also accompanied by a progressive reduction in the vessel calibers and an increase in the number of vessels (called Murray's Law [29]). Current top-down fabrication tools can create large millimeter-scale conduits the size of arteries, but creating the entire network of vessels spanning arterioles (~500 μm) to capillaries (5–10 μm) has been a long-standing challenge [30]. Engineered vasculogenesis-based microvascular networks [31] and blood vessel organoids [32] can address some of the challenges in creating the intricate network of capillaries. New emerging methods are beginning to construct simple (two-level) hierarchies of the microvasculature, by combining preformed, endothelialized microvessels and photoablation-guided angiogenesis [33]. Expanding on such efforts to achieve the many-level hierarchies that exist in native tissues will require additional innovations. This is part of the broader challenge for engineered models in reconstructing the spatially varying vessel architecture, multicellular compositions, and matrix organization at once: Large-caliber arterioles have a layer of smooth muscle cells that surround the endothelium, and the endothelial cells in the capillaries are in close proximity to pericytes that are critical to maintaining the vascular barrier.

There are substantial hurdles in re-creating the subtle forms of the individual vascular elements that limit our understanding of the processes that drive their formation. Vascular elements such as bicuspid valves in small venules (<100 μm diameters) that prevent backward flow *in vivo* [34] cannot be re-created with anatomical accuracy either by top-down fabrication methods or through self-organization. Likewise, although much attention has been paid to sprouting angiogenesis, comparatively little is known about the mechanisms behind splitting angiogenesis (or intussusceptive angiogenesis) – the splitting of a capillary into two by the formation of an intraluminal post (intussusception) [35]. It is likely that one may learn more by creating models of the intermediate stages of splitting angiogenesis and observing their progression. Our inability to engineer ECM pillars inside lumens with submicrometer resolution has been a limiting factor. Therefore, structural engineering will play a central role in fundamental and translational pursuits in vascular biology.

~five to ten times cell size) to prevent clogging. This imposes lower bounds on the smallest achievable features as well as the build time. By contrast, subtractive processes such as laser-based photoablation can work at fine micrometer-scale resolutions but are restricted to small volumes (utmost millimeter scale) due to the limits on the depth of light penetration and degradation time [20]. Therefore, photoablation can be used to define any small complex section of a vascular network, but it is limited when it comes to forming the entire network with multiple hierarchical branches or with a dense parenchymal cell population (Figure 1A). Future advances will therefore have to focus on fabricating structures with multiple materials and cells at adaptive resolutions, choosing between coarse resolutions for creating the bulk tissue and finer resolutions for defining features such as conduits and intricate cell arrangements. If controlling all the spatial features (that span multiple orders of magnitude) using a single process proves to be a major hurdle, it would be prudent to focus on hybrid approaches. Multiprocess 3D printing is being explored for creating more complex synthetic parts by transferring a printed part between disparate printing processes that each have their unique capabilities [28], but implementing this for constructs with live cells and hydrogels/ECMs may prove to be more difficult. However, one can easily envision using top-down fabrication tools to realize macroscopic architectures and rely on cellular self-assembly and organoid morphogenesis to evolve the finer structures. This is part of the broader paradigm of 4D fabrication, where the final structure is evolved over time (the fourth dimension), such as by leveraging shape-changing materials or the self-organization of cells. In this case, it would be critical to learn the input–output spatial relationships (i.e., how predefined constructs morph to the final organoid-driven architecture) to achieve better control of the final structure.

## Next-generation challenges: engineering dynamics and autonomy

Tissue homeostasis in adult human tissues is characterized by stable cell numbers and compositions that are tightly regulated through control of cell proliferation, death, fates, and maturity. This enables the maintenance of tissue structure and size through controlled changes in the ECM architecture (at least for a time). As a result, such stable tissues exhibit consistent functional

performance and metabolic loads that could be thought of as a signature of 'static' tissues. From an engineering perspective, however, these systems are not truly static, and the field would benefit immensely from a more quantitative consensus framework in benchmarking what constitutes stability of engineered tissue constructs and how to provide nondestructive measurements for that framework.

By contrast, there are times when cells experience dramatic changes in their microenvironment: During development, there are rapid variations in cellular organization, fates, and composition; tissue repair immediately following an injury often involves extensive deposition of provisional matrices; and tumors that become malignant trigger fast vascularization by signaling through their microenvironment. These dynamics are often coordinated by changes in multiple environmental cues and regulated by multiple cell types (and may involve specialized cells such as immune cells). Engineering the extreme dynamics seen during development, regeneration, and at the onset of disease *in vitro* is one of the main frontiers for next-generation microsystems.

New efforts should focus on ways to tune structures and environmental cues noninvasively mid-experiment. Engineering approaches to mimic tissue folding through patterned cell-generated tractions [36] could be adapted to initiate folding on demand by linking the cell contractile machinery to **optogenetic** triggers [37]. Magnetic actuators that can rapidly and controllably deform ECMs wirelessly [38] could be expanded to large arrays and controlled using spatially selective actuation schemes [39] to generate highly anisotropic strain fields in a tissue construct. This would allow us to investigate the first cellular responses in conditions that simulate the extreme dynamics in development. Activating ECM stiffness in a spatially confined region could also help in studying the implications of prepathological increases in matrix stiffness, offering a window into the onset of pathological states [40]. Likewise, spatial patterning of the mechanical stiffness of the environment could be used to drive robust tissue patterning [41]. Stiffness patterning through multilayer fabrication has already offered a window into local cellular responses to sharp stiffness gradients [42]; however, new techniques are needed to achieve such patterning in 3D with both spatial and temporal control.

The orientation, spreading, and migration of cells that are bioprinted cannot be directly regulated once they are deposited at the onset of the experiment. One solution could be the use of optogenetic and **magnetogenetic** methods that have recently been developed to direct cell behaviors in a time-varying manner [43,44]. Controlling this diverse cast of environmental cues at once will provide an explicit handle on the wide range of factors that determine the tissue microenvironment, but assembling and tuning all stimuli may prove to be as much a control systems problem as a fabrication hurdle. Therefore, focusing on varying the minimal set of microenvironmental cues needed to study dynamics will be critical. For example, with rapid progress in 3D embryo organoids or 'gastruloids' [45], it would be useful to explore if high-fidelity tissue folding or improved organ specification can be achieved *in vitro* by predefined temporal variations of ECM stiffness alone – inspired by *in vivo* tissue stiffening programs [46].

Instrumenting engineered constructs with sensors to continually evaluate tissue function will help expedite studies on the role of different environmental cues in enhancing function. Using data from tissue-embedded sensors to feed back and dynamically regulate environmental cues would then allow us to begin to uncover the ever-changing processes and programs necessary to drive maturation of engineered constructs or to model the development of disease. Robotic microsystems that autonomously sense function and optimize the microenvironment through control of growth factor concentrations, matrix properties, and biophysical stimuli such as forces would be a substantial advance. This would help us better mimic the interorgan crosstalk that is

responsible for system-level homeostasis as well as better simulate humoral regulation of tissue function in the next generation of engineered microsystems. It would be prudent to first engineer cells themselves to be sensors and actuators with desired input–output behaviors to reduce potential interference from synthetic materials within a tissue construct. These cells can then be directed to certain locations without physical tethers through optogenetics or magnetic fields if desired. Although robotic closed-loop control of the tissue microenvironment is currently an aspirational goal for engineered microsystems, the bevy of technologies required is advancing in parallel (e.g., optogenetic [47] or electrical [48] control of transcriptional circuits as a means of controlling cell fates and function, tunable materials [49], and automated fluid processing [24,50]).

## Next-generation opportunities: complex models, personalization, and information processing

Developing models of the immune system is critical to understanding natural immunity, diseases, and their resolution. Yet, the complexity of the immune system poses diverse challenges for engineered microsystems: the immune system consists of a plethora of immune cells (including T cells, B cells, natural killer cells, macrophages, neutrophils) that work in tandem; they are derived from hematopoietic stem cells and myeloid stem cells that have complex niches; immune cells reside in different immune organs such as lymph nodes with distinct architectures; and the efficacy of immune response is determined by other structures, such as the lymphatic network, that aid immune cell migration. With new ways to engineer the individual components of the immune system, such as the hematopoietic niche [51], immune organoids [52], and lymphatic vessel networks [53] (see [54] for a focused review), there are emerging opportunities in designing systems that integrate these elements.

In conjunction with advances in induced pluripotent stem cell technologies, gene editing (that has enabled isogenic cell lines), and high-throughput fabrication, there is now a realistic opportunity for engineered microsystems to enable personalized medicine. With the growing awareness of the impact of age, sex, and genetic diversity of cell sources [55] used in engineered microsystems, they can be used to study disease onset, progression, and resolution for finer subsections of society. The effort required for screening drugs tailored for smaller subgroups is also substantially reduced, such as screening for cystic fibrosis treatments by tracking the swelling of patient-specific organoids [56].

There is a general trend to scale engineered microsystems to large numbers to test multiple groups and conditions. This, compounded with our ability to collect high-resolution volumetric images and quantify the single-cell **transcriptome** and **proteome** (including subsets such as the secretome and matrisome), results in a vast amount of data. Classes of machine learning algorithms such as convolutional neural networks are well suited for dealing with high-dimensional biological image data [57]. Using a range of dimensionality reduction and clustering methods [58], the integration of data from these diverse sources (called 'integrative omics') is already yielding new biological insight [59]. The collective design of experiments and data analysis is likely to prove a powerful strategy and represents one of the biggest opportunities for the next generation of engineered microsystems (Figure 2).

## Concluding remarks

There is an inseparable link between tissue organization and cells' perception of all microenvironmental cues that drive their function, including how they sense and respond to forces. As a result, a mechanobiologist's view on engineering new microsystems is centered on creating accurate structures and spatial arrangements *in vitro*. With our improving ability to compose biomaterials and cells into complex constructs, the aspirations for engineered microsystems are becoming

## Outstanding questions

How do we fabricate tissue constructs controlling features that span multiple orders of magnitude (such as hierarchical vascular networks ranging from arterioles to capillaries)?

3D engineered microsystems cannot yet match traditional planar cell culture in ease of use or experimental/imaging throughput. Can we develop patterned 2.5D assays that faithfully merge the benefits of both strategies by incorporating cell–ECM interactions typical in 3D systems and the simplicity of 2D culture? Specifically, can these systems then generate high-throughput data demanded by emerging machine learning algorithms?

Will the combination of coarse top-down fabrication with organoid morphogenesis succeed in generating all organotypic constructs? If so, what level of structural detail is required for the initial structure?

Can we achieve complex tissue-like structures with cells and natural ECMs by merging multiple bioprinting processes (with coarse and fine resolutions) and subtractive processing?

What physical mechanisms can allow us to fabricate biological structures at extreme resolutions (<1 μm), such as to model complex basement membrane architectures or intraluminal pillars seen in intussusception?

How can we control multiple environmental cues – cells, ECMs, growth factors, and physical forces – simultaneously and rapidly to simulate highly dynamic microenvironments? Can we develop tissue-folding programs that can be triggered to mimic rapid folding seen during morphogenesis?

Are there general means to develop tissue-embedded sensors to noninvasively sense tissue function and maturity that are modular and work across many tissue types?

Eventually, can we build robotic microsystems by instrumenting platforms with sensors that report tissue function and actuators that regulate microenvironmental cues to then (autonomously) learn the programs that drive maturation and disease?

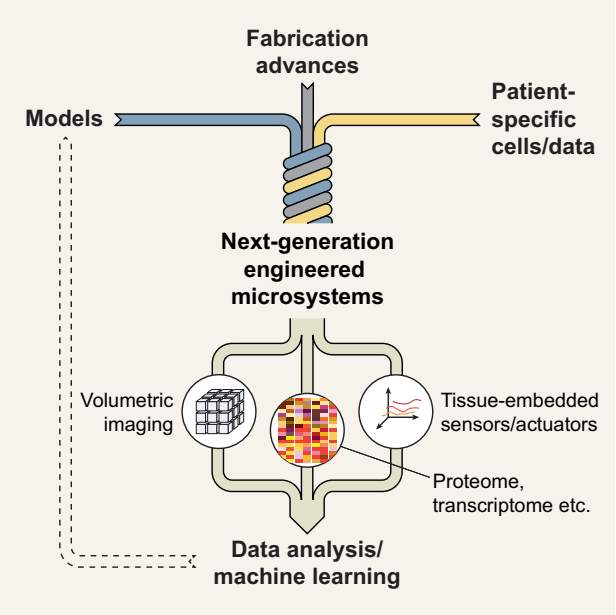


Figure 2. Next-generation engineered microsystems. The design of next-generation engineered microsystems ties together advances in many areas: new 3D fabrication methods are enabling better mimicry of tissue microenvironments; continually improving understanding of biological processes, and the ability to build complex mathematical and numerical models, are guiding experimental design; and patient-specific cells and patient stratification data are driving focused questions with better cell sources. New engineered microsystems also readily lend themselves to more detailed characterization, resulting in a vast amount of data, including high-resolution volumetric imaging data, feedback from tissue-embedded sensors and actuators, and gene and protein expression profiles at the single-cell level. Emerging machine learning and dimensionality reduction tools are offering collective insights from these data and thereby refining our current map of biological processes. A holistic design of devices, experiments, and data analysis schemes is one of the key opportunities for future engineered microsystems.

much broader: to effectively model development and diseases, functionally mimic complex organs, faithfully model drug responses, and foster the right environment for engineering large-scale tissues for eventual transplant. The current set of engineered microsystems is far from meeting all these ambitious goals (see Outstanding questions). In the realm of tissue fabrication technologies, inventing ways to build structural features spanning multiple orders of magnitude at once will be a key priority; this includes designs such as hierarchical vascular networks with ~5–10 μm capillaries to >500 μm arterioles. This may be possible by combining multiple fabrication processes or by merging coarse top-down fabrication methods with the self-organization capabilities of cells and organoids. Even as modern 3D microsystems better mimic the tissue microenvironment, new engineering advances are needed to match the throughput and ease of use of planar and patterned cell culture. We envision that focusing on ways to control multiple cues mid-experiment, through optogenetic or magnetogenetic triggers, will aid the study of tissue dynamics. More extreme dynamics, such as in development, can be simulated in future engineered microsystems by augmenting tissues with synthetic sensors and actuators that enable autonomous and rapid control. Such robotic platforms with closed-loop control will leapfrog our capabilities in addressing new questions in cell biology. Engineered microsystems are increasingly interfacing with many diverse tools: Their design is driven by advances in fabrication methods, patient-specific cells, and mathematical models, and the vast amounts of data generated are being analyzed collectively through machine learning tools. A holistic consideration of all these different interfaces represents a new opportunity for next-generation applications of engineered microsystems.

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## Declaration of interests

The authors declare no competing interests.

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