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Engineering Tissue-Scale Properties with Synthetic Cells: Forging One from Many

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Abstract

In metazoans, living cells achieve capabilities beyond individual cell functionality by assembling into multicellular tissue structures. These higher-order structures represent dynamic, heterogeneous, and responsive systems that have evolved to regenerate and coordinate their actions over large distances. Recent advances in constructing micrometer-sized vesicles, or synthetic cells, now point to a future where construction of synthetic tissue can be pursued, a boon to pressing material needs in biomedical implants, drug delivery systems, adhesives, filters, and storage devices, among others. To fully realize the potential of synthetic tissue, inspiration has been and will continue to be drawn from new molecular findings on its natural counterpart. In this review, we describe advances in introducing tissue-scale features into synthetic cell assemblies. Beyond mere complexation, synthetic cells have been fashioned with a variety of natural and engineered molecular components that serve as initial steps toward morphological control and patterning, intercellular communication, replication, and responsiveness in synthetic tissue. Particular attention has been paid to the dynamics, spatial constraints, and mechanical strengths of interactions that drive the synthesis of this next-generation material, describing how multiple synthetic cells can act as one.

Graphical Abstract

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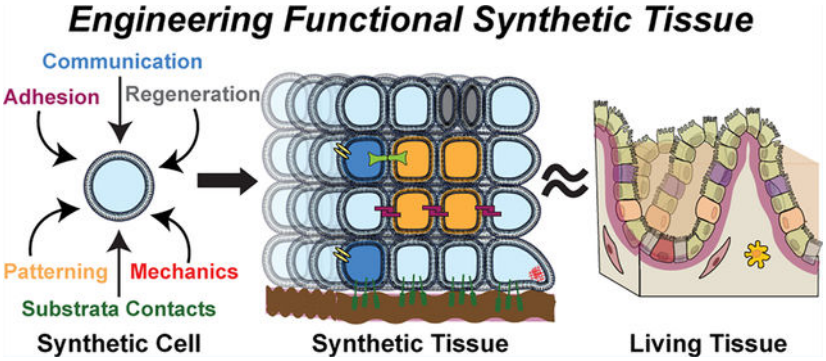

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synthetic cells; tissue; adhesion; intercellular communication; regeneration; cell mechanics

INTRODUCTION

Scientists have long marveled at the structure and dynamic properties of animal tissue. High-surface-area tissue—including the small intestine, lung, and mammary gland, among others—have provided ample inspiration for porous materials,[1] while tissue's regenerative capacity has helped guide the development of self-healing systems.[2] With the introduction of synthetic cells, scientists and engineers marked a new era of mimicry, one that more closely relies on biological products and mechanisms for synthesis. For the purposes of this review, we define synthetic cells as micrometer-sized vesicles with living cell-like functionalities whose components are reconstituted from natural or artificial sources. Processes, such as transcription, translation, and metabolic pathways, common among all single living cells, were some of the first features engineered into synthetic cells.[3] Constructing these synthetic processes relied on decades worth of biochemical knowledge, which provided an abundant blueprint for the necessary enzymes and metabolites required for reconstitution. As a result, synthetic cells encapsulating genomic sequences and possessing advanced biochemical circuitry have come to the forefront over the last two decades and have transformed our thinking of origins-of-life and minimal cells for advanced technology.[4] Yet, the focus on single synthetic cells obscures context. Living tissue in animals relies on cells working together, not in isolation. Consequently, attention has turned to synthetic multicellular systems built from synthetic cells to emulate the impressive functional feats and material properties of living tissue.[5] Applications of synthetic tissue abound as multicellular assemblies can be designed for biomedical purposes, for controlled release, and as new purification systems, adhesives, protective coatings, and storage devices.

The seeming simplicity of tissue—cells bound to each other—belies the innovations that allow tissues to adopt convoluted topologies, establish barrier function, maintain homeostasis in the face of numerous insults, and adapt to their surroundings. The small intestine is one example, among many, of the emergent capacity of tissues to establish long-range anisotropies and achieve complex functions (Figure 1A). Arranged into finger-like villi, where high-surface area epithelial cell sheets regulate absorption, and crypts, where

stem cells continuously renew the tissue, the small intestine represents one example of a dynamic three-dimensional structure with patterned niches. Turnover rates are critical for maintenance and repair in proliferating tissue,[6] and as such, cell death is tightly coupled to a process known as extrusion in epithelial tissue,[7] wherein neighboring cells eject apoptotic cells,[8] to clear damaged cells. Final tissue patterns depend on proper localization of each cell type, and membrane protrusion-dependent migration from the crypts to the villi help position new villus cells correctly.[9] Once localized in space, membrane proteins determine the absorption potential of cells and ensure cohesion of the tissue.

Multicellular tissue can be defined as a composite material, formed not just with cells but also with nonliving materials, the basal extracellular matrix (ECM), which provides structural support, and the apical mucus layer, which helps protect tissue from invading pathogens. Cells secrete these materials,[10] shape their morphology,[11,12] and receive signals from them.[13] In fact, before the emergence of animals, an extracellular matrix may have been a significant driver of cell aggregation, as has been shown for choanoflagellates.[14] For synthetic tissue to approach the potential apparent in its living counterpart, synthetic cells endowed with proper molecular modules, materials, and mechanisms that govern these tissue properties would need to be realized.

Fortunately, recent advances in cell and developmental biology have yielded key insights into the protein structures and mechanisms responsible for tissue-scale properties. The junctions that interface cells with their surroundings, their neighbors, and the cytoskeleton all play an outsize role in generating tissue-level organization and function.[15] A far cry from simple noncovalent connections, these cellular structures evolved unique forms of mechanics and mechanosensitivity to imbue tissue with surprising resiliency,[16] and even super-elasticity,[17] in the face of stress and fluid flow. Junctional and cytoskeletal structures form and reorganize to enable tissue to adapt and maintain physiological homeostasis and to reseal broken connections.[18] Synthetic cells will need to capture these biophysical characteristics to approach tissue's dynamic responses. New work shows that junctions, themselves, can guide organ development. For instance, the organization of junctional proteins can discern self from nonself in neurons,[19] a hallmark of neuroplasticity, and junctional adhesive forces can lead to robust sorting and patterning of cells during spinal cord development.[20] With molecular knowledge and understanding of junctional and cytoskeletal organization, engineering these structures or mimics thereof within synthetic cells has become a priority and an intense area of research activity.

In this review, we describe advances toward the goal of generating tissue-level properties with synthetic cells. While several groups have demonstrated remarkable control of tissue with engineered living cells and ECM,[21–23] here our focus will be on synthetic cell-based tissue as they provide the clearest opportunity for complete control over composition and function. We posit that engineering synthetic multicellular systems depends on the implementation of six key features of tissue: adhesion between synthetic cells, synthetic cell–substrata contacts, tissue-wide mechanics, regeneration, intercellular communication, and patterning of synthetic cell assemblies (Figure 1B). Within this framework, we outline recent findings and achievements in addition to outstanding goals and unrealized needs in the synthetic tissue field. We limit ourselves to synthetic tissue considerations as

comprehensive reviews on single synthetic cells have been published elsewhere.[24–27] We also attempt, where appropriate, to mention quantitative aspects of the systems since tissue properties are sensitive to molecular binding strengths and forces. We hope that this review acts to galvanize the field to fill in the gaps on the road to engineering synthetic cells with tissue-scale behavior.

ADHESION BETWEEN SYNTHETIC CELLS

In forming higher-order tissue structures, individual living cells must contact their surrounding neighbors, and *in vivo*, they do so through cell junctions.[28] Components of cell junctions—adhesive membrane proteins and cytosolic proteins, which include adaptor proteins, signaling proteins, and cytoskeletal proteins—work in concert to form dynamic but stable membrane structures.[29] Adhesive membrane proteins physically link cells together via binding in the extracellular space and, as such, have been a significant focus for the synthetic cell community. For living tissues, strengths of adhesive membrane protein interactions can vary and are often reflective of the underlying function of the junction. For example, claudins, which are found at the tight junction (TJ) in epithelial tissue and regulate paracellular flux, were found to have rupture forces of 21–48 pN,[30,31] whereas E-cadherin, the major adhesive membrane protein responsible for tissue cohesion, displayed significantly higher rupture forces between 40 and 70 pN.[32] Beyond mere adhesions, cellular junctions can also serve as specialized channels for communication and exchange between cells, which is the case for gap junctions that form intercellular pores for cell-to-cell material transfer.[33] In contrast to their living counterpart, synthetic cells need not be bound by nature's constraints, and to date, synthetic cell adhesion has been accomplished through a variety of means, including non-natural linkages and nonspecific interactions in addition to taking advantage of natural receptors (Figure 2A). In the section below, we summarize the progress in synthetic cell adhesion through these three mechanisms.

Synthetic Cell Adhesion through Non-natural Molecular Interactions

Some of the first efforts to drive adhesion between synthetic cells focused on forming strong and specific interactions. An early example was the use of streptavidin–biotin, a widespread noncovalent linkage in biotechnology (bond rupture force of 75 pN at 1000 pN/s loading rate, with a range of 5–70 pN depending on the loading rate).[34,35] By incorporating a phospholipid–biotin conjugate into synthetic cell bilayers, the multivalent receptor, streptavidin, can bridge multiple synthetic cells together, resulting in a synthetic cell assembly. Although untested, strong individual interactions, such as biotin–streptavidin, could, in theory, give rise to rigid and plastic synthetic tissue. Similarly, lectins that recognize glycans (bond rupture force ~47 pN at ~10,000 pN/s for ConA–mannose interactions)[36] have been used to adhere synthetic cells containing glycoconjugates.[37,38] Both of these systems induce cell–cell adhesion via exogenous receptors, and consequently synthetic cells will aggregate after receptor introduction. This type of aggregation provides an easily accessible technique to produce synthetic tissue. These methods are, however, both indirect, relying on the exogenous receptor to adhere two ligands together that are attached to different synthetic cells. Indirect binding schemes, while having bond strengths and high

specificity similar to cellular junctions, are not well suited for patterning synthetic cells in defined locations since the aggregation process is often stochastic.

To offer more control over adhesion, researchers have made use of receptor–ligand combinations that directly adhere synthetic cells together. Complementary DNA strands have seen extensive use to form aggregates of synthetic cells or emulsion droplets.[39–42] The unbinding force of these complementary DNA strands have been characterized within 20–50 pN at loading rates of 16–4000 pN/s for a range of 10–30 complementary base pairs.[43] Not only can DNA strength be fine-tuned between synthetic cells, but also duplex-based adhesion can be used in combination with free strands to engineer competitive binding,[40] generating more dynamic multicellular structures. More recently, triggering dynamic and reversible assembly without a third component was demonstrated with photoswitchable adhesions. By decorating vesicles with the protein ligand, Nano, and a photosensitive receptor, iLID,[44] Wegner and co-workers showed that synthetic tissue could be formed in response to blue light. While dynamic, this receptor–ligand pair is weaker than the examples described above, having a characterized bond rupture force of ~10 pN (at a loading rate of ~0.5 pN/s).[45] As the bond rupture force is lower, one might expect the synthetic tissue to be more flexible and have more potential for reorganization as was shown for GFPuv-based adhesion.[46] The Nano–iLID interactions, complementary DNA pairing, and GFPuvs all provide unique benefits toward adhering synthetic cells together in a dynamic and tunable manner.

Other direct adhesion schemes using cysteine reactivity, click chemistry, functionalized peptides, and rhodium–bipyridine have also been reported for vesicle aggregation.[47] The first three adhesion methods are of note as they generate covalent adhesions. Disulfide bridges with a cholesterol–cysteine peptide conjugate, triazole production between lipidated BCN and azide species, and C–N bond formation between synthetic cells with esters and hydrazines all successfully ligated synthetic cells together.[47] Covalent bonds have been shown to exceed bond strengths of 100 pN to >1 nN.[48,49] While these bonds are considerably stronger than typical cell junction adhesions, they will lead to highly specific and strong connections that will most likely strengthen the overall mechanics of synthetic tissue, although at the cost of possible reorganization, which is a critical feature of living tissue. In sum, synthetic cell adhesions from 10 pN to >100 pN have been demonstrated in synthetic cells by utilizing non-natural specific receptor–ligand pairs and chemistries. This, in turn, may enable a wide range of synthetic tissue properties to be built into synthetic cell–synthetic cell adhesions.

Synthetic Cell Adhesion through Nonspecific Interactions

In addition to the highly specific interactions explored in the previous section, nonspecific interactions, especially between charged functionalities, have been exploited to adhere synthetic cells together. One advantage of nonspecific interactions is that the aggregation of synthetic cells can be more sensitive to the chemical environment around it, potentially achieving sensing properties of tissue. When the synthetic cell bilayer is doped with charged surfactants, aggregation of synthetic cells via an electrostatic force has been achieved by adding charged molecules to the outside solution. In one example, sodium

oleate was used to dope the outer membranes of vesicles with a negative charge,[50] and positively charged poly-L-arginine was then added to the solution to induce vesicle aggregation and establish primitive cell communities. Didodecyl dimethylammonium bromide and cetyltrimethylammonium bromide have also been used to engineer positive surface charges.[51] Vesicle aggregation can then be induced with negatively charged tRNA or poly-L-glutamic acid. Recently, synthetic cell adhesion was shown with optical tweezers via nonspecific means.[52] When the salt concentration in the solution was increased or decreased, adhesion and dissociation was observed, respectively. These synthetic cell adhesion forces are typically governed by a balance between attractive van der Waals interactions and repulsive electrostatic, hydration, and thermal undulation forces.[53] Complex synthetic cell assemblies stabilized by nonspecific interactions still achieved high spatial organization by precise placement with an optical tweezer. Differing from specific receptor–ligand methods of linking cells together, nonspecific interactions offer potential for large-scale cell adhesion while also enabling control over the extent of adhesion by manipulating the surrounding chemical environment. However, translation to more complex applications may be challenging as the chemical environment, such as pH, can vary across a single tissue.[54] Thus, while electrostatic and nonspecific adhesions may assist in facile assembly of synthetic cells, the interactions are susceptible to environmental perturbations.

Synthetic Cell Adhesion through Natural Protein Receptors

Reconstituting cell adhesions using endogenous adhesive membrane proteins presents an opportunity to take advantage of the emergent features of native cell junctions. While limited examples exist, below we highlight how adhesive proteins from epithelial tissue can drive synthetic cell adhesion. Transmembrane proteins have been successfully reconstituted into synthetic cells through a variety of methods,[55] and we point readers to key methods in the field, such as proteoliposome recombination (e.g., picoinjection, detergent solubilization), lipid film swelling or electroswelling, cell blebbing, and microfluidic jetting.[56–62] Recently, we have successfully reconstituted the transmembrane claudins from an epithelial tight junction in synthetic cell membranes. After producing multiple synthetic cells using microfluidic jetting, claudin–claudin interactions assembled synthetic cells into tissue form and recapitulated *in vivo* features, such as membrane diffusion barriers.[63] Researchers have also constructed proto-adherens junctions between synthetic cells and supported lipid bilayers by decorating lipids with the extracellular domain of E-cadherin.[64–66] Yet, fully reconstituting the adhesome, which would include other critical components of cell junctions, e.g., adaptor and cytoskeletal proteins, in synthetic cells has not been fully realized.[67] The incorporation of various adhesive proteins into and onto synthetic cells bodes well for constructing synthetic cell junctions that approach cellular capabilities, some even interfacing with living cells themselves.[61,68]

A wide range of methods have been leveraged to adhere synthetic cells together that incorporate native and non-native features into synthetic tissue. The rupture force of some cell junctions is on the order of 20–70 pN.[30–32] Interactions used between synthetic cells take a similar, but wider range of bond strengths from 10 pN to over 100 pN,[34–36,43,45,48,49] and many of the non-native interactions, such as electrostatic adhesions, provide chemical sensitivity that enables dynamic tissue, an essential trait of natural systems. Each form of

adhesion offers distinct benefits that may be combined to yield properties beyond those of living tissue. For instance, if a synthetic tissue would need to be rigid and to interface with living cells, then a combination of covalent bonding and native transmembrane proteins could be implemented. Since tissues are heterogeneous in nature, highly specific and tunable adhesions can be used to pattern tissue with complementary DNA by varying the base pairs and length of each strand. Still, to date, a fully reconstituted cell junction has not been achieved. In the future, a goal would be to add molecular machinery into synthetic tissue that will allow mechanical sensing and signaling across membranes (see sections below), which can be achieved through transmembrane linkages and lumenal connections. We anticipate that future studies will also start to incorporate multiple cell junctions in tandem to mimic the pleiotropic functions of living tissue.

SYNTHETIC CELL–SUBSTRATA CONTACTS

Cells adhere not only to each other in tissue but also to a secreted material, known as the extracellular matrix (ECM). The ECM plays a critical role in determining tissue shape and mechanical properties as well as mediating other cellular processes, such as migration within tissue.[69] Fibrous proteins, such as collagens, elastins, fibronectins, and laminins, make up the structural network of the ECM.[69] Cells typically bind to these fibrous proteins through integrin transmembrane complexes, although other ECM receptor proteins also contribute to ECM contacts.[70] Integrins can interact with an arginine-glycine-aspartic acid (RGD) peptide sequence commonly found in ECM proteins and are sensitive to ECM surface density and spatial arrangement.[71] Integrins are also involved in various signaling pathways and other cell–cell adhesions,[72] but for the purposes of this review, we will focus on synthetic cell adhesion to ECM-like materials.[73,74]

In early studies, successful reconstitution of αIIBβ3 integrin in synthetic cells was achieved through detergent solubilization.[75] Synthetic cells 20–40 μm in diameter exhibited adhesion to an ECM-like fibrinogen surface (Figure 2B, top) but no adhesion to a control casein surface, as shown via reflection interference contrast microscopy. An alternate method based on sequential picoinjection of synthetic cells also incorporated integrins into the membrane and had similar selectivity for fibrinogen against a BSA surface (Figure 2B, bottom).[59,76] Here, water-in-oil droplets stabilized by polyethylene glycol and perfluorinated polyether polymers initiate the formation of droplet-stabilized giant unilamellar vesicles (dsGUVs). Passing through a microfluidic channel, the dsGUVs are exposed to an electric field that destabilizes the membrane and allows injection of proteoliposomes containing integrins into the GUV. The proteoliposomes then fuse with lipids of the dsGUV before the polymer shell is removed to yield a reconstituted integrin protein. However, in both cases above, the orientation of the reconstituted integrins is not unidirectional; i.e., only a subset of integrins is properly oriented and can bind to the ECM-like substrate. In another work, adhesion of an RGD-decorated synthetic cell on an integrin-coated substrate has also been reported,[77] and very recently, a new method of reconstituting integrins into synthetic cells based on gel-assisted swelling was described.[78] In the latter case, synthetic cells were reconstituted from a poly(vinyl alcohol) substrate using proteoliposomes, and protein functionality was demonstrated using an RGD-functionalized supported lipid bilayer. These studies not only show that functional integrins can be successfully reconstituted in synthetic cells but also

suggest that synthetic cells can attach to ECM-like materials to mimic the composite nature of tissue.

To impart tissue-like three-dimensionality, encapsulation of synthetic cell or synthetic-cell-like structures into soft materials may prove advantageous. Synthetic cells have been suspended in a sodium alginate matrix cross-linked with calcium ions.[79] Because the synthetic cells encapsulated urease, the amount of cross-links within the alginate gel could be tuned, and consequently, a soft microscale actuator was produced. This actuator was shown to have reversible extender and contractile properties similar to a spring. Moreover, Schwille and co-workers have reported work encapsulating synthetic cells in 3D-printed hydrogel shapes, leading to user-defined composite geometries.[80] Hence, recent progress toward encapsulating synthetic cells in biocompatible polymeric materials offers another fruitful path to the production of synthetic tissue.

Establishing biomimetic synthetic cell–substrata contacts depends on receptor–ligand binding to an ECM, which in turn can aid in shaping synthetic tissue in 3D. To date, most studies have focused on using natural integrin receptors and natural ECM materials as highlighted above.[81] Other approaches use biocompatible fibrous polymers with engineered ligands to build ECM-like contacts or by encapsulating synthetic cells in soft polymeric materials. Decorating ECM-like surfaces with ligands that can interface with cognate receptors (see Adhesion between Synthetic Cells section above) on synthetic cells would also lead to ECM-like contacts. Soft polymeric materials, such as hydrogels, offer the benefit of controlling viscoelastic responses by varying the underlying cross-linking chemistries.[82] However, no reports to date have described such strategies with synthetic cells. Additionally, programming bacterial cells to secrete ECM-like materials in and around synthetic cells could produce living cell control over tissue-like structures to support 3D morphologies.[83,84] As above, incorporating native ECM receptors such as integrins into synthetic cells and subsequently incorporating lumenal signaling pathways[76] will be an important next step for imparting environmental sensing, biomimetic tissue mechanics, and migratory potential into synthetic tissue.

ESTABLISHING TISSUE MECHANICS WITH SYNTHETIC CELLS

Cells impart critical dynamics and mechanics onto tissue through intracellular polymers, known as the cytoskeleton, which are linked subcellularly with flexible, semirigid surfaces, including the cell membrane. With these molecular parts, cells are able to withstand shear, tension, and compression within a tissue and impart mechanical driving forces for long-range organization. Forming a complex network within cells, cytoskeletal components —actin, microtubules, intermediate filaments, and septins—regulate a cell's shape and morphology[85,86] and contribute to an endless array of cellular processes.[87] Tissue-level mechanics are often governed by unique combinations and structures of the cytoskeleton working in concert across cells.[85] With an eye to the future, we expect that incorporating synthetic cells with different mechanical properties into a multicellular tissue will be a critical step for reconstructing tissue-level mechanics *in vitro* and for stabilizing synthetic cells against external stresses. In the following section, we detail single synthetic cell examples that could be, in principle, adapted to emulate the mechanical properties of tissue

(Figure 3). As cells must migrate and move collectively to repair and organize themselves within tissue,[88] we also highlight synthetic cell movement focusing on cell membrane deformation, a pivotal step in cell extrusion[89,90] and wound healing.[91]

Reconstituting Internal Cytoskeletal Structure within Synthetic Cells

A wide variety of synthetic cell fabrication techniques have been used to encapsulate cytoskeletal structures, particularly filamentous actin (F-actin), within a phospholipid bilayer. In fact, incorporation of F-actin is often used as a gold standard for showcasing lumenal encapsulation, which was the case for continuous droplet interface crossing encapsulation[92] and sequential assembly via microfluidic picoinjection.[59] Recently, successful encapsulation of cytoskeletal polymers has extended beyond actin. Keratin, a structural protein found in intermediate filaments, was coencapsulated with actin and subsequently polymerized in vesicles via ionophores.[93] Interactions of the two-protein polymeric network led to stabilization of keratin filaments by an actin-mediated steric resistance mechanism. More dynamic cytoskeletal structures, such as contractile actomyosin rings, have also been encapsulated in synthetic cells and were shown to produce localized membrane deformations when attached to the membrane.[94] Local control over actin's organization would be beneficial for defining activity in different synthetic cells within a tissue, and photoinducible systems hold potential for building a dynamic internal structure in synthetic cells.[95] As more cytoskeletal polymers are encapsulated in synthetic cells, the question of how to link their structures—either naturally or through non-native means—becomes even more important to build the necessary dynamics and mechanics for synthetic tissue.

Reconstituting Membrane Cortex Attachment within Synthetic Cells

The cell cortex—a thin (~50–100 nm), cross-linked actin network beneath the inner leaflet of the plasma membrane[96]—provides structure and support to the plasma membrane[97] and plays pivotal roles in morphogenesis, cell division, cell polarization, and movement.[98,99] Within living tissue, the cortex can influence cell sorting during tissue patterning[100] and overall cavity shape.[101] Reconstruction of the cortex, therefore, offers multifold benefits, including imparting tissue-level mechanics, maintaining the integrity of its constituent synthetic cells, and generating defined 3D structure.[102,103]

Early efforts toward reconstituting the cell cortex made use of extracts to anchor actin to membrane proteins through ankyrin and spectrin.[104] Later, a purely bottom-up reconstitution was achieved by anchoring actin to the membrane through a His-tagged WWA subdomain that recruits the Arp2/3 complex.[105] Synthetic cells containing a functional cortex required greater membrane tube pulling forces compared to empty synthetic cells, indicating that cytoskeletal-to-membrane linkages provide a molecular handle for defining synthetic cell and global tissue mechanics.[102] Cytoskeletal organization can also dictate synthetic cell shape. Invaginations via actin bundles were observed when tethered to the exterior of synthetic cells,[106] and interior cortices led to predictable directional deformation, outward versus inward, depending on the capping protein's concentration within the actin network (see Membrane Deformation and Movement in Synthetic Cells section below).[107] After the addition of nonmuscle myosin II to interior cortices, complete membrane fission ensued.[107]

This behavior may prove advantageous for building syncytial tissue in the future. Beyond His-tagged attachments, biotin–avidin linkages have been applied in synthetic cells to tether cytoskeletal structures to the membrane.[108] Carvalho et al. showed that contractile actomyosin clusters only remained tethered to membranes under strong linkage strength.[109] As well, cross-talk between membrane adhesions and synthetic cortex architecture was observed during synthetic cell adhesion to a solid substrate,[110] suggesting engineering feedback between the membrane and the cortex will be a critical design feature for adaptability and dynamics of synthetic tissue.

Synthetic cell cytoskeletons need not be confined to natural polymers. One possibility relies on alternative self-assembling systems to establish synthetic cytoskeletons. DNA origami with its geometric programmability and addressability offers exquisite 3D control over both nm features and μm lengths. In some of the first test cases, DNA nanostructures were found to bind cationic lipids within synthetic cells and once bound stabilize cells against osmotic shock.[103] More recently, Göpfrich and co-workers have pioneered the assembly and disassembly of DNA nanostructured filaments within synthetic cells to mimic features of the natural cytoskeleton, pointing to a future where a synthetic cortex can be manipulated to vary its mechanics within tissue dynamically.[111,112]

Membrane Deformation and Movement in Synthetic Cells

For tissue maintenance and regeneration, living cells must undergo complex mechanical programs to rid the body of damaged cells and to close wounds.[90,91] In both of these processes, membrane deformations play indispensable roles. To remove dead cells within the epithelium, healthy cells extend their membranes to apply force and extrude dead cells.[89] Similarly, for wound repair, cells migrate across a matrix via membrane protrusions to achieve re-epithelization.[113] Below, we describe how synthetic cells have been adapted to achieve membrane deformation and movement.

We will focus our discussion on membrane deformation from internal encapsulated factors, although notable examples of external deformation of the membrane have been reported through the application of septins, actin, and DNA origami (see above).[114–119] Over 2 decades ago, foundational work suggested that actin polymerization can deform membranes and create protrusions *in vitro*.[120–122] More recently, Tanaka et al. reported that synthetic cells displayed spindle-like morphologies when the high density of actin filaments align after encapsulation.[123] Either osmotic pressure differences or photoactivation triggered morphological changes in membrane deformation. Capping proteins (CP) can lead to membrane protrusions or intrusions depending on CP concentration,[107] and actin's interaction with different lipid phases may also provide a means of perturbing the membrane locally.[95] Membrane deformation through cytoskeletal elements in synthetic cells is not limited to actin encapsulation, however. Tubulin-mediated membrane deformation has been observed in the presence of kinesin motors.[124,125] DNA nanostars have also been leveraged to induce membrane deformations by Dekker and co-workers.[126] Stomatocyte-like deformation occurred by adding DNA-cholesterol into the membranes of the synthetic cells and encapsulating nanostars. In another study, a micrometer-sized molecular robot was developed through a complex of kinesin, microtubules, DNA, and biotin–avidin linkages

and upon actuation deformed synthetic cell membranes.[127] By inclusion of a photosensitive DNA element, active membrane deformation could be turned on or off with the addition of light. This study highlights the advantages of developing membrane deformation systems that can be controlled externally but act internally. These dynamic systems can be leveraged for detecting external signals and responding with mechanical deformation, a critical step for reconstituting death-induced cell extrusion.

Cells must migrate within tissue to heal wounds and injuries—a long-standing goal for synthetic cells. While cell crawling is a complex, multistep process involving multiple molecular machines, motility in synthetic cells currently relies on relatively primitive mechanisms, although inroads have been made. Light-guided cell motility was first achieved by decorating a synthetic cell with the Micro ligand and coating a substrate with the iLID photoswitchable receptor.[128] In this way, blue light localized to the “leading edge” of synthetic cells was able to guide movement through photoactivating receptors on the substrate. While not an autonomous process, this study nonetheless demonstrates the prospect of long-range synthetic cell movement. Cells also undergo collective cell migration in tissue.[88] Recently, DNA-ligated synthetic vesicles were found capable of leader-dependent movement via a DNA toehold mechanism.[129] Coating a substrate with a DNA hairpin structure, a lead vesicle activates the substrate by DNA pairing, enabling a subsequent vesicle to follow. Encoding this mechanism into synthetic tissue would allow for synthetic cells to follow the path of one leader cell, giving rise to collective migration. Another form of cellular movement is directional motion in solution. By decorating synthetic cells with enzymes, Somasundar et al. were able to program movement in concentration gradients.[130] More recently, two studies that mimic the first steps in cellular movement involved synthetic cells binding to surface-bound dynamic cytoskeletal proteins and DNA,[131,132] which might ultimately translate to migration across a fibrillar structure in tissue.

Emulating the mechanics and the movement of cells within synthetic cells remains a significant challenge given the complex machinery underlying a cell’s internal structure. To mimic living tissue, synthetic tissue would need to achieve anisotropic distributions of mechanics and movements. While this level of complexity has not yet been achieved for synthetic tissue, progress toward reconstituting cellular movement and mechanics has been made on the single synthetic cell level with encapsulated filamentous polymers. Internal cytoskeletons and membrane cortices have been reconstituted in various configurations, yet much is to be done on connecting different cytoskeletal architectures and components together. Cytoskeletons have also been leveraged for membrane deformation. However, more work is needed to generate higher magnitudes of forces to allow for cell extrusion, which may be on the order of nanonewtons.[133] To move and achieve cell migration similar to tissue, integrins and other membrane receptors would need to be functionally coupled to the actin cortex to allow for outside-in and inside-out signaling. A synthetic cell demonstrating three steps of cell movement across a substratum (extension, adhesion, and pulling) would show enormous potential for synthetic collective cell migration. Yet, a gap remains experimentally as little work has been done to mimic the forces and movements on a multicellular level.

TISSUE REGENERATION

Long-range tissue function depends on the coexistence of different cell types and on cell division for long-term cell survival. Cells in tissue are constantly dying and regenerating. A delicate balance between cell division and death ensures tissues maintain form and function throughout the life span of an organism.[134] For instance, rapidly dividing $Lgr5^+$ stem cells replenish the intestinal epithelium every 3–4 days by producing differentiated daughter cells that move up the crypt-villus axis from the crypt base.[135] What lies at the center of tissue regeneration is cell proliferation.[136] While synthetic replication has been pursued in isolation, in the context of a tissue, the synthetic replication process must unfold in a way that maintains synthetic tissue's structure (Figure 4). Here, we limit our discussion to strategies for DNA replication, DNA segregation, growth, and division in synthetic cells that satisfy this physical constraint (Figure 5A). We refer readers to excellent recent reviews elsewhere on cell division in individual synthetic cells.[4,137–139]

DNA Replication

DNA replication ensures that daughter cells inherit a copy of genomic content from the parent cell. In recent work, van Nies et al.[140] achieved replication in synthetic cells by employing phage replisomes. In this strategy, DNA polymerase (DNAP) and three associated proteins replicate linear genomic DNA capped with the Phi29 terminal protein in the confines of a synthetic cell. Similarly, Sakatani et al.[141] showed that self-encoded Phi29-DNAP and Cre recombinase can enable rolling circle amplification and recombination for *in vitro* self-replication of circular DNA. Although the Phi29 DNA replication systems are attractive for driving DNA replication in synthetic cells, they suffer from a lack of regulation since DNA segments are amplified by these viral replicative systems continuously. Control over the initiation and completion of replication would be an essential design feature for maintaining the balance of cell growth and death and is discussed in more detail later in this section.

DNA Segregation

After replication, duplicated DNA must be properly partitioned between daughter cells prior to division. DNA segregation represents a synthetic cell organizational problem that has eluded reconstitution to date. Even so, researchers in the field have sought inspiration from the bacterial world and from polymer behavior to solve the segregation problem. The bacterial actin-like partitioning (Par) system can push apart coupled plasmids[142] and space plasmids regularly along a nucleoid,[143] thereby leading to equal partitioning of DNA segments. Long-chain polymers, on the other hand, offer the possibility of spontaneous segregation under spatial confinement due to the concomitant increase in conformational entropy.[144] While both mechanisms above may lead to segregation in synthetic cells, there are obstacles that need to be addressed first. Segregation in the Par system is thought to be driven by dynamic anchoring to sites at the membranes of growing cells or by directional biases in either replication or transcription,[145–148] and if implemented in synthetic cells would demand cellular asymmetries, particularly in the membrane. In that respect, entropy-driven segregation may be a preferred mechanism for synthetic cells since it is a general physical phenomenon. While the shape and size of confinement are important parameters

that would need to be optimized to drive segregation,[149] we imagine that mostly spherical synthetic cells would rely on symmetry breaking machinery (see next paragraph) to enhance segregation. Very recently, Tran et al.[150] designed a DNA segregation module in synthetic cells by employing principles from liquid–liquid phase separation to form DNA droplets that can segregate in response to enzymes and light. This method provides control over the initiation of segregation, although the daughter droplets not being identical is a caveat. Clearly, examining these DNA segregation mechanisms and others in multi-synthetic cell systems is much needed to achieve synthetic cell reproduction within synthetic assemblies.

Membrane Growth

Prior to cell division, cells expand their membrane to accommodate the increased surface area needed for cytokinesis. Likewise, synthetic cells will have to be equipped with the necessary membrane growth machinery to prevent daughter cells from becoming too small in size.[151] To date, various bottom-up approaches have focused on the expression of lipid synthesis machinery inside synthetic cell systems. In early efforts, lysophosphatidic acid and phosphatidic acid were produced by expressing *sn*-glycerol-3-phosphate acyltransferase and lysophosphatidic acid acyltransferase, respectively, inside liposomes,[152,153] and fatty acids have been incorporated into the membrane by encapsulating Fatty Acid Synthase Type I enzyme inside POPC liposomes.[154] Despite these achievements, a low yield of lipid species from encapsulated enzymes might place limits on membrane growth in micrometer-sized synthetic cells. Other approaches to address this shortcoming have focused on providing lipids or reactive precursors externally to effect membrane growth.[155–157] Notably, recent work from the Devaraj group[158,159] has demonstrated the *de novo* formation and growth of phospholipid membranes by using a soluble mycobacterial ligase, FadD10. FadD10 catalyzes the conversion of fatty acids, ATP, and $Mg2^{+}$ into fatty acyl adenylates (FAA). FAAs can then react with amine-functionalized lysolipids to form phospholipids. While providing lipids to synthetic cells externally has been shown to induce cell division (see below), this strategy may not be ideal in the context of synthetic tissue. In a synthetic tissue, only a subpopulation of cells would undergo the division process during regeneration, making internal replication machinery desirable and warranting further work to improve the synthesis and recruitment of lipids inside synthetic cells.

Cell Division

The final step in the cell cycle is division into daughter cells. Cell division proceeds through a multistep process: symmetry breaking, membrane deformation, and membrane abscission. Symmetry breaking in synthetic cells has already been demonstrated via a reaction-diffusion mechanism at the membrane, leading to the formation of protein gradients and polarity.[160] Pole-to-pole oscillations in the bacterial Min system led not only to symmetry breaking in synthetic cells but also to subsequent splitting. Membrane deformation, too, has been realized as of late (see Establishing Tissue Mechanics with Synthetic Cells section above for additional examples). The bacterial division protein FtsZ has attracted much attention for this purpose.[161] Recently, Kohyama et al.[162] exploited the interplay between Min proteins and FtsZ ring assembly to produce pronounced deformations (aspect ratios (diameter/length) of ~0.75) in synthetic cells. Actin in combination with actin-processing motor proteins can also lead to strong deformations in synthetic cells. Litschel et al.[94] successfully reconstituted

contractile actomyosin rings in synthetic cells that led to furrow-like deformations. These approaches represent essential steps toward synthetic cell division and may generate constrictive forces sufficient to cause abscission. Alternatively, membrane growth via fusion with lipids generated from inside or outside the synthetic cell can initiate the deformation process (see Membrane Growth). The deformation can be further catalyzed by chemical stimuli,[163] temperature changes,[164] osmotic pressure,[165] membrane-bound proteins,[166] or enzymatic reactions[167,168] to produce synthetic cell division.[159] Toward the final step in division, Litschel et al.[160] observed abscission in osmotically deflated vesicles that make use of the Min system. External mechanical devices like microfluidic splitters have been used for membrane abscission,[169] although this latter method may have limited applicability in synthetic tissue. Light-based methods present a way to introduce spatiotemporal control over synthetic cell division. To this end, Dreher et al.[170] used an externally added photosensitizer and UV light to trigger local lipid peroxidation and subsequent membrane abscission in osmotically deflated vesicles. However, further exploration into a completely internal light-sensitive synthetic cell division machinery is needed for application in synthetic tissue. In this vein, more recently, Franceschi et al.[171] encapsulated Dynamin A in dumbbell-shaped synthetic cells to produce membrane hemiscission and scission. Despite this recent progress, consistent homogeneous membrane abscission has yet to be achieved through autonomous mechanisms and therefore requires further research into dedicated division machinery for use with synthetic tissue. A better understanding of synthetic cell division under mechanical stress and pressure would also help facilitate successful regeneration in synthetic tissue since division will be constrained by other nearby cells.

In this section, we have discussed efforts to reconstitute distinct steps of the cell cycle in synthetic cells. In synthetic tissue, the ultimate goal is to equip cells with the machinery to undergo the entire cell cycle at defined positions within a confined geometry. The machinery for each step is certain to influence subsequent steps and as such processes in tandem must be investigated. For example, the DNA segregation methodologies discussed above break symmetry and hence may play a role in both symmetry breaking and membrane deformation steps. As well, the method for DNA replication might need to be coupled to a strategy for segregation. Another area that requires further work is control over the initiation of growth. Synthetic cell growth should ideally occur only when and where there is a need for repair of a damaged portion of synthetic tissue. Therefore, cells in synthetic tissue need to be able to sense damage to their neighboring cells. If a defect is detected, then the undamaged cells would rid the synthetic tissue of the damaged cells and also communicate to certain cells to begin reproduction (see section below). This entire process would operate while ensuring that the synthetic tissue's structural organization remains intact. Consequently, the coordinated process of regeneration requires spatially constrained synthetic cell division, resulting in minimal disruption to nearby undamaged cells. Successful efforts to replicate the different steps of the cell cycle in synthetic cells discussed above serve as starting points toward a fully synthetic cell cycle in tissue.

INTERCELLULAR COMMUNICATION

Comprised of communities of cells, tissues perform specialized functions based on coherent actions. Cells in the body detect and respond to environmental cues using

distinct forms of cell signaling. Signaling encompasses diffusion-mediated communication (paracrine signaling, autocrine signaling, and endocrine signaling) and communication by direct contact. Within tissue, signaling plays a significant role in coordination and communication between groups of cells. In collective migration discussed above, migrating cells communicate with each other, thereby allowing for collective decisions that ensure tissue structure remains continuous while remodeling takes place.[88] As well, aggregates of cells—which can communicate with their neighbors and divide labor costs—may display enhanced growth rates compared to noninteracting cells.[172] Within living tissue, signaling molecules work in conjunction with adhesive contacts to coordinate action over long distances.[173] Signaling molecules enable diffusion-mediated intercellular communication of positional information among cells in tissues, while adhesive molecules allow the exchange of information through mechanical coupling or through direct pore formation in neighboring cells. These communication networks in cells sort unorganized clumps of cells into well-ordered tissues and allow tissues to regulate their activity as a whole, contributing to tissue homeostasis.[174] For example, a minor population of cells expressing estrogen and progesterone signaling receptors coordinate growth and morphogenesis during different development stages (prepubertal, postpubertal, and pregnancy) via an intricate paracrine signaling network.[175] To regulate collective action, synthetic cells endowed with communication networks will be indispensable for the development of synthetic tissue (Figure 5B).

Diffusion-Based Communication

Individual cells execute short- and long-range functions in tissue by sending and receiving information from other cells in the form of signaling molecules or through cell–cell contacts. The process for the former in synthetic cells has often depended on triggering enzymatic or chemical reactions in receiver synthetic cells by membrane-diffusible small molecules[168] or by diffusion of larger molecules from the sender cells through membrane protein pores, e.g., alpha-hemolysin (αHL),[176–180] melittin,[181,182] perfringolysin O,[183] and mechanosenstive channel of large conductance (MscL).[184] The product of the reaction acts as a transduced signal that leads to modification of the receiver cell. While this reaction-dependent scheme represents an efficient form of communication in isolation, there are a few drawbacks that would need to be addressed for translation to synthetic tissue. Diffusion of large molecules across the membrane is a challenge that is often resolved by the formation of membrane pores or large channels.[185] However, in most cases these pores and channels are permanent modifications made to the membrane and can lead to undesirable leakage of internal contents, which would impact the long-term compartmentalization of tissue. Making use of different membrane materials may offer one answer. For instance, larger molecules such as TetR-sfGFP (50.1 kDa) and T3 RNA polymerase proteins (98.8 kDa) are able to cross porous acrylate membranes,[186] although pinpointing a membrane material with suitable transport properties for different signaling molecules of varying physicochemical properties may prove challenging. The development of new membrane pores and channels that can open reversibly and selectively offers perhaps the most elegant solution to the problem. In this vein, Langton et al.[187] have developed a signal transducer that is embedded in the lipid bilayer membrane. The transducer reversibly catalyzes the formation of surfactant molecules based on the external pH, thereby modulating the global permeability of the

membrane. While inducible surfactant production presents a facile alternative to controllable pores and channels, the surfactant-containing membrane leads only to the release of small molecule cargoes, highlighting the need for more work on controllable pores and channels in synthetic cells. Very recently, we reported a light-based method for controlling the assembly and activity of connexon nanopores in synthetic cells.[188] By engineering connexin's assembly to be protease-sensitive, we triggered nanopore activity by uncaging a protease with light, ultimately leading to rapid signal release across the membrane. This type of strategy—re-engineering natural membrane pores with synthetic regulatory mechanisms—takes advantage of the bottom-up and tailorable nature of synthetic cells for diffusion-based communication.

Contact-Based Communication

While signaling molecules can provide long-distance communication between synthetic cells, contact-based communication can be relied upon for short-distance communication. We refer the reader to the engineering Adhesion between Synthetic Cells section above for a detailed discussion of strategies to reconstitute adhesion in synthetic cells. Briefly, asymmetric adhesion between synthetic cells has been realized using complementary DNA-linkers[189] or light-activated protein adhesion pairs,[44] and direct-contact-based communication is possible through membrane fusion[177] or through the formation of gap-junction-like structures.[61] In order for tissues to act collectively, the coexistence of short- and long-distance communication machineries will be pivotal to achieve tissue-like function.

As introduced above, synthetic cells must be able to sense and rid the tissue of damaged cells. The first step in this process would rely on intercellular communication, wherein healthy cells detect the presence of a damaged cell through a signal. Light-based communication has been reported previously,[190] and two recent studies describe synthetic cell-produced bioluminescence as one possibility for a transducible signal. Adir et al.[191] employed bioluminescence for the activation of synthetic cells, leading to induction of protein expression and membrane localization, and Chakraborty and Wegner[192] took advantage of bioluminescence-based adhesion between two synthetic cells, resulting in the lysis of one partner cell. These studies present a strategy to induce signaling-based communication and subsequent lysis of damaged cells for tissue repair. Beyond repair, position-specific signals may also be used to direct self-organization of synthetic cells. The various diffusion-mediated and contact-based communication networks discussed above, coupled with synthetic tissue-guided modifications, are needed for elevating synthetic tissue from a static group of adhering cells to a dynamic collective that can adapt to biological stimuli and insults over long distances and over time.

SYNTHETIC CELLULAR ASSEMBLIES AND PATTERNING

The sections above detail approaches engineering individual synthetic cell behavior, and, in this section, we extend our discussion to current advances in manipulating synthetic cell assemblies, i.e., synthetic tissues. How are synthetic tissues different from synthetic cells and their behaviors? Synthetic tissues—also called prototissues for their underlying compositional simplicity—describe an ensemble of cells that work in concert to produce

a functional property or properties that no individual unit alone can accomplish. Current properties that are being targeted fall under three central themes: patterned and controllable morphology, information transfer, and responsive capabilities (Figure 6). Patterning signifies the ability to achieve defined spatial heterogeneity in cellular assemblies, while controllable morphology is the capability to engineer a three-dimensional shape. Patterning enables synergistic activities by combining multiple cell types together, for instance, in the small intestine, where enteroendocrine, enterocyte, goblet, Paneth, and tuft cells are precisely positioned within the tissue.[193] As well, tissues assume a wide variety of morphologies within the human body. State-of-the-art engineering methods, such as nozzle-based printing, present opportunities to tailor synthetic tissue shape with high resolution.[194] Next, information transfer refers to the capacity to exchange material between cells, similar to channel function in living tissue.[195,196] Lastly, we define responsiveness as changes to synthetic tissue properties in the presence of new environmental conditions or stimuli, such as light, temperature, or molecular signals. For example, constriction of the epithelial apical surface in response to the ligand, Fog, leads to a new morphological feature, the ventral furrow, in *Drosophilia* mesoderm tissue.[197] Work described in the sections above present experimental options to tune properties of synthetic cells. Yet, eliciting prescribed tissue-level properties from assemblies of synthetic cells remains challenging, and this section aims to highlight forward-thinking solutions for engineering functional assemblies and the need for more innovative work to form fully functional tissue synthetically.

Patterning and Morphology

As the basic building block of synthetic tissue, one overarching goal in patterning is to achieve single-cell resolution during assembly. The Bayley group has made significant contributions in this area, spearheading work on droplet bilayer interface (DIB) assembly with 3D printing methods.[198–201] Using lipid monolayer-stabilized water droplets as a bioink, Villar et al. achieved single-droplet resolution when using a nozzle in aqueous solution.[198] Controlling the equilibrium contact angle between droplets allowed defined packing of DIB assemblies.[201] The authors were ultimately able to demonstrate a five-layer DIB assembly with two independent signaling pathways. Additionally, DIB patterning with defined morphology was achieved by Elani et al. by patterning 2D rows of DIB assemblies with different lipid compositions and dispensing droplets row-by-row to yield a four-row arrangement.[202] Other means of controlling DIB assembly morphology have also been introduced, including magnetic droplet levitation and direct injection into gels.[203,204] In each of these methods, DIBs can take on a patterned configuration with controllable morphology in structures of ~10–100s of DIBs. Of note, these assemblies may be difficult to translate for specific applications since they are formed in oil and may be incompatible with certain aqueous environments.

Both patterning and morphological control of synthetic tissue are desirable but can be challenging to implement simultaneously. Optical tweezing of GUVs holds great promise for accomplishing both, though the technique has the drawback of assembling only a few (~2–10) synthetic cells at a time. Recently, Ces and Elani and co-workers positioned synthetic cells using optical tweezers with both single-synthetic cell resolution and morphological control.[52] Subjecting synthetic cells to external fields provides another

opportunity for assembly. Acoustic waves and magnetic fields have recently been implemented to generate 1D/2D and 2D/3D spatial arrangements of ~10–100 synthetic cells, respectively.[181,205] In the case of acoustic wave manipulation, the number of vesicles per pressure node, lattice spacings, and 2D geometry appear highly tunable, but this level of patterning comes at the expense of facile morphological control.[181,182] In contrast, imposing magnetic fields led to remarkable morphological definition—even assembling synthetic cells into the figure of words—although at the sacrifice of single synthetic cell spatial resolution. Laser cutting of proteinosome assemblies by Gobbo and co-workers offers another alternative to generate complex 3D architectures composed of a large number of proteinosomes, ~100–1000.[206] In this example, the authors use click chemistry to ligate a proteinosomal tissue-like structure on a mold that is then subsequently laser-cut. In a recent paper by Casas-Ferrer et al., the authors report a series of methods that rely on strong linkages, biotin–streptavidin interactions or DNA adhesion, and different modes of mixing to selectively build either flat 2D or irregular 3D morphologies of synthetic cells.[42] These assemblies had ~10–100 synthetic cells. Each method described above displays distinct advantages for patterning and 3D structure, e.g., high spatial resolution, tunable morphologies, or increased throughput (Figure 6A). Still, the obstacle of defining both patterning and morphology at the same time stands, but creative combinations of techniques have led to outstanding progress over the past decade, perhaps most apparent with optical tweezing efforts.

Information Transfer

Material exchange represents a rapid mode of communicating information within an integrated tissue. Here, we discuss tissue as a complex network of both independent and interconnected signaling pathways, focusing on distances and spatial separation (for a longer discussion on Intercellular Communication, see section above).

Long-range signaling in response to an epithelial wound occurs on the order of >10 mm.[207] The various distances of communication present in tissue allows for multiple cells to have a tissue-level response to a stimulus. In DIB assemblies, short-range and long-range signaling has been demonstrated via αHL incorporation in the membrane[199,204,208] and takes place on a scale of a few droplets to >10 droplets (~500 μm) (Figure 6B).[198,201,203,209] At present, significant room remains for achieving information signaling over longer distances within lipid bilayer synthetic cell assemblies. As mentioned above, αHL has seen extensive use in synthetic cells since the protein spontaneously inserts and creates a pore within a lipid bilayer.[210] Recently, direct communication between two synthetic cells with lipid bilayers was established by forming a αHL channel between the two cells.[52] Including an αHL channel blocker, TRIMEB, outside the cells, two synthetic cells could be adhered together via electrostatic forces and were shown to exhibit exchange of calcium ions. More recently, information transfer between synthetic cells and living tissue was shown by expressing basic fibroblast growth factor in synthetic cells. By implanting the synthetic cells in both cell cultures and live mouse models, Schroeder and co-workers were able to induce remodeling of vascular structures on the order of ~10 mm.[211] Another form of long-range information transfer that occurs in living cells involves the propagation of electrical signals. For instance, in bacterial cells, the propagation of ion waves through ion channels

has been implicated in long-distance metabolic coordination.[212] In living tissue long-distance electrical communication involves membrane depolarization, neurotransmitters, and synapses between neurons. Such systems are yet to be incorporated into synthetic cells and warrant further exploration.

Responsiveness

Over the past decade, DIB ensembles have shown response profiles to external stimuli. In one of the first examples, DIB assemblies were engineered with lipids that undergo phase changes in response to different pHs or temperatures, allowing the assemblies to release internal compartments upon changes to these stimuli.[213] Hydrogel DIB structures have also been shown to respond to temperature and light through shape changes, such as curling, and when encapsulating magnetic particles, the structures moved in response to magnetic fields.[214] This enabled DIB structures to grip an object via a morphological change and then navigate through a maze. Single cell resolution responsiveness was achieved using DIB structures that encapsulated a light-dependent expression system,[199] and 3D folding has also been shown.[198] Rather, Gobbo and co-workers encoded thermoresponsive properties into assembled proteinosomes by making use of PNIPAM to induce contraction upon temperature change,[215] and Mueller et al. decorated colloids with photoswitchable ligands to enable self-sorting in response to light (Figure 6B).[216] While these latter two examples are not in lipid-bilayer-based synthetic cells, their work informs future methods seeking to produce tissue-level responses. Very recently, membrane deformation of synthetic cell colonies through ATP introduction and subsequent actin polymerization has been shown.[217] This is a demonstration of built-in mechanical responses to a chemical stimulus, bringing the idea of responsive synthetic tissue closer to fruition.

Judicious patterning of single responsive synthetic cells within larger structures provides an option for anisotropic actuation. Beta cell-like features have been reconstituted in single synthetic cells, ultimately leading to insulin secretion after glucose-dependent stimulation and membrane fusion. Incorporation of these synthetic cells into diabetic mice tissue resulted in normoglycemic blood glucose levels.[218] Other synthetic cell responses that could reasonably be integrated into tissue and provide benefit include forms of mechanosensing,[219] inflammatory chemical sensing,[220] and photosynthesis.[221] Still, synthetic cell assemblies have yet to reach the precise responsiveness of DIBs and proteinosomes, and future work will hopefully address this critical gap.

CONCLUSION

Significant advances in synthetic cell technology have paved the way for building synthetic tissue using bottom-up approaches. Essential to the field’s advancement has been the combination of new engineering methodologies for synthetic cell formation and encapsulation with bespoke molecular tools that enable dynamic and evolvable multicellular systems. Progress toward each of the key features of living tissue, namely, adhesions, mechanical responses, spatial patterning of individual cells, regeneration, and intercellular communication, has been notable and promising. Adjacent research areas have also contributed to synthetic tissue development, especially in patterning synthetic cells by 3D

printing, laser-cutting, and optical tweezing techniques. Yet, the integration of multiple tissue features into a single synthetic tissue remains an outstanding goal. Continued investment and work in coupling extracellular recognition with intracellular cytoskeletal reorganizations will help close the gap to tailored and controllable tissue-wide properties.

Looking to the future, focus will need to be placed on interfacing synthetic tissue with living tissue, giving rise to new devices, implants, and graft materials (Figure 6C). We expect that hybridization, rather than biocompatibility, will play a crucial role in this direction as hybrid junctions allow the communication between abiotic and biotic tissue, which will be crucial to heal and treat injuries and disorders. Hybridization need not rely solely on natural components but may involve natural biomolecules outfitted with unnatural parts that give user-defined control over their functionality and, in turn, synthetic tissue properties. However, special attention will need to be paid to synthetic cell integrity, such that synthetic tissue can withstand the aqueous environment of living tissue and the biological and mechanical insults therein. In this way, synthetic tissue can act and respond to living tissue. With new technologies emerging at a rapid pace for synthetic cells and tissues, the future is bright for forging one from many.

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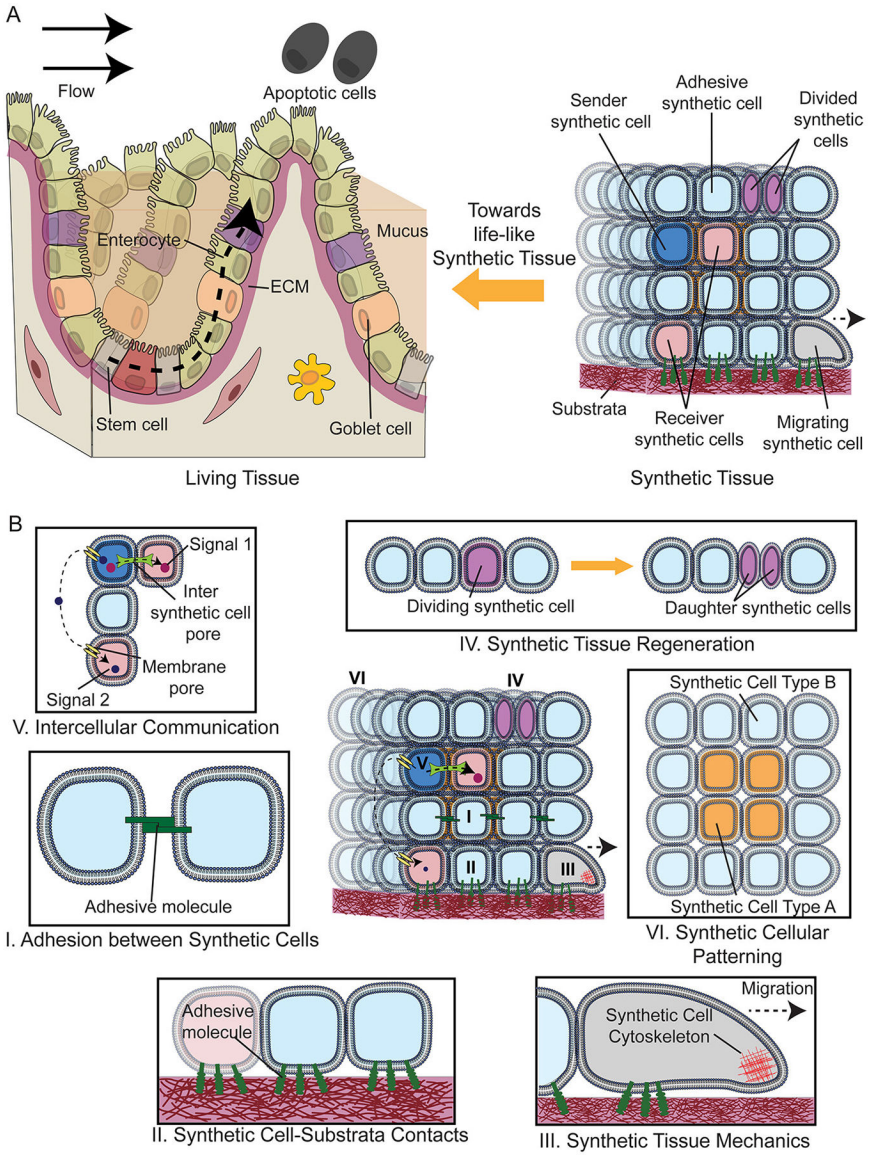
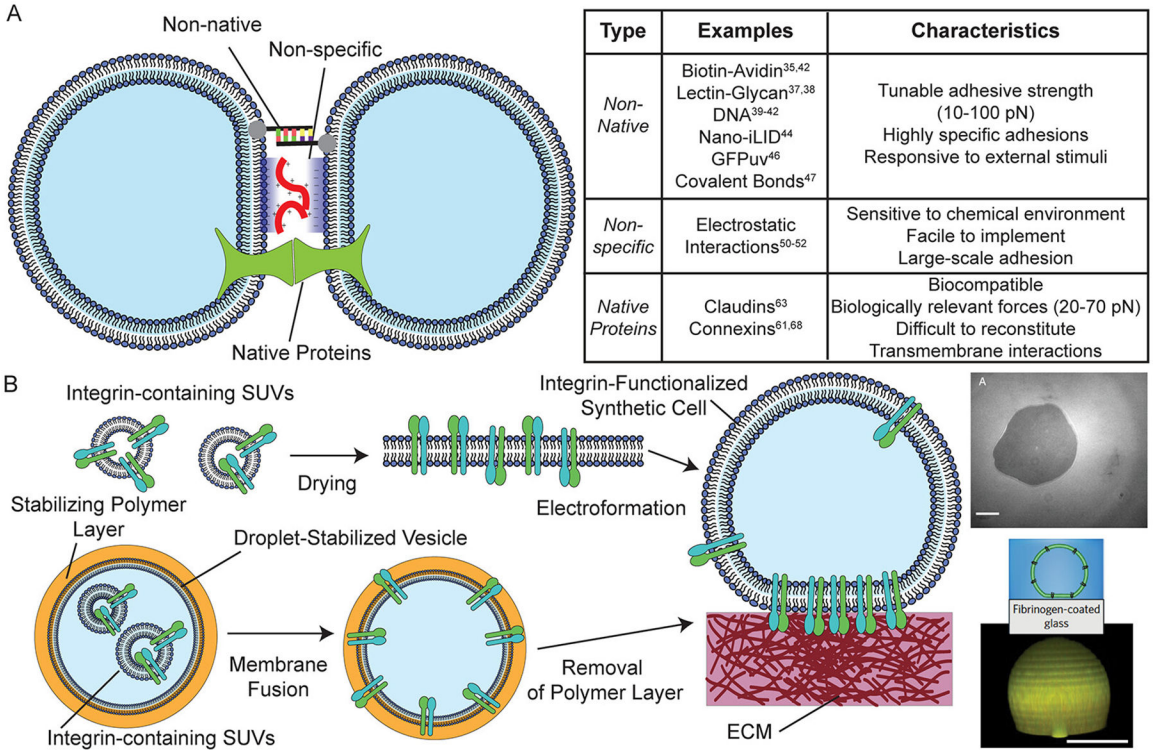


Figure 1.

Synthetic tissue is inspired by the organization and dynamics of living tissue and seeks to emulate key features and functions of its living counterpart. (A) Tissue of the small intestine (left) contains (i) different cell types patterned within the villi and crypts and (ii) noncellular structures, including the extracellular matrix (ECM) and the mucus layer, that shape and contribute to the tissue and its function. Signaling-dependent differentiation and migration maintain the functional capacity of the organ for absorption under fluid flow. Synthetic tissue (right) requires the precise combination of distinct synthetic cell building blocks and substrata materials to approach the capabilities and resiliency of living tissue. (B) Six desired functionalities of synthetic cells for the construction and maintenance of synthetic tissues include adhesion between synthetic cells, synthetic cell–substrata contacts, synthetic tissue mechanics, regeneration, intercellular communication, and synthetic cell patterning.

These functionalities either depend on or must operate in the presence of synthetic cell neighbors.



Type	Examples	Characteristics
Non-Native	Biotin-Avidin[35,42] Lectin-Glycan[37,38] DNA[39-42] Nano-iLID[44] GFPuv[46] Covalent Bonds[47]	Tunable adhesive strength (10-100 pN) Highly specific adhesions Responsive to external stimuli
Non-specific	Electrostatic Interactions[50-52]	Sensitive to chemical environment Facile to implement Large-scale adhesion
Native Proteins	Claudins[63] Connexins[61,68]	Biocompatible Biologically relevant forces (20-70 pN) Difficult to reconstitute Transmembrane interactions

Figure 2.

Linkage chemistries for adhering synthetic cells to each other and to the extracellular matrix (ECM). (A) Non-native adhesions, nonspecific interactions, and native proteins have been used to interface synthetic cells into multicellular assemblies (left). This large repertoire of interactions enables engineering of the strength and dynamics of synthetic tissue assemblies. Examples and characteristics of each type of adhesion are summarized in a table (right). (B) Reconstitution of integrin heterodimers, which engage the ECM, has been achieved via electroformation (top) (reprinted from ref 75, with permission from Elsevier) and membrane fusion (bottom) (reprinted from ref 59, Copyright 2017 Nature Publishing Group). Both methods lead to synthetic cells contacting the substrata to build composite materials, analogous to living tissue.

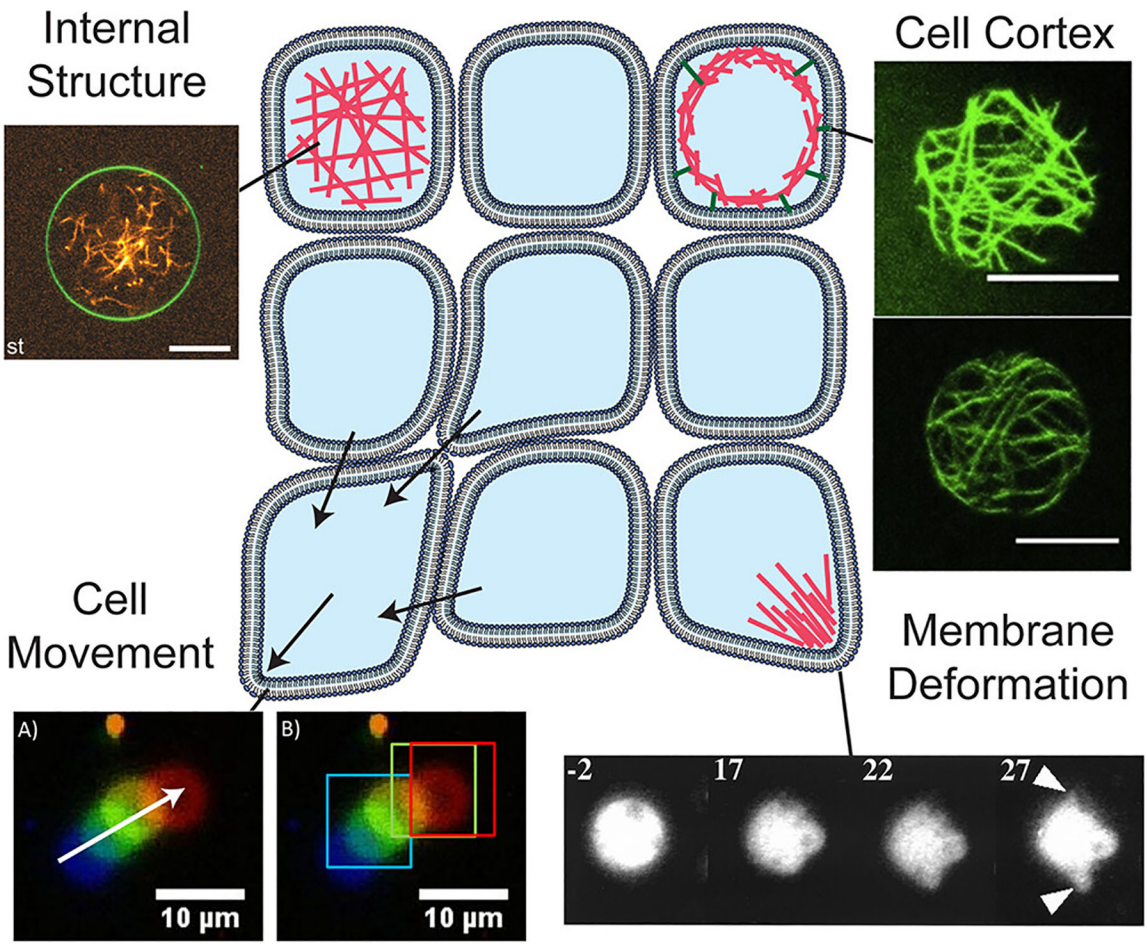


Figure 3.

To engineer defined mechanical responses in synthetic tissue, incorporating cytoskeletal proteins, their resulting networks, and connections to the membrane remains critical and has been an intense area of focus. Successful examples include building internal structures by ligating multiple cytoskeletal elements together (top left) (from ref 111, CC BY 4.0), generating artificial cell cortices (top right) (from ref 110, CC BY 4.0), generating synthetic cell movement with photosensitive proteins (bottom left) (reprinted with permission from ref 128, Copyright (2018) American Chemical Society), and inducing membrane deformations with molecular motors (bottom right) (reproduced with permission from ref 121, Copyright (1999) National Academy of Sciences, U.S.A.). Placement of anisotropic mechanical properties within synthetic tissue can give rise to convoluted morphologies, often found in living tissue, and may provide access to collective phenomena, like collective cell migration.

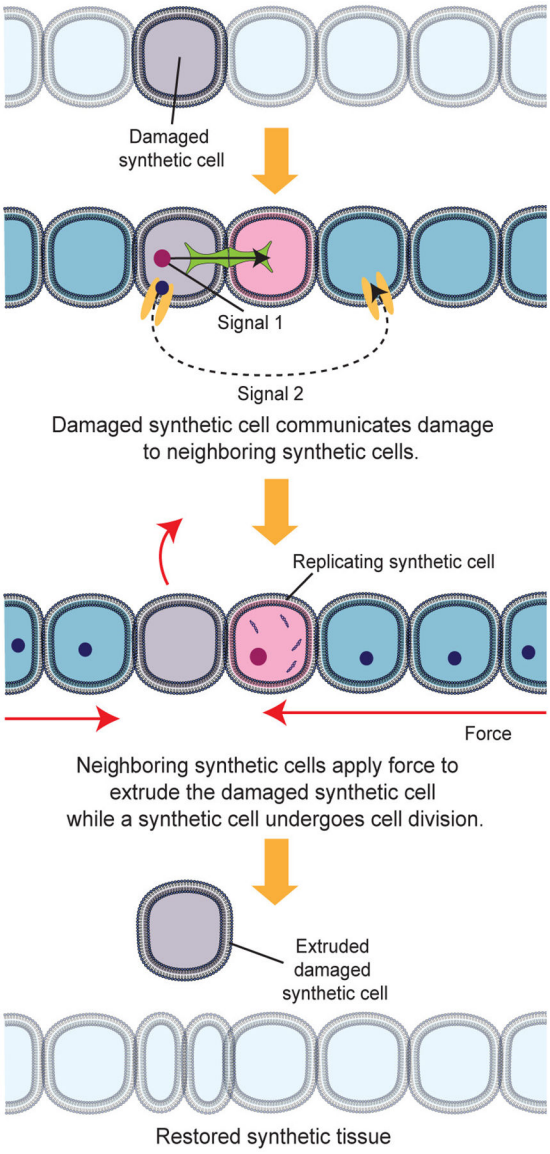


Figure 4.

Hypothetical path for regenerating damaged synthetic tissue. Damaged cells within synthetic tissue must be recognized, extruded, and replaced to maintain the function and integrity of synthetic tissue. After receiving damage-specific signals, neighboring cells respond by initiating cell replication and tissue-wide compression. The neighboring cells extrude the damaged cell while simultaneously dividing to fill the gap left by the damaged cell. Ultimately, the damaged cell is extruded, and the synthetic tissue is repaired.

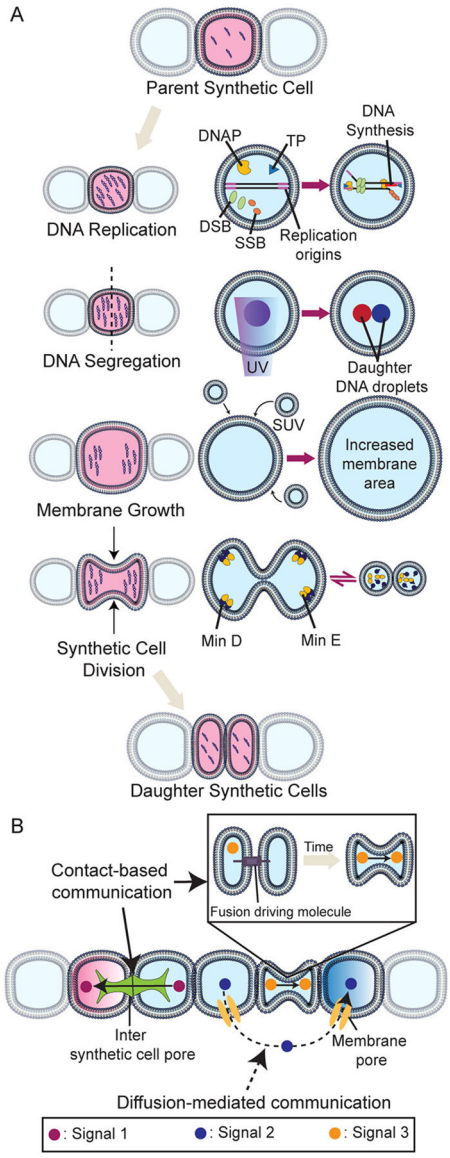


Figure 5.

Recent methods of synthetic cell replication and communication. (A) For replication within synthetic tissue, synthetic cells must undergo similar steps of cell division to those of living cells under the constraint of surrounding neighbors and their cumulative pressure. First, DNA must be replicated, which has been accomplished with encapsulating machinery from the phi29 replication complex. Double-stranded binding protein (DSB) and single-stranded binding protein (SSB) stabilize DNA while terminal protein (TP) and phi29 DNA polymerase (DNAP) initiate and catalyze DNA replication, respectively (from ref 140). DNA can then be segregated. A DNA droplet with photolabile sites formed through liquid–liquid phase separation undergoes segregation in response to UV light (from ref 150). Next, small unilamellar vesicles (SUVs) are fused with a parent GUV to mimic membrane growth (from ref 156). Finally, the membrane must deform and split, creating two separate synthetic cells, which has been partially demonstrated with encapsulating Min proteins in osmotically

deflated vesicles (from ref 151). (B) Two general forms of communication in synthetic tissue. Contact-based communication is governed by direct cell–cell channels or through fusion of synthetic cells and mainly occurs between proximal cells, while diffusion-mediated communication relies on release of soluble signaling molecules via membrane pores, which can reach more distant neighbors.

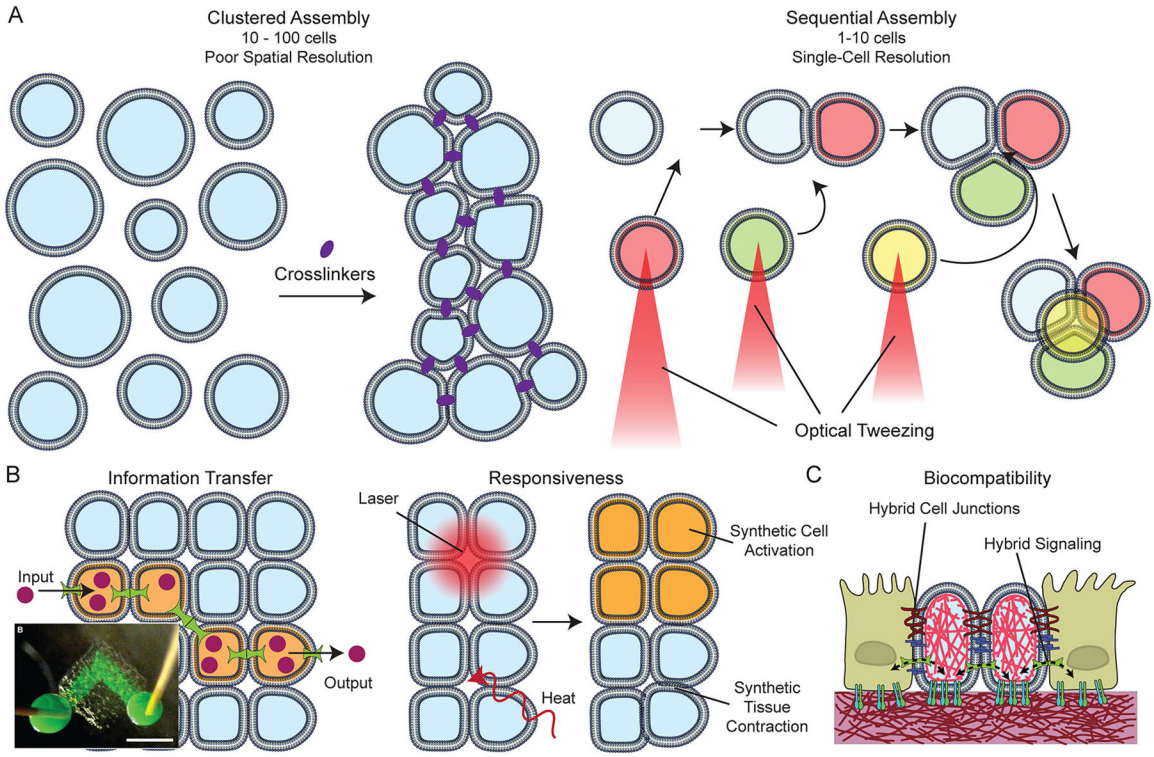


Figure 6.

Synthetic cells can be patterned spatially to yield assemblies capable of information transfer and responsiveness. (A) Two general methods of assembling synthetic tissue have been shown in the literature. One, clustered assembly (left) relies on external forces and/or chemical cross-linkers to group cells together. While this method can organize many cells together (10–100), single-cell spatial patterning is difficult to achieve with this approach. Sequential assembly (right), on the other hand, occurs either through optical tweezing or 3D printing of single synthetic cells. Optical tweezing is capable of only patterning up to ~10 cells at once, a limitation for building large systems. (B) Transfer of information and responsiveness are defining features of living tissue. In synthetic tissue, transfer of information (left) has been implemented by endowing synthetic cells with the ability to transmit signals over long distances, requiring both spatially defined processing and transport machinery (from ref 198, Copyright 2013 The American Association for the Advancement of Science). Responsiveness to external stimuli (right) has also been achieved, allowing synthetic tissue to adapt to various environmental conditions, mechanical states, and to external user-defined inputs, such as light pulses and temperature changes. (C) In the future, we envision synthetic tissue–living tissue interfaces, where biocompatible synthetic cells are engineered to signal to neighboring living cells directly through hybrid junctions.