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Engineering native biological complexity from the inside–out and outside–in

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The balance of 'outside–in' and 'inside–out' signaling is critical in tissue development and regeneration. This Comment highlights emerging strategies to engineer and manipulate this delicate equilibrium and fine-tune cellular responses using complementary tools in biomaterials design and synthetic biology.

In living organisms, cells are surrounded by a tissue-specific, three-dimensional (3D) microenvironment that presents a complex milieu of soluble and insoluble factors to the resident cell populations. These outside–in signals are transmitted through the extracellular matrix (ECM) and direct and coordinate cellular functions (for example, proliferation, migration and differentiation) that are critical during development, homeostasis and tissue regeneration. Adding to this complexity, a dynamic reciprocity exists whereby cells are innately programmed to respond to and remodel their microenvironment, leading to feedback and feedforward mechanisms between 'inside–out' and 'outside–in' signaling. However, diseases arise when this delicate balance goes awry, which has motivated the development of new interventions based on tissue engineering principles.

When considering strategies to regenerate tissues, the multifarious tissue microenvironment goes well beyond presenting an initial condition. The boundaries and processes involved in bottom-up tissue construction are dynamic, occurring in series and parallel over many time and length scales, rendering efforts to control these events an archetypal engineering problem. Information abounds from advances in multi-omics (for example, RNA-seq, ATAC-seq and metabolomics), and imaging tools have evolved to visualize biological processes in striking detail across orders of magnitude, from translation of single molecules to development of entire organs. Computational models and machine learning enable expedited mining of these data, which informs the field and provides critical insights for new strategies in biomaterials design, cellular engineering and engineering tissues. We ask how can engineers use this plethora of information to control intracellular reactions, design the ECM microenvironment to coordinate multicellular behavior, and orchestrate these biological events towards the emergence of tissue-level function? In this Comment, we provide a perspective as to how manipulating transformations at the molecular level in both cells and biomaterials might be integrated with the modular thinking of unit operations and process control to ultimately engineer and direct cells to (re)generate functional tissues (Fig. 1).

Outside–in customization of the cellular microenvironment

Within the intricate dance of outside–in signaling, biomaterials serve as both the stage and choreographer. The advent of biocompatible (and eventually bioorthogonal 'click') chemistries made possible the encapsulation and long-term culture of cells in well-defined, synthetic 3D matrices, enabling direct study of cellular responses to isolated ECM signals. Better still, the structural parameters of physical confinement imposed on cells by encapsulating polymeric networks are both predictable and highly tunable, allowing for derivation of useful models to describe how bulk properties (for example, stiffness) are controlled by the underlying nanoscale network architecture. Early progress in the field centered on identifying the minimal features for the design of synthetic ECM, focusing on those that could promote desired cell responses including proliferation and deposition of nascent proteins towards regenerating tissues. Strategies from materials science and peptide engineering coordinated to yield synthetic hydrogels bearing only simplified mimics of binding domains or enzymatic cleavage sites of native ECM proteins such as fibronectin and collagen[1]. These elastic matrices, defined by their fixed initial conditions, were designed to offer tunable degradation rates and mechanical properties by way of hydrolysis or cell-mediated enzymolysis. Germinating from these results, investigations into materials-based mechanobiology transformed cell culture capabilities, even supporting the development of stem-cell-derived 'organoids' with similar symmetry breaking and maturation events to those found in vivo[2]. Remarkably, experimental analysis of cellular sensitivity to matrix degradation kinetics revealed that the genesis of these elaborate tissue models requires delicate balancing of mechanics and mass transport, highlighting the necessity of process optimization in bioengineered living systems.

Although the static or unimodal properties achieved by elastic hydrogels enabled unprecedented modes of study, native tissues have distinct time-dependent responses. Controlling these temporal properties, including viscoelasticity, peristaltic action and flow, has been an essential step and one focus in the design of the next generation of bio-instructive matrices. Biological stress relaxation profiles vary across many orders of magnitude and distinguish tissues from the highly compliant (for example, fat and brain) to structurally integral (for example, bone and connective tissue). Tailoring of viscoelastic properties and the timescales of relaxation is one lever to study cellular responses to temporal changes in their microenvironment and has established models to study tissue-specific responses in vitro, including in organoids[3]. The use of adaptable chemistries and their reversible bonds affords temporal modulation of cell–matrix interactions (for example, forces) akin to feedback controllers in process design. Towards more complex and integrated biological processes, microphysiological systems integrate multiple biomaterial–cell interfaces interconnected with channels that control fluid dynamics and/or peristaltic stretch. Such systems use unit operations principles (that is, separations, pumps and reactors) to simulate individual organs and processes in a minimal 'human body,' achieving more realistic pharmacokinetics and pharmacodynamics for testing drug safety, efficacy or delivery methods[4]. These devices illustrate one synergy

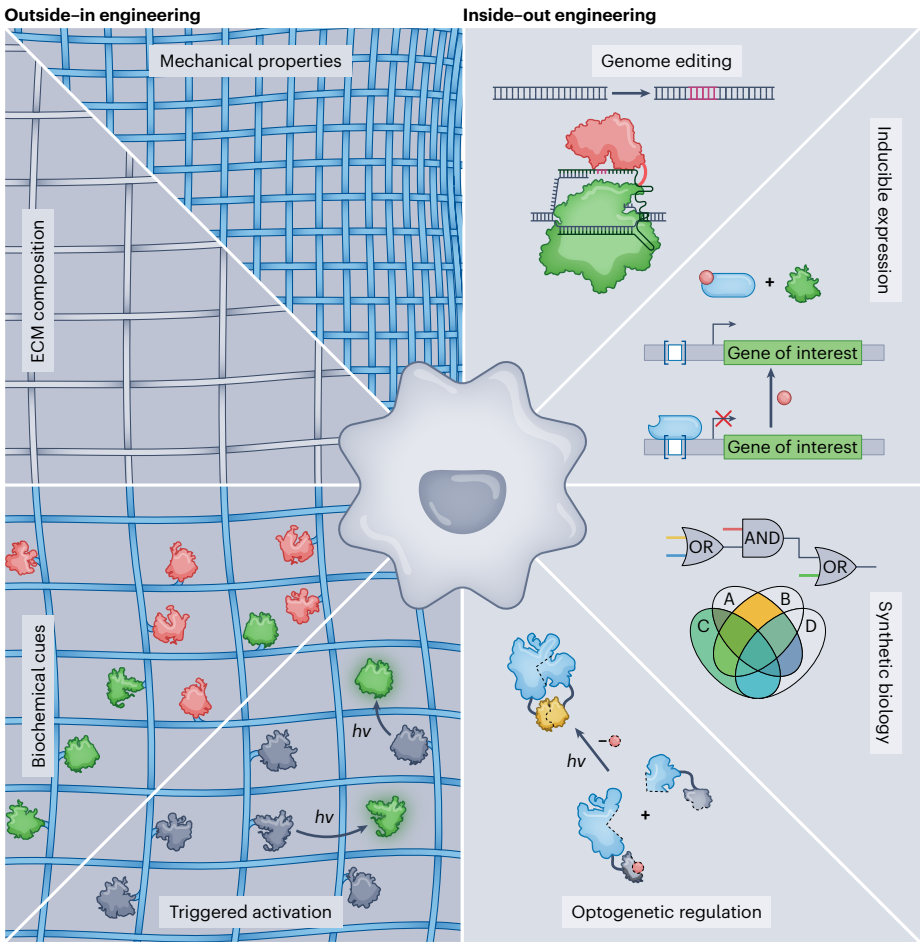

Fig. 1 | Engineering cell fate via inside–out and outside–in methods. User-defined and real-time modulation of ECM composition, mechanics and viscoelasticity, biofunctionalization, and degradability drive changes in cell phenotype and function; engineered tools for gene editing, inducible protein expression, synthetic biology and optogenetics enable direct intracellular customization that in turn drives changes in cell-mediated matrix remodeling. In both outside–in and inside–out methods, light (*hv*)-induced transformations can afford spatiotemporal control over physical, chemical and biological processes. A gray cell schematic is shown in the image center; meshed lines depict the ECM; activated biomolecules are colored, whereas inactive species are shown in gray; colored circles correspond to molecular photocages or transcriptional inducers.

between chemical engineering, biomaterials science and cell biology to create physiologically relevant tissue models that could obviate the ethical and practical drawbacks of animal testing.

If temporal control sets the pace, then spatial control provides the roadmap, directing cells through the complex landscape of a synthetic matrix. As one example, substantial progress has been made in the field of biofabrication, providing researchers with tools for generating spatially defined scaffolds composed of granules or fibers and featuring micro- or macroporosity, which enhances transport to resident cells and facilitates otherwise inaccessible scalability[5]. Owing to their temporal and spatial modulation capabilities, photochemistries have become indispensable tools for the development of bioinks and printed matrices, enabling real-time (and sometimes reversible) control over the cellular microenvironment. Offering the ability to write, erase or modify physicochemical cellular instructions, photochemical approaches represent versatile technologies that have underpinned numerous biomaterials innovations[6]. For example, while organoid culture in viscoelastic or degradable elastic matrices allows stochastic symmetry breaking events, photoadaptable systems empower researchers to deterministically sculpt multicellular morphologies[7] and gain a deeper understanding of the fundamental 'rules of life' directed by outside–in signaling. Collectively, biomaterials design through the application of chemical engineering concepts to tune initial conditions, temporal changes and spatiotemporal adaptability in cellular microenvironments represents a dynamic nexus that is advancing new tissue-engineered products. These pivotal achievements lay a fertile ground for the continued convergence of traditional engineering disciplines with life sciences, forming a collaborative framework that is ripe for exploring potential synergies with emerging technologies in synthetic biology for regulating cells from the inside–out.

Inside–out specification via cellular reprogramming

Directly complementing efforts to guide cell fate from the outside–in via dynamic ECM customization are those that inversely approach the problem, specifying cellular states from inside–out through intracellular

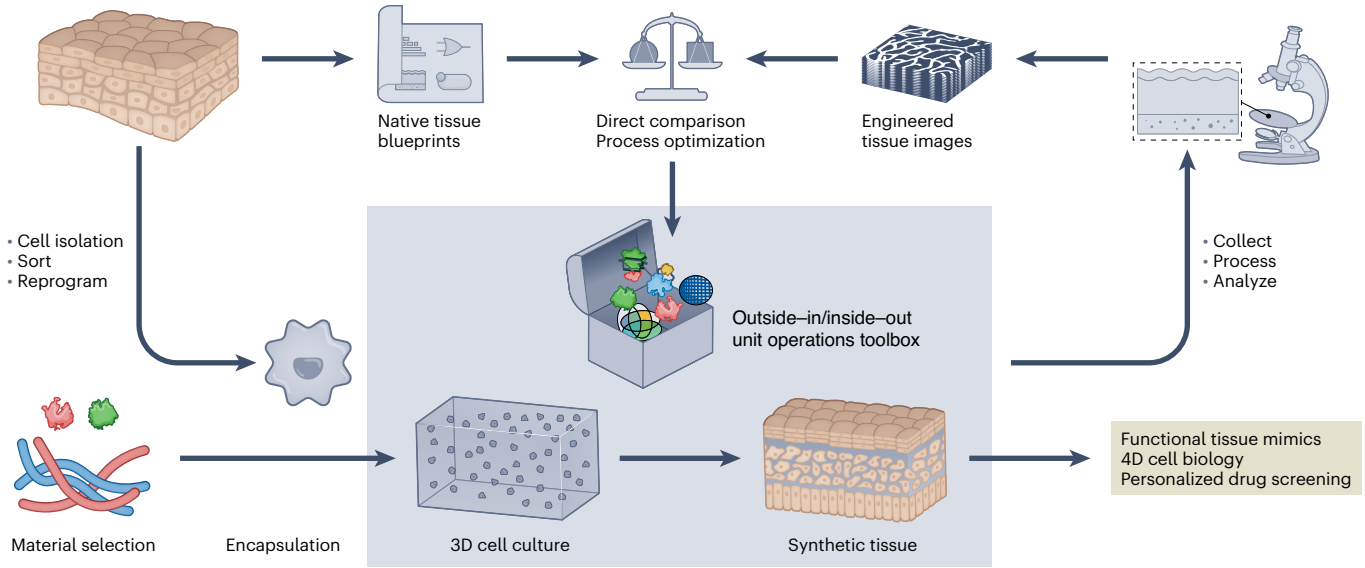

Fig. 2 | Engineering synthetic tissue. Native tissue provides both a structural blueprint and a source for primary cells, offering a functional roadmap and the living components for engineering functional tissue units via outside–in and inside–out methods. Integral to these strategies are innovative materials, cutting-edge synthetic biology tools, and advanced quantitative imaging and biological assays in 4D.

manipulation. Doing so effectively requires a well-defined starting point from which to deviate. This has been traditionally accomplished through direct isolation and/or cytometry-based sorting of desired cell populations from living organisms or with immortalized lines obtained from community-stocked cell banks. Although these 'native' cells have proven useful in many contexts, engineers and biologists alike have gravitated towards methods that permit tailored redefinition of the cells' initial state through gene knock-in/knock-out and overexpression/underexpression of natural and recombinant proteins. Beyond transient transfection in which gene-encoding plasmids are temporarily introduced into cells through chemical or physical methods (for example, lipofection and electroporation), techniques enabling stable genome modification are particularly powerful in redefining the cellular landscape for long-term and repeatable study. In this context, viral transduction[8], transposases (for example, piggyBac and Sleeping Beauty) and integrases have each shown tremendous utility in introducing designer DNA into the host-cell genome. While these techniques readily permit large genetic elements to be efficiently and stably inserted, instrumental for installing the four Yamanaka factors to create induced pluripotent stem cells[8], a lack of control over where such random insertion occurs can lead to undesired gene silencing or oncogene activation. More recent tools, especially those based on transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPR)[9], enable highly targeted genome editing with high – but still not perfect – fidelity. Such tools are best suited for creating point mutations within native genes, enabling direct study of a diseased phenotype or complete gene knock-out through stop codon introduction, although newer extensions combining the genome-targeting capacities of CRISPR with the insertional power of fused enzymes (for example, integrases and reverse transcriptases) are beginning to allow site-directed gene integration and mutagenesis[10]. The imperfect nature of these techniques still requires users to introduce modifications, separate clones with desired phenotypes, and then genomically characterize before more extensive study.

Since biology is intrinsically dynamic, great effort has been placed in developing tools that permit temporally controlled intracellular perturbation of a starting biological equilibrium through triggered gene expression or silencing. The majority of these systems involve small-molecule inducible promotors (for example, doxycycline) that enable direct 'turn-on' of recombinant genes of interest, in an increasingly multiplexable manner[11] that could be used to replicate and drive key developmental signaling cascades ex vivo. Triggered genomic modifications – changes that are inherently irreversible – are also possible with engineered cell lines; inducible expression of Cre recombinase capable of deleting a *loxP*-flanked portion of an engineered genome has proven indispensable for on-demand knock-in/knock-out. These and other emerging tools in synthetic biology[12] now permit precisely programmed customization of biological circuits, providing external control over cell development, growth and death.

Complete specification over multicellular function requires both control over when and where a biological process occurs. From an intracellular perspective, this has been most effectively accomplished with stimuli-inducible promoter platforms with inputs that can be spatiotemporally introduced. Similar to methods highlighted for ECM modulation, light-based platforms that form the basis of 'optogenetics' offer the most precise 4D regulation of protein expression and activity, ion transport, and subcellular translocation[13]. As light can penetrate native tissue, at least to some degree, optogenetics can allow dynamic and heterogenous control over cell signaling in living organisms. Heat-inducible promotors have also been used, with higher potential for multiplexed spatial activation, but with more limited resolutions dictated by the laws of heat transport. Since the SynNotch system enables overexpression of arbitrary genes via cell receptor engagement with an immobilized extracellular ligand[14], spatial control over gene expression becomes an exercise in spatially controlling the tethered cue. User-directed intracellular regulation that is both irreversible and spatiotemporally controlled is less developed. Notable examples in this regard involve coupling reversible photoactivation to

irreversible genome editing (for example, Cre and CRISPR) or through direct activation of photocaged proteins expressed using tools involving genetic code expansion[15].

Engineering for life

With modern analytical tools unraveling biological complexity with unprecedented resolution and scale, the blueprints of life have never been clearer. Almost by definition, chemical engineers thrive in their ability to draw upon such drafted blueprints in creating high-value products (for example, functional tissue, disease models and high-throughput screening platforms) from lower-value starting materials (for example, adult cells, polymers and plasmid DNA) via a coordinated collection of biochemical transformations (for example, proliferation, differentiation and gene activation) performed in both parallel and in series. Applying meticulously chosen techniques to manipulate cells and their ECM using both outside–in- and inside–out-type unit operations, the engineer first defines the starting condition of the system (for example, cell populations, ECM composition and structural arrangement) and the initial reaction kinetics and thermodynamic trajectory (Fig. 2). Precisely tailored monitoring systems (for example, genetically encoded reporters, fluorescent imaging and enzymatic readouts) provide multiplexed information underlying reaction progress in real time, enabling informed and intervening perturbation to further attenuate or enhance specific outcomes. Intracellular- and extracellular-based control systems can be put in place such that stimulating interventions occur autonomously, enabling creation of living tissue models that ultimately engineer themselves ('development') and subsequently maintain a dynamic and homeostatic equilibrium characteristic to life itself. Integrating advances in synthetic and cell biology, organic and materials chemistry, control theory, imaging, and multi-omics, the era of engineering native biological complexity is very much upon us.

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Competing interests

The authors declare no competing interests.

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