REVIEW

The emerging era of cell engineering: Harnessing the modularity of cells to program complex biological function

Wendell A. Lim

A new era of biological engineering is emerging in which living cells are used as building blocks to address therapeutic challenges. These efforts are distinct from traditional molecular engineering—their focus is not on optimizing individual genes and proteins as therapeutics, but rather on using molecular components as modules to reprogram how cells make decisions and communicate to achieve higher-order physiological functions in vivo. This cell-centric approach is enabled by a growing tool kit of components that can synthetically control core cell-level functional outputs, such as where in the body a cell should go, what other cells it should interact with, and what messages it should transmit or receive. The power of cell engineering has been clinically validated by the development of immune cells designed to kill cancer. This same tool kit for rewiring cell connectivity is beginning to be used to engineer cell therapies for a host of other diseases and to program the self-organization of tissues and organs. By forcing the conceptual distillation of complex biological functions into a finite set of instructions that operate at the cell level, these efforts also shed light on the fundamental hierarchical logic that links molecular components to higher-order physiological function.

As we explore ways to harness our knowledge of biology to solve diverse problems in medicine, one of the most innovative emerging concepts is that living cells can be engineered to execute therapeutic functions (*1*). Such efforts have been inspired by the successful use of engineered immune cells to treat hematological cancers (*2*–*4*). We now also have powerful suites of technologies to rewrite genomes and to transfect DNA. But what biological code should we write with these methods? How can we learn to program cells so that they carry out useful functions with the same degree of precision and reliability as naturally evolved cells? How can we expand the range of therapeutic problems to which cell engineering can be applied, beyond cancer or immunity? Perhaps there is a set of more universal principles, components, and approaches that can be used to functionally program cells, whether the goal is designing an immune cell to eliminate an evasive tumor or designing a set of cells that can regrow into a new organ.

Programming new biological function: Connecting cellular building blocks

Evolution has generated an inspiring array of organismal functions through gradual genetic changes. So how do we rationally alter biological behavior? One of the great barriers to understanding the relationship between genetic information and physiological function is the complex multiscale nature of biological systems (Fig. 1). Although all function is ultimately encoded in genes and their control elements, the expression of higher-level function depends on a diversity of contexts. For example, the simple expression of a receptor gene in a cell is not sufficient to generate "function" unless: (i) that cell has the necessary downstream components to mediate receptor response and (ii) that cell is localized, by history or migration, to a site where it can access signals from a partner cell expressing the cognate ligand. Thus, genetic components that contribute to any one physiological function often act at different spatial and temporal scales and in different cells. Given this multiscale complexity, where does one pragmatically focus when trying to create new function?

Although all scales of biology are important, there are many reasons to believe that the intermediate scale of cellular function and connectivity may prove to be the most pragmatic scale at which to intervene to create new biological functions (Fig. 1). Cells, after all, are compartmentalized agents that function as the fundamental units of life, providing a strong argument for viewing cells as the key "building blocks" for complex function. Much of complex biological function is ultimately determined by how cells interact and communicate with one another within multicellular systems, and thus, logically, many substantially new functions likely result from changes in the interactions between cells rather than the development of new cell-intrinsic functions. Notably, there are also a finite number of channels through which cells communicate with one another (send and receive systems) and finite types of core state changes that cells can undergo as a response (grow, die, secrete, express, migrate, adhere, etc.) (Fig. 2). Thus, focusing on reconnecting the external input and output properties of cells, whether in evolution or engineering, provides a conceptually simpler framework in which to link molecular-scale parts with physiological function.

Focusing on cells in terms of their external input and output properties provides a way to abstract the high molecular complexity of a cell but still productively manipulate how it works within a multicellular context. This abstraction is analogous to focusing on valance electrons in chemistry to understand

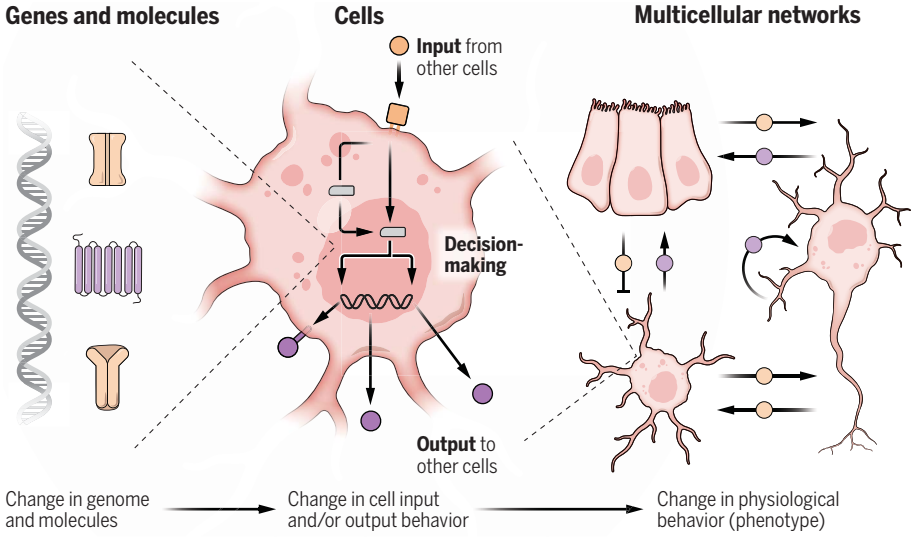

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Fig. 1. Cell-centric perspective of programming complex biological function. Biological systems are encoded as genetic or molecular components, but their higher-scale functions ultimately manifest through the way the resulting cells interact with one another. Thus, focusing on how to rewire cell inputs and outputs may provide a particularly useful perspective from which to strategically guide engineering of biological function.

Cell Design Institute and Department of Cellular and Molecular Pharmacology, University of California, San Francisco, CA, USA.
Email: wendell.lim@ucsf.edu

the functional potential of an atom (*5*). Although atoms have complex nuclear components and structures, from the perspective of understanding bonding and reactivity with other atoms, one can largely focus on only those electrons in the outermost shell, as these are the ones that can interact to form chemical bonds. This is a substantial abstraction, yet it is sufficient to rationally guide the understanding and synthesis of a universe of larger chemical structures. An analogous concept of "cellular reactivity"—how individual genes, molecules, pathways, and organelles contribute to shaping the potential external interactions of a cell—could be similarly useful in understanding and synthesizing larger-scale biological systems.

Toward a universal tool kit for cell engineering: Learning to push a cell's buttons

Most cell–cell interactions are highly complex, but they can still be broken down into a core set of common primitive cell-state changes. For example, when a chimeric antigen receptor (CAR) T cell recognizes a cognate antigen on a cancer cell, a combinatorial response is triggered: The T cells secrete payload molecules that cause target cell death (from granules), they secrete cytokines that promote their autocrine T cell proliferation, and they undergo changes in adhesion, migration, and cell state. Although this composite response may be specific to T cells, each of the individual component responses can be found in many other cell types. For example, an analogous combination of core responses are observed in neurons, which respond to signals by changing their axon shape, precisely adhering to their target cells to form synapses, and expressing transmission or receiving signaling molecules localized to these termini (neurotransmitters and receptors). Even though T cells and neurons are very different cell types, their behaviors consist of similar core elemental cellular responses.

From this perspective, another way to frame a major goal of cell engineering is to be able to controllably "push a cell's buttons" to trigger the appropriate suite of core regulatory changes (Fig. 2). A chimeric receptor, such as that used in T cells, provides an orthogonal way to link detection of a user-specified surface antigen to induction of the endogenous T cell killing response. Optogenetically or chemogenetically controlled receptors provide a way to use orthogonal light or small-molecule inputs to trigger downstream responses.

Over the past several years, a growing set of orthogonal cell–cell linkage components have been developed, and more are in the pipeline. These include orthogonal cytokine-receptor systems that can controllably induce T cell proliferation without significant cross-interference with native cells (*6*). Other orthogonal cell–cell signaling ligand and receptor

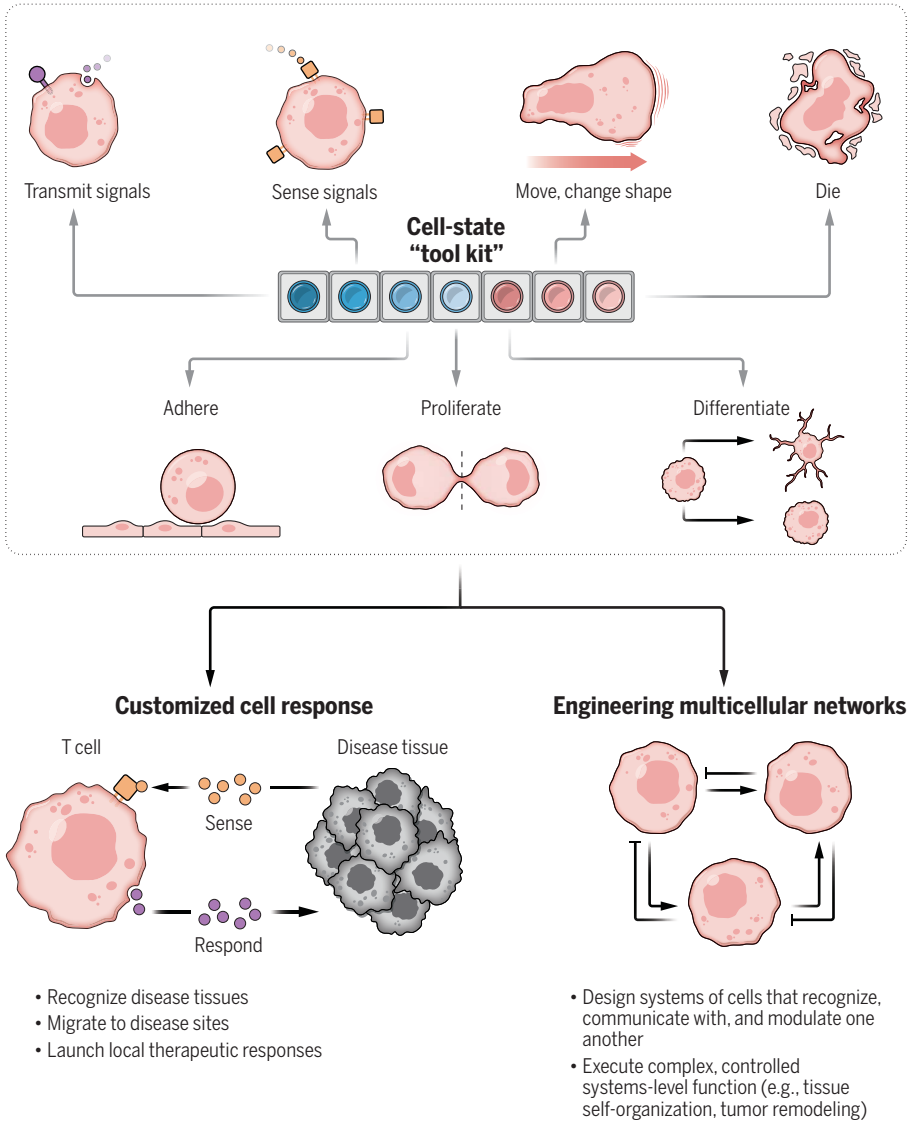

Fig. 2. Learning to push a cell's buttons: Fundamental tool kit for cell engineering. (Top) Nearly all cells can undergo a finite set of core types of state changes, a few of which are illustrated here. Many advances in cell engineering are based on developing new molecular tools that allow connecting new extracellular input to these core cellular outputs. (Bottom left) These types of tools for reconnecting cells with their environment can be used to engineer a T cell to recognize and kill a tumor. (Bottom right) The same tools can also be used to engineer more complex multicellular networks, which can involve both engineered and endogenous cell communication. Engineering tissue self-organization or remodeling of a tumor microenvironment likely requires thinking about cell engineering from a multicell-network perspective.

systems have also been developed (*7*, *8*). Synthetic Notch (synNotch) receptors and other related binding-triggered transcriptional receptors provide a particular flexible way to rewire cell–cell regulation (*9*). These receptors can be programmed with extracellular single-chain antibody domains to recognize a user-defined input ligand. When engaged, the receptors undergo transmembrane cleavage, releasing an intracellular transcriptional domain that then enters the nucleus to induce expression of a user-defined gene. The system can be used to induce expression of diverse payloads, including receptors, ligands, adhesion molecules, proliferative cytokines, master regulators, or apoptotic inducers. In short, the system can be used to rapidly create a host of completely new regulatory linkages, yielding engineered cells that respond to user-specified inputs by generating desired outputs.

So far, many endogenous cell–cell communication systems, such as those described above, have proven to be surprisingly engineerable platforms in which relationships between extracellular inputs and intracellular

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responses can be flexibly altered (*10*). In addition, these engineered receptors can function equally well in diverse cell types, as the downstream intracellular response machinery is often nearly universal. These findings speak to the highly modular structure and function of cell–cell communication molecules, many of which have undergone extensive modular recombination during the course of evolution (*11*).

Immune engineering and cancer: Lead applications

This growing tool kit of cell engineering components can be deployed in different contexts to engineer therapeutically useful behaviors, as summarized in Fig. 3. By far the most advanced and well-studied systems are those in which immune cells are engineered to treat cancer (*2–4*). The advent of the CAR first demonstrated that it was possible for a user to rationally redirect a powerful cellular immune response.

Although a primary focus of immune engineering in cancer remains on identifying antigens to target with a CAR, there is growing appreciation that this is a multifaceted problem (*12–14*). Not only must an antigen that is present at sufficiently high levels in the tumor be targeted, but it must also be homogeneous enough to avoid tumor escape. Furthermore, the antigen, or related cross-reactive antigens, must not be present in any critical normal tissues, lest it lead to toxic off-tumor killing. It is also critical to consider how the CAR T cells interact, not only with cancer cells but also with other cells in the tumor microenvironment. Especially in solid cancers, which have suppressive environments, regulatory T cells (T_{regs}), myeloid cells, and cancer-associated fibroblasts can have powerful suppressive functions that need to be overcome. Thus, to effectively treat solid tumors in the long term, it is likely necessary to view CAR T cell engineering more as a multicell interaction network problem than one focused solely on simple cellular retargeting.

To overcome this set of challenges, anticancer immune cells will likely require a combinatorial approach, in which multiple components in the cell engineering tool kit are used together to simultaneously address specificity, tumor heterogeneity, and tumor immune suppression. For example, with current tools, it is, in principle, possible to target a brain cancer such as glioblastoma with a multistep genetic program: T cells could be programmed to recognize that they are in the brain (via a synNotch receptor) and then induce the expression of a killing molecule, such as a CAR or a secreted bispecific engager (*15*, *16*). The specificity of these killing molecules need not be perfect, as long as their target antigens are not expressed in the brain. Moreover, these cells could be induced by tumor recognition to locally deliver proinflammatory payloads. A more in-depth perspective on immune engineering is given in the accompanying Review by Irvine *et al.* (*17*).

Expanding the therapeutic scope of cell engineering

As the engineering of immune cells for cancer turns toward more-combinatorial solutions, an obvious question is how the cell engineering tool kit can be applied to other classes of diseases (*18–22*). In Fig. 3, we summarize a host of potential and emerging areas of application and the types of modular therapeutic cell programs that could be useful. Engineering cells to treat autoimmunity and inflammation is already a growing area and involves

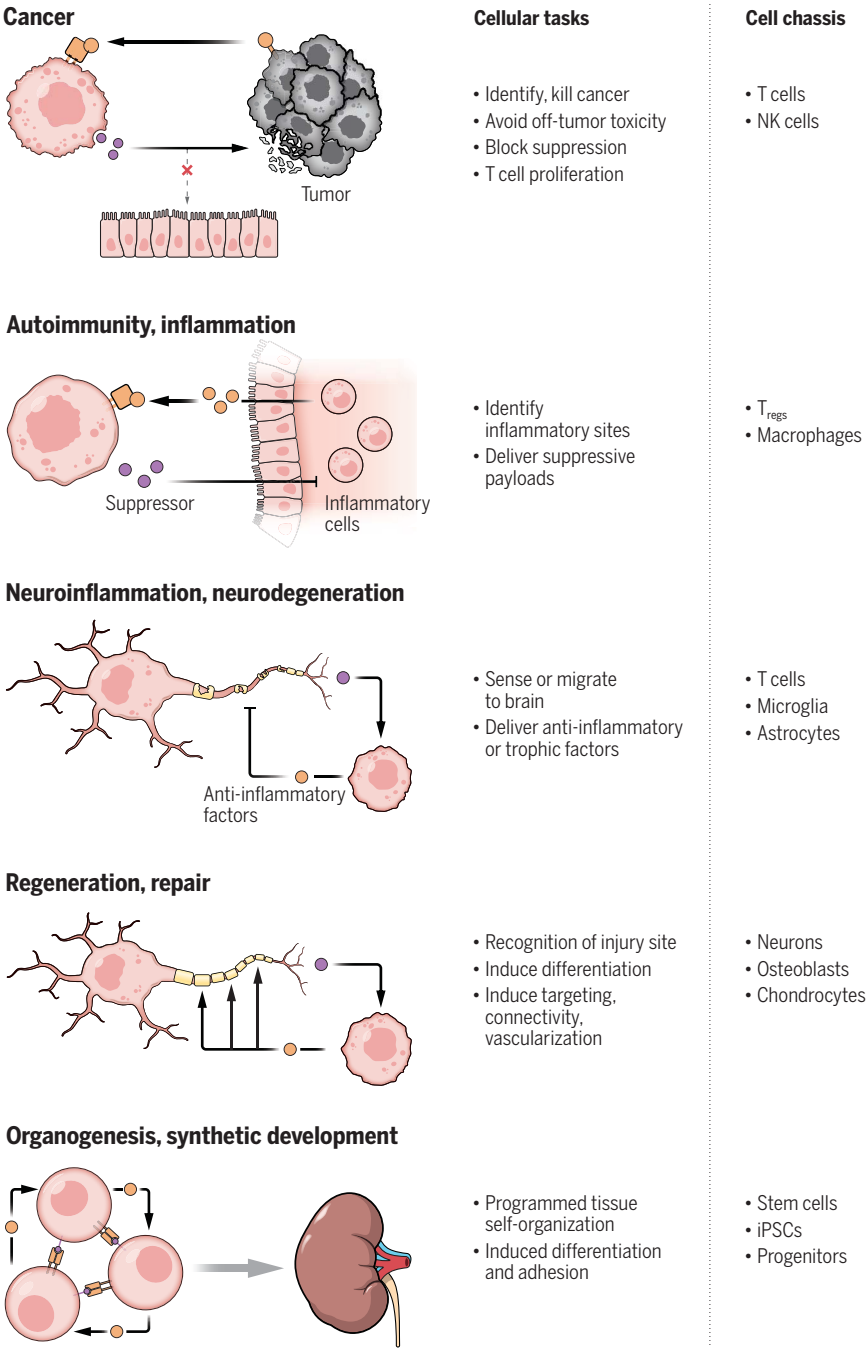

Fig. 3. An expanding set of potential therapeutic areas of application for cell engineering. Examples of fundamental cellular tasks that could be useful in each area, as well as cell types that could be useful chassis for these functions. NK cells, natural killer cells. Many of these examples are forward-looking, but see the following: cancer (*2–4*, *12–17*); autoimmunity and inflammation (*18–20*); regeneration, repair, and synthetic development (*23–28*).

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strategies ranging from killing the attacking immune cells to redirecting the action of suppressor cells, such as T_{regs}, to the targeted delivery of suppressive payloads (*18*–*20*). Other tissue-based diseases, such as fibrosis, could benefit from cells designed to sense fibrosis and to produce responses that disrupt fibroblast activation. Neurological diseases, including neuroinflammation (e.g., multiple sclerosis) and neurodegeneration, are also potential targets. In this case, it might be possible to harness brain-sensing cells to migrate to the brain and locally deliver anti-inflammatory or trophic factors. Engineered immune cells might provide previously unexplored strategies to overcome the challenges faced by molecular agents in crossing the blood-brain barrier, if their active mechanisms of transmigration can be redirected.

Another growing focus in cell engineering is synthetic development, where the spotlight is on learning to program the self-organization of functional multicellular structures, a topic reviewed in this issue by Martínez-Ara *et al.* (*23*). The tools and approaches of cell engineering are being used to explore the logic of self-organizational circuits and to potentially guide and improve organoid growth or regeneration (*24*–*28*). Combining engineered morphogen and juxtacrine signaling with control over differentiation and cell adhesion can provide the basis for complex spatiotemporal organization.

Neuroengineering is an intriguing future area in which one might apply the same approaches and tools to wire, self-organize, or repair brain circuits. Advances will require learning how to trigger the localized differentiation of neural stem cells into specific cell types, such as neurons and oligodendrocytes, as well as how to synthetically control the connectivity of these cells (Box 1).

Another frontier is the engineering of bacteria as agents programmed to interact with mammalian cells and disease tissues. Changes in the microbiome have been linked to many diseases, and thus systematically engineering the microbiome might provide powerful therapeutic avenues. Such engineered bacterial/mammalian systems are also very relevant to treatment of cancer and inflammation, a topic reviewed in this issue by Gurbatri *et al.* (*29*).

Advantages of engineered cells over molecular-scale interventions

The realization that many different diseases and different areas of biology could be affected by these general cell engineering tools and approaches speaks to the fact that so many biological challenges (including therapeutic ones) require solving the same types of spatiotemporal problems that cells are uniquely well suited for. Say, for example, that a secreted factor that plays a role in a disease state is identified and proven to block a key disease mechanism in vitro. In most cases, the problem of how to precisely deliver this molecular factor to the right place at the right time will still need to be solved. Because this factor likely also functions in other normal processes throughout the body, it cannot simply be administered systemically, given the potential danger of inducing toxicity. Cells—which can move, send out processes, and conditionally secrete factors—provide an ideal platform for precision local delivery of factors (Fig. 4). It is the fact that cells can act in such a localized manner that allows multicellular organisms to repeatedly reuse the same molecular systems to control different aspects of biology (consider, for instance, how many distinct biological systems in a single organism use Wnt or transforming growth factor–β). Cells also have the power to act more as organizers, orchestrating decision-making and calling in other endogenous cell types to carry out their functions in a coordinated and spatially localized fashion (*30*).

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Another potential advantage of engineered cells as therapies is that they have the potential to respond to a disease in a homeostatic manner. For example, when a molecular therapeutic such as an anti-inflammatory drug is given, the system is pushed in one direction and does not necessarily reestablish a stable state. A hallmark of living systems is their ability to achieve homeostatic, self-adjusting, and balanced responses. In nearly all cases, cellular systems are used to achieve these behaviors (Box 1), relying on a network of opposing positive and negative regulation (e.g., inflammatory versus anti-inflammatory responses), coordinated through feedback control (*31*). Similarly, regenerative processes must achieve a balance between proliferation and cell death, so that new tissue can emerge, without resulting in cancer. How to engineer robust homeostatic cellular circuits remains a great future challenge and opportunity.

Cell engineering also provides an opportunity to invent novel workarounds to roadblocks inherent to biology. There are cases in which therapeutic problems might be better solved by replacing, rather than repairing, a function (akin to surgical replacement or reconstruction). For example, consider a disease in which a gene involved in the early development of a tissue is disrupted. In the context of a mature organism, replacing this gene in a stem cell or pharmacologically interfacing with it would likely be useless, given that the critical developmental period has past, that is, the context of morphogens that guide development is likely long gone. In this case, it may be more effective to try to reinvent development—for example, to design a stem cell that can recognize specific signals present on the defective tissue and to use these as a trigger for establishing new synthetic morphogen fields that can guide regeneration. In such cases of creating new functions, it must be known how to apply the principles and modules of cellular networks, but in novel, nonnatural combinations.

Outlook

Although the broader field of cell engineering is still in its infancy, several clear lessons have emerged. First, it is certainly possible to engineer novel complex biological functions: Cells can be programmed to carry out highly precise synthetic multistep and precisely localized functions in vivo, especially as demonstrated in engineered cell systems developed to treat

Box 1. Examples of potential future challenges in therapeutic cell engineering.

Homeostatic therapeutic responses: Can we engineer therapeutic cellular systems that use feedback and counteracting positive versus negative control to achieve balanced, self-correcting therapeutic responses?

Neuro- and endocrine-engineering: Can we program neural connectivity to construct self-assembling neural circuits? Can we create new diffusible regulatory systems to exert homeostatic control over diverse diseases and/or organs?

In situ regeneration: Can endogenous signals of injury or damage be leveraged to prime synthetic developmental programs?

Bidirectional integration of extracellular matrix (ECM) into cell engineering: Can we artificially induce synthesis of ECM to drive formation of physically rigid tissues (e.g., epithelia)? Can we create orthogonal ECMs that are still genetically encoded, such that the ECM can serve as both input signal and output signal?

Cell modification and manufacturing: How do we upload larger genetic programs into cells? How do we reduce costs of manufacturing? How can we make allogeneic or induced pluripotent stem cells (iPSCs) that alleviate problems of immunogenicity and/or rejection?

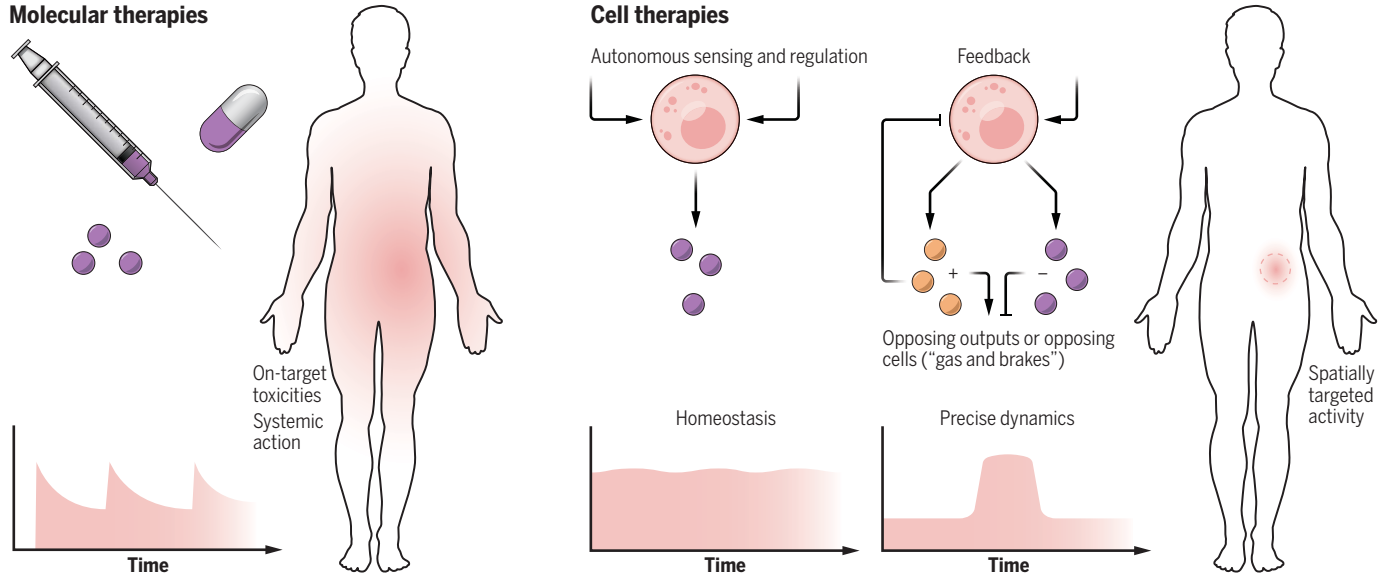

Fig. 4. Advantages of cell therapies over conventional molecular therapies. Molecular therapies (left), including small molecules and biologics, are given systemically, leading to toxicities that arise from multiple normal functions of most target molecules. Such therapies have limited dynamic and amplitude dosage control (see graphs). In contrast, cellular machines (right) can be engineered to execute a function or deliver a molecular payload in response to very specific sets of inputs, allowing for much more spatially targeted therapeutic action (with reduced toxicities). Additionally, cells can, in principle, coordinate multiple opposing outputs. This type of "gas and brakes" control is observed in most examples of natural homeostatic regulation. Feedback and cross-regulation in such systems could thus yield engineered therapeutic actions that are far more precise, robust, and autonomously controlled.

cancer. Cellular systems are generally far more flexible to rewiring than had been previously thought. Second, many of these new functions have been enabled by engineering new cell–cell connectivities. Extracellular signaling systems are, by nature, highly modular, and we can exploit this modularity to create user-specified orthogonal cell–cell communication systems. Third, there is growing appreciation that a key to accelerating advances in cell engineering is the development of a more comprehensive and robust tool kit of parts for rewiring fundamental cell behaviors. These cell–cell communication and interaction components are highly universal in their function, such that many of them are likely to prove useful in diverse future areas of application, well beyond oncology and inflammation. Regeneration and development represent such an area in which there is a growing interest in using these types of cell engineering tools to reprogram self-organization. Fourth is the recognition that rather than simply fixing or redirecting natural cellular programs, cell engineering offers the possibility of deploying new combinations of modular cellular responses to construct complex nonnatural cellular systems that can solve challenging medical problems.

Perhaps most importantly, trying to solve these problems in engineering higher-order functions forces us to grapple with the underlying logic and hierarchy of biology (*32*). The approaches of the molecular biology era have tended to focus on identifying and studying the genes or genetic elements involved in a process or disease. But while such information is critical to know, it alone does not necessarily give a holistic multiscale understanding of how a system works or a concrete and actionable path to treating a disease. Genes can only exert their functions in the context of living cells, and what may be most relevant is ultimately how those genes alter the interactions that a cell participates in. Thinking about parsing biological function through the lens of a more cell-centric perspective may help unleash the ability to better interface with and to modulate complex biological systems.

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