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Engineering multicellular systems: Using synthetic biology to control tissue self-organization

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Abstract

The control of multicellular systems in general and of tissue formation in particular is a frontier for regenerative medicine and basic biological research. Current manipulations of multicellular systems such as tissue engineering, *in vitro* organoid development, and stem cell differentiation are revolutionizing the field, yet remain confronted with difficulties controlling precision, complexity, and functional integration. New methodologies and tools are needed to address these issues before the ambitious goal of building complex, customizable organs and tissues can be achieved. One promising approach is starting to make gains in this area: the genetic engineering of cellular signaling to directly or indirectly affect cellular self-organization. This review will focus on genetic manipulations that make use of, and/or are modeled after, the self-organization programs that multicellular systems use during development and regeneration. In particular, current examples and future directions of the following three areas will be explored: (i) Engineering developmental trajectories in non-developmental systems, with an example for epithelial patterning; (ii) Engineering control in developmental systems, with an example of increasing cellular composition complexity in stem cell differentiation; (iii) Engineering regeneration in non-regenerating systems, with an example from limb regeneration with engineered cells. The use of synthetic biology to control the genetic layer of these three areas will undoubtedly uncover important rules dictating cellular self-organization, putting us one step closer to a powerful approach for building multicellular systems, one we will call **synthetic tissue development**. In the future, we anticipate that convergence of this approach with more established approaches to multicellular system control will lead to improved functional tissue formation *in vitro* and the possibility of transformative advances in regenerative medicine.

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Introduction

Building tissues is a fascination of modern biomedicine that is evident in the rapid expansion of tissue engineering for transplantation and regeneration, stem cell based therapies, advances in organoid development, and organ-on-a-chip disease modeling [1,2]. These revolutionary approaches face challenges in precision, complexity, control, and functional integration [3–5]. These are formidable challenges, as cells do not often behave in predictable or easily controllable ways. New approaches are therefore necessary to accomplish the lofty goal of building complex, customizable, self-organizing organs that can seamlessly replicate or augment endogenous organ function and integrate with host systems.

Yet, a technology that builds tissue with high precision already exists naturally: embryonic development. During embryonic development, undifferentiated progenitor cells are directed to build all the various tissue types of the mature organism. As revealed by developmental, regenerative, and stem cell biology, many of the programs that direct this self-organization are encoded at the genetic level in the form of gene regulatory networks and cell signaling cascades. It has been proposed that synthetic biology approaches at this level could be transformative [6,7]. Until recently, the tools necessary for this kind of direct genetic engineering and control were unavailable, but recent technological advances, deepened scientific understanding, and social paradigm-shifts have created an opportunity for the growth of an entirely new engineering discipline focused on multicellular systems [8–10]. Specific synthetic biology tools such as synthetic receptors, synthetic transcription factors, and engineered communication pathways are described in more detail in the Toolkit section.

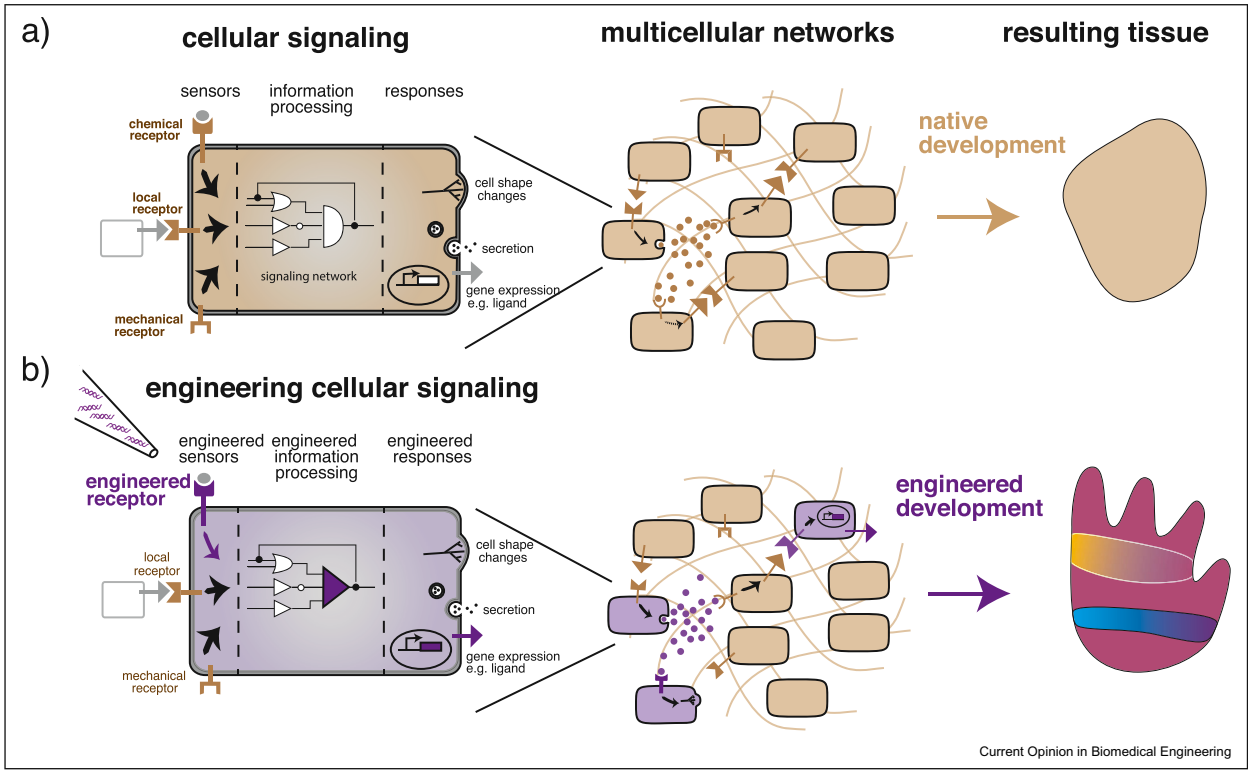
Recent technological advances in genetic manipulation have provided a favorable background for engineering

multicellular systems. Of particular importance are genetic manipulations that allow for either the stable introduction of large **exogenous** signaling circuits in the genome or the ability to engineer changes directly in **endogenous** loci to rewire native signaling. Synthetic DNA of even very large size can now be produced relatively cheaply and landing pads can be used to integrate these larger synthetic constructs into the genome in site-specific ways [11]. Entirely artificial chromosomes can also be used as carriers of exogenous DNA, either alone or in combination with transposon-based technology [12]. In addition, advances in CRISPR based technologies have allowed for unprecedented manipulation of endogenous loci allowing both genetic replacement and nuanced gene control [13].

Concurrently, deepening biological knowledge of the principles relating to cellular differentiation have led to a greater understanding of the plasticity of cell fate via reprogramming [14] and to the development of protocols for *in vitro* organoid generation [15]. Many points of genetic control on cell differentiation have been identified and can be used as targets for the creation of synthetic circuits driving changes in cell fate. Recent breakthroughs in tumor immunotherapy and the FDA approval of CAR-T cells have changed the social landscape and have had the bystander effect of lowering perceived risks of engineered cells in the clinic [16].

Taken together we believe a new discipline is being defined. Described alternately as synthetic development, synthetic morphogenesis, or tissue development engineering, we will call it **synthetic tissue development.** Synthetic tissue development is based in the tools of synthetic biology and in the scientific underpinnings of developmental biology and it can be defined broadly as

Fig. 1



Cell signaling networks as the key to engineering multicellular self-organization. a) Representation of a native cell (represented as beige throughout this review) as a computational unit made up of sensing, processing, and response subunits. Receptors capable of sensing secreted chemicals, locally bound ligands, mechanical changes, or other environmental cues are an example of the sensing subunit of cells. An information processing subunit then uses signaling networks to transform the incoming signal into a response. Responses are computational outputs and can take many forms such as alterations of gene expression leading to morphological changes, molecule secretion, ligand production, or a variety of other behaviors. In a multicellular context, the computational output of any one cell both influences and is influenced by its neighbors. Each cell both senses and responds and the collection of cells creates its own signaling network. These complex networks generate, at the multicellular level, the emergent properties of self-organization, patterning, morphogenesis, differentiation, and decision-making that ultimately combine to build complex tissues. **b)** Engineering efforts (represented in purple throughout this review) can be directed towards modifications of cell sensing, processing, response or any combination of these subunits to suit the needs of the engineered system. In a multicellular context, the presence of even a few properly engineered cells can then change the computational output of the system as a whole. Engineering cell signaling at the level of the individual cell can thereby result in increased complexity and control in the multicellular context of tissues. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)