

REVIEW

Combination of versatile platforms for the development of synthetic biology

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Background: Synthetic biology has attracted enormous attention in recent years. A key focus of synthetic biology is to utilize modular biological building blocks to assemble the cell-based circuits.

Results: Scientists have programmed the living organisms using these circuits to attain multiple, delicate and well-defined functions. With the integration of tools or technologies from other disciplines, these rewired cells can achieve even more complex tasks.

Conclusions: In this review, we will focus on the recent achievements in new materials and devices assembly, next generation therapeutics development and versatile manufacturing by combining the synthetic gene circuits, various tools and technologies from multiple fields, such as printing technology, material engineering and electronic engineering.

Keywords: synthetic biology; material engineering; printing technology; electronic engineering

Author summary: Synthetic biology has achieved large strides in recent years. In this review, we discuss the integration of synthetic biology with technologies from multiple fields including printing technology, material engineering and electronic engineering.

INTRODUCTION

With the development of systems biology, a rigorous engineering biological branch was raised to create, control and program cellular behaviors, resulting in the field known as synthetic biology in the late 1990s. In the past two decades, synthetic biology has undergone remarkable growth in scope, expectation and output as a well-known discipline [1]. The roots of synthetic biology can be dated back to the insights and envisions of *lac* operon in *Escherichia coli* system [1,2]. In the beginning of the year 2000, two pioneer studies reported the engineering of genetic circuits to program the cells with desired functions [1]. Collins and colleagues constructed a genetic toggle switch in *E. coli* and provided a simple theory that predicted the conditions necessary for biostability, while Elowitz and Leibler constructed a repressilator with a triple negative-feedback loop of

sequential repressor–promoter pairs to build an oscillating network in *E. coli* [3,4]. These pioneer studies play a significant role in testing and engineering synthetic cellular behaviors using the minimal design principles postulated by mathematics equations and computational simulation, which may lead both to the engineering of new cellular behaviors and to an improved understanding of naturally occurring networks.

In the relevantly early stages of synthetic biology, the artificial gene circuits were mostly designed and created in prokaryotes and some simple eukaryotes, such as *E. coli* and yeast, including toggle switches, oscillators, timers, counters, clocks, pattern detectors, band-pass filters, and intercellular communication systems [5–14]. The gene circuits provide cells with new biological behaviors, including dynamic gene expression and logic control [15–17]. Connecting smaller functional circuits, higher-order networks could be realized with predictable

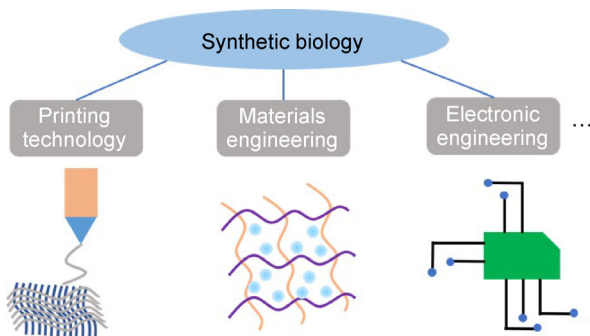


Figure 1. Synthetic biology integrated multiple disciplines to achieve various applications.

behaviors [18]. The *de novo* design of genetic circuits with biological modules for synthetic pathways is beginning to address crucial issues like biosensors, diseases therapy, green fuel, cheaper drugs and natural products in practical trials [19–24]. Focusing on “design, construction, and characterization of biological systems using engineering design principles” [25,26], synthetic biology by far has accumulated tremendous tools and engineered cells ranged from *E. coli*, *Bacillus subtilis*, yeast, drosophila to mammalian cells [27–33].

Despite of increasing sophisticated gene circuits, attaining complex tasks remains a big challenge for engineered cells themselves. For instance, although the engineered cells can develop a defined pattern from a single cell or a cell colony, it is still hard for cells to align themselves to assemble a functional material [34,35]. In another example, scientists have engineered cells with sensing and responding capability so they can release the therapeutics at a certain condition. However, these engineered cells need further confinements: to restrain the cells mobility and also protect them from the immune system [36]. Therefore, the integration of tools between synthetic biology and other technologies will benefit the scope and depth of the application as shown in Fig. 1. In this review, we will focus on these recent integrations and their achievements in the production of new material and devices, the development of next generation therapeutics and versatile manufacturing.

INCORPORATION WITH PRINTING TECHNOLOGY

The inkjet printers have been widely used in our daily life. Due to its simple set-up, cost effectiveness, and non-contact nature, inkjet printing technology has become one of the popular ways for micro/nano fabrication [37]. It has also been introduced to the synthetic biology field for the printing of bacterial living materials owing to its more accurate control, higher precision and faster processing

speed [38,39]. In a recent study, Cao *et al.* used inkjet printing to assemble the programed cells to build up a pressure sensor. They programed the cells using the genetic circuit so these engineered bacteria could grow and form a dome structure on a permeable membrane autonomously [40]. These dome structures were then labeled with gold nanoparticles. By pressing a pair of dome structure facing each other, the dynamic response to pressure was determined by the distribution of the gold nanoparticles, resulting in a kind of resettable pressure sensors responding to the pressure intensity and duration.

The three-dimensional (3D) printing technology, also known as additive manufacturing, is an advanced technology for rapid prototyping. On the basis of digital model, 3D printing constructs objects in a layer-by-layer fashion using adhesive materials such as powdered metal or plastics. It has been widely used in a variety of fields including industrial design, architecture as well as medical engineering [41–45]. Using the 3D printing technology, the engineered biofilm-forming cells can be printed to construct an ordered structure with versatile functionalities. *Bacillus subtilis*, the gram-positive bacteria can form the biofilm with the TasA amyloid export machinery on various surface. The extracellular protein TapA acts as a protein nucleator for the TasA assembly, resulting in the formation of amyloid fibers. Zhang *et al.* presented a kind of functional cellular glue formed by *Bacillus subtilis* biofilms [46]. The rewired cells integrated three natural marine adhesive system including barnacle, mussel and sandcastle worm. The adhesive system contained an engineered hydrophobin-like protein and an engineered amyloid protein functionalized with a mussel foot protein, inducing tight associated bacteria in extracellular matrix. The study points out a new method for the preparation of smart living glue.

In a following study by Huang *et al.*, they took use of 3D-printing technology to print the engineered bacteria. In these engineered bacteria, the TasA domain was genetically fused with different functional domains, including His-tag and MHETase, so the programmable nanofibers could be modified with gold nanoparticles, or catalyze the biodegradation of organophosphate pesticides. With the help of 3D-printing, the engineered bacteria could be constructed to versatile morphologies [47].

The 3D printing technology provides an efficient method to manipulate the assembly and shape of bacteria as living materials [48]. In a recent study by Schaffner and co-workers, 3D printing was used to approach the creation of bacteria-derived functional materials, combining the natural diverse bacteria and the shape design freedom of additive manufacturing together. Two types of “living materials” that are capable of degrading pollutants and producing medically relevant bacterial cellulose,

were printed using embedded bacteria as a functionalized 3D printing ink [49]. Moreover, in the publication by Lehner *et al.*, a novel methodology was developed. The capability of bacteria was applied to new materials with the reproducibility and tailored advantage. A commercial 3D printer was modified and new alginate-based bioink chemistry was developed for printing bacteria. Printing temperature, printhead speed, and bioink extrusion rate were all adapted and customized to maximize bacterial health and spatial resolution of printed structures, leading to a sustainable way for the production of numerous new materials [50]. In another study by González, a new tool was created to provide living cells in materials with continuous water and nutrients [51]. A 3D printer was used to mix agarose hydrogel with living cells to build 3D objects. *Bacillus subtilis* spores were printed within the hydrogels and germinated on their external surface. The material was tolerant to a series of tough conditions including desiccation, solvents, osmolarity, pH, ultraviolet light, and γ -radiation. The programmed cells in the materials were able to produce chemicals on demand or give response to stimuli, illustrating the applications of living functional materials resistant to environmental stresses.

COMBINATION WITH MATERIAL ENGINEERING

Materials engineering is as old as human history but updates quickly with the exploding of human knowledge. New materials, such as organic or inorganic materials, have always been among the greatest achievements of every age. In the past few decades, polymer material, especially hydrogels have attracted much attention due to its versatility and biocompatibility. Hydrogels are polymeric materials formed by cross-linked three-dimensional (3D) network. Owing to its unique structure, porosity and water content ability at high level, hydrogels are usually used as ideal matrices for cell encapsulation, controlled release of biomolecules, drug delivery, and formation of scaffold in tissue engineering [52–56]. Additionally, hydrogels have the ability to response to external stimulus by shrinking or swelling, such as temperature, pH, ionic strength, and light [55–59].

In a recent publication by Collins and coworkers, they presented a series of stimuli-responsive materials actuated by biological signal. A group of hydrogels containing DNA as structural or anchorable elements were developed with the ability to respond the programmable nuclease Cas12a [60]. Activated by the guide RNA-defined inputs, the DNA linker was cut by Cas12a in the hydrogels and therefore connected biological signals to material properties. Such model has been used in four different

hydrogels: branched poly (ethylene glycol) hydrogels, degradable polyacrylamide-DNA hydrogels, conductive carbon-black-DNA hydrogels and polyacrylamide-DNA hydrogel. A series of applications were presented including release of DNA, encapsulation of nanoparticles, and remote signaling.

Cell-based therapies offer a promising method for treating diseases that cannot be directly addressed by pharmaceuticals and has been considered as an outstanding method for the next generation medicines [61]. A number of designed gene circuits have been used to treat different diseases, such as cancer, metabolic disorders or immune diseases with cooperation of biocompatible materials [62,63]. Integration with material can further strengthen the system capability. For example, encapsulating the cells with the materials provides a number of supplementary functions like supporting cell viability, encapsulating substrates (*e.g.*, growth factors) and protecting cells from the hosts immune system [64]. For instance, Ye *et al.* developed a self-adjusting synthetic gene circuit to correct the resistance of insulin in mice. The programmed HEK293 cells were encapsulated inside coherent alginate-poly-(l-lysine)-alginate beads at a density of 200 cells per capsule. The mitogen-activated protein kinase (MAPK) signaling pathway was functionally rewired to produce a hybrid transcription factor. The tetracycline repressor, TetR, was fused to the human ELK1-derived transactivation domain (TetR-ELK1) [65]. A synthetic insulin-sensitive transcription control device was then constructed to distinguish physiological and increased blood insulin levels. Accordingly, the reversible expression of therapeutic transgenes was able to be fine-tuned from the synthetic TetR-ELK1-specific promoters. This device shed light on the gene and cell-based treatments of multifactorial metabolic disorders in the future.

The smart biosensing vesicles are promising platforms for the development and application of new therapeutic or theragnostic methods [66]. In a study by Ding *et al.*, a new artificial cells (ACs) was presented using liposomes made of phospholipid and cholesterol. Genetic circuits were encapsulated inside the ACs to protect cells from the extracellular chemical contexts [67]. Furthermore, these designed ACs could detect, interact with, and kill bacteria in simulated external environments with different chemical complexity, leading to a new frontier in controlling stability of artificial systems using bioinspired mechanisms.

In most of the previous studies, the engineered cells could sense the external stimulus and respond accordingly. However, this dynamic remains in one-direction since the material just functions as a physical barrier. Recently, Dai *et al.* constructed two-direction dynamics between the engineered cells and the materials and

achieved versatile biomanufacturing [68]. The core of the system acted as the microbial swarmbot consisting of a stimulus-sensitive polymeric microcapsule encapsulating engineered bacteria. By sensing the confinement, the bacteria underwent programmed partial lysis at a high local density. Conversely, the encapsulating material shrunk in response to the changing chemical environment caused by cell growth and death, squeezing out the protein products released from bacterial lysis. This platform was then integrated with downstream modules to enable quantification of enzymatic kinetics, purification of diverse proteins, quantitative control of protein interactions, and assembly of functional protein complexes and multi-enzyme metabolic pathways through division of labor. This platform demonstrated the use of cell-material feedback to engineer a modular and flexible system with well-defined functions.

The engineered bacteria can also be integrated with the inorganic material, such as nanoparticles. First discovered from *E. coli* strain in the late 1980s, Curli were found as the major proteinaceous extracellular matrix in many *Enterobacteriaceae*, which mediated cellular adhesion, aggregation, invasion and biofilm formation [69]. The outer-membrane curli-specific gene B (CsgB) proteins functioned as nucleators and guided the polymerization of the major protein subunit CsgA into curli fibrous networks on the cell surface [70,71]. Curli has also been a representative member among numerous living materials. In a publication from Lu's group in 2014, the protein-based amyloid fibrils were shown from living cells [72]. With the control of genetic cellular communication circuits, *E. coli* curli amyloid production was able to assemble into different length, leading to amyloid-based materials. The designed curli fibers were found to be interfaced with various inorganic materials, such as gold nanoparticles and quantum dots (QDs). Using the programmed materials, a number of novel devices were created, including a biofilm-based environmentally responsive electrical switch, gold nanowires and nanorods, modulation of QDs fluorescence lifetimes, together with nucleation of fluorescent QDs.

Besides binding to nanoparticles, the programmed bacteria can also be endowed with specific catalytic properties derived from those inorganic components. Wang *et al.* published a strategy to control biofilm spatially and assemble nano-objects (NOs) at the same time [73]. The programmed dynamic biofilm was formed together with the assembly of discrete inorganic NOs or hetero-nanostructures on various interfaces in a dynamic, scalable, and hierarchical fashion. Engineered *E. coli* could sense blue light and respond to produce biofilm curli fibers, leading to simultaneous formation of biofilm and NOs. QDs were patterned with a minimum resolution of 100 μm . Through controlling the NOs addition, layer-

by-layer assembly of hetero-structured thin films were achieved. In another publication by Wang *et al.*, the immobilization of nanoscale was used to overcome the limitation of toxicity and nanomaterial pollution [74]. The curli nanofiber system was genetically engineered to spatially and precisely anchor NOs. Three scalable, tunable and reusable catalysis systems were shown in this study: biofilm-anchored gold nanoparticles to reduce nitro aromatic compounds, biofilm-anchored Cd_{0.9}Zn_{0.1}S QDs and gold nanoparticles to degrade organic dyes and biofilm-anchored CdSeS@ZnS QDs in a semi-artificial photosynthesis system for hydrogen production [75]. Their work demonstrated the ability of biofilms to form scalable and complex spatial arrangements and their integration with inorganic particles for multiple potential applications.

INTEGRATION WITH ELECTRONIC ENGINEERING

Electronic engineering utilizes nonlinear and active electrical components, such as semiconductor devices to design electronic circuits, devices and their systems. The development of electronic engineering has greatly changed the way we live and work, facilitated the use of computer network and bring us a new era. The integration of the engineered cells and the electronics can potentially utilize the advantages from the both sides, such that cells can sense a certain chemical or biological signal, process the signal and give the output, while the electronics can transmit the signal and also calculate to give a final decision.

In a recent study by Shao and coworkers, a smartphone-assisted semi-automatic treatment of diabetes was created in mice that incorporated with electronic engineering, software design and synthetic biology [75]. The wireless signal was processed by a programmed custom-designed home server. The smartphone was used to control the hormone level with the help of engineered cells implanted in diabetic mice by a far-red light (FRL)-responsive optogenetic interface. The engineered cells and wireless powered FRL light-emitting diodes (LEDs) were both implanted in hydrogel capsules. The production of mouse insulin by engineered cells or a short variant of human glucagon-like peptide 1 (shGLP-1) *in vivo* was able to be remotely controlled by smartphone programs or a custom-engineered bluetooth-active glucometer in a semiautomatic, glucose-dependent manner. This study gave a stride towards engineered cell-base therapies into clinical use.

In another study by Justus *et al.*, a soft gripper was presented using engineered bacteria to detect chemicals in

the environment [76]. A flexible LED circuit was applied to the soft gripper for converting biological to electronic signals. Meanwhile, soft pneu-net actuators were induced to convert the electronic signals to the movement of the gripper. Results showed that the bio-LED-actuator assisted the gripper to detect chemicals by applying pressure and releasing an IPTG-submerged hydrogel. The gripper then gave actionable decisions according to chemical sensing and feedback during a pick-and-place operation, shedding light on the integration in soft matters, synthetic biology, and interfacial robotic systems.

Using synthetic biological tools, researchers created new methods to detect diseases. Mimee *et al.* constructed a miniaturized, fully integrated, wireless readout capsule for targeted sensing of bleeding in the gastrointestinal tract based on environmentally resilient biosensor bacteria and miniaturized luminescence readout electronics. They first engineered probiotic bacteria so it could sense the heme liberated from lysed red blood cells. The lysed situation was detected through a synthetic promoter regulated by the *Lactococcus lactis* heme-responsive transcriptional repressor and an outer-membrane transporter that allowed for the transit of extracellular heme through the cell envelope. The engineered probiotic gave the luminescence as the output. Accordingly, the scientists assembled the device combined nanowatt-level time-based luminometer chip with a microprocessor, wireless transmitter, and a set of phototransistors inside a molded capsule. Bioluminescence from activated cells was sensed by phototransistors. The detected luminescence signal was then converted to a digital code by the low-power luminometer chip and transmitted wirelessly outside the body for calibration, display, and recording. Consequently, an ingestible micro-bio-electronic device (IMBED) was constructed. In the following, the swine was treated with administer blood in neutralization solution. After the deposition of capsule, the signal was detected and sent to a commercial receiver connected to a laptop or a cellular phone via wireless transmission. The designed biosensors gave an accurate diagnosis of gastrointestinal bleeding in swine, providing a new sight for gastrointestinal biomarkers discovery [77]. This integration of biological engineering and semiconductor electronics offers opportunities to transform diagnosis, management, and monitoring of health and diseases.

SUMMARY AND PROSPECTIVE

The integration of synthetic biology and tools from versatile disciplines together has brought the cells with more delicate functions and provided new insights for various critical issues, including novel materials, next generation medicine and advanced molecular devices. Although industrial or clinical trials are still pending for

many of these applications, we believe that the integration between synthetic biology and various disciplines will shed light on multiple fields in our daily life, or even change it fundamentally.

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COMPLIANCE WITH ETHICS GUIDELINES

The authors Baizhu Chen and Zhuojun Dai declare that they have no conflict of interests.

This article is a review article and does not contain any studies with human or animal subjects performed by any of the authors.

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