Synthetic Biology Cellular Devices: Mammalian Cell Based Theranostic Systems

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Introduction

One of the many fields that has 'come of age' with the acceleration of engineering methods in biology has been synthetic biology. While this term is exceedingly broad and has many multidisciplinary applications in a variety of other fields as well, it has generally come to refer to the engineering of organisms from basic molecular building blocks found either in nature, or synthesized de novo. To date, synthetic biology approaches have been applied successfully towards a myriad of problems ranging from agriculture, biofuels, food, and plastics (Slomovic 2015). However, most importantly for patients with complex diseases that are currently untreatable using current therapeutic strategies, synthetic biology approaches represent a powerful and promising therapeutic platform that can be used to treat more sophisticated disease pathologies (Kojima 2020).

Synthetic biology cellular devices are one particular platform onto which human designed control systems have been able to impact biological environments. Using cell components like DNA, RNA, and proteins, gene regulatory circuits that mimic electrical circuits, logical functions can be built into cells, and are able respond to various cellular stimuli and produce various outputs. These cellular 'programs' can be specifically designed and tailored towards specific diseases and even specific patients, allowing exceedingly precise and targeted treatments (Bai 2016, Saxena 2016).

One approach to implement these cellular devices is through mammalian-cell-based systems. A significant advantage of using mammalian-cell-based systems is the availability of a larger variety of biomolecular components such as membrane receptors or signaling molecules that can be used to sense input molecules. These components allow for the development of more sophisticated and programmable therapies. Viral and bacterial systems, on the other hand, are usually dependent on transcription factor based switching that can sense only the presence or absence of small molecules. Further, mammalian-cell-based systems are expected to be safer than viral or bacteria based systems due to their lower immunogenicity and higher compatibility with the human body.

Specifically, these cellular devices can be implemented as cell-based theranostic devices, which are a combined diagnostic and therapeutic treatment approach that can sense disease markers and generate therapeutic molecules in response (Kojima 2020). These theranostic devices can maximize therapeutic effects while minimizing any undesired side effects (Kojima 2016), as the therapeutic molecules are generated in the vicinity of the target, and avoid any unwanted targeting of otherwise healthy tissue (Haellman 2017). Cell-based theranostic devices respond specifically to the disease microenvironment in situ, generating a therapeutic response in a targeted manner. Appropriate treatments for each patient may be different and cell-based theranostics allows for personalizable treatment that is tailored for each individual. Theranostic systems, once implanted, can be engineered to deliver automatic treatments, and thus do not need continuous external treatments. Thus, chronic disease such as those related to inflammation, and

control systems based diseases, such as those related to metabolism, represent promising initial use cases where such cell based systems could have an immediate impact (Kojima 2020).

Cell based systems exhibit inherent scalability, as cells are mobile, highly sensory, and capable of producing a variety of biomolecules (Haellman 2017). As a result, they are strong enablers of specific and targeted delivery of therapeutic payloads that can be tuned using a variety of structural, molecular, and cellular environmental prompts. While this provides a wealth of available options for disease pathologies to target, it also introduces significant risks, such as immunogenicity, dysregulation of circuit function, or off target effects. Effectively controlling and mitigating these risks while preserving functionality of these cells will be central to the future success of this platform (Korima 2016).

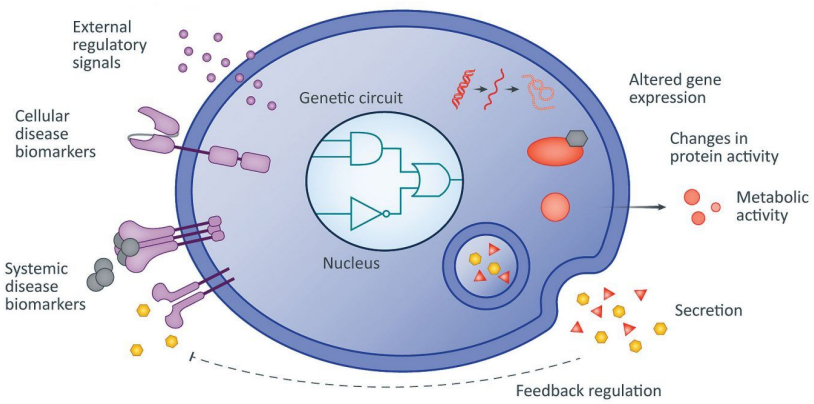

Figure 1: Depiction of a model programmable cell[1]. Cell based theranostics can take in a variety of input stimuli and through programmable genetic circuits, produce a regulated set of outputs. Common input stimuli may include disease biomarkers, while common outputs might include secreted proteins.

Composition of Cell Based Theranostics

Cell based theranostic systems utilizing synthetic biology can be broadly broken down into three component systems: the control logic, the hardware and cellular architecture, and the delivery and encapsulation system. Each component must be specifically engineered according to the target disease pathology and requires significant reliance on existing understanding of disease biology and microenvironment conditions (Kojima 2020).

The gene circuitry responsible for the control logic is the foundation for the rationale of using cells as theranostic systems. The sophistication offered by cells and their existing signal transduction architectures provide a substantial baseline onto which genetic engineers can augment with new functions (Haellman 2017). Current research work has investigated methods for manipulating genetic architectures to create complex logic gates and methods for basic computation. For example, Matsuura and colleges developed AND, OR, NAND, NOR, and XOR gates using microRNA (miRNA) and protein-responsive mRNAs as decision-making controllers (Matsuura 2018).

[1] Figure edited from Senti Biosciences

However, while there has been progress in developing gating technologies, these are often case specific, and gating technologies that are successful in certain cell lines may not have significant activity in other cell lines from different organs (Ye 2017). Central challenges to genetic circuitry are the entropy driven nature of intracellular signal transduction, natural biological variation between cells, and the effects of natural interplay between cells and their microenvironment (Haellman 2017).

In addition to genetic circuitry within the cell, the inputs and outputs of these genetic circuits are equally important. Cells can be thought of as Input/Output machines where the programmed inputs to the cell and outputs created by the cell are how they interface with their cellular microenvironment. Cells are highly communicative, not only in their local neighborhood but potentially also far away at various sites throughout the body, with tremendous variety in communication systems ranging from receptor based mechanisms to exosomal systems (Kojima 2020). Thus, it is essential to closely monitor inputs and especially outputs, in order to ensure these devices have minimal off-target effects. Both inputs and outputs of cellular machines are highly varied and are summarized in Table 1.

Table 1: Inputs, programs, and outputs of programmable cells[2]

Inputs	Programs	Outputs
Antigens	Optimized Expression	Cytokines
Chemokines	Logic Gates	Antibodies
Cytokines	On/Off Switches	Proteins
Hormones	Analog Rheostats	Transcription Factors
Transcription Factors	Kill/Safety Switches	microRNAs
microRNAs	State Machines	Peptides
Gases	Memory	Reporters
pH	Genetic Erasers	Hormones
Small Molecules	Counters	
Proteins	Feedback Circuits	

Current Development and Implementation Strategies

Current development and implementation of cell based theranostics is highly variable with many steps requiring customized experimentation and validation. However, a generalized workflow described by the Fussenegger group (Ye 2017) is described as follows:

1. Large-scale manufacturing of patient-specific designer cells
2. Frozen storage of the designer cells, either before or after encapsulation inside a vascularizing immunoprotective container

[2] Adapted from Senti Biosciences

3. Implantation of the encapsulated designer cells, preferably subcutaneously where they can easily be replaced at regular intervals by minimal ambulant intervention in the event of fibrosis

The first step has taken advantage of the vast acceleration of gene and cell therapy development over the past decade. There are now a variety of methods of stable transfection of DNA to cells such as the Sleeping Beauty transposase expression vector, that is able to integrate a DNA sequence into host cell chromosomes (Ye 2017). This allows cells to stably express the required genetic circuitry without repeat transfection. Vector design has been significantly accelerated by advances in sequencing and the availability of sequencing data (Dunbar 2018).

The success of the second and third steps in the workflow will significantly impact logistics and the potential for theranostic devices to successfully translate as a clinical therapy. 'Off the shelf' designer cells that are able to be thawed and implanted must be thoroughly validated using trials for immunogenicity and viability (Kojima 2020). More immediate application of cell based theranostics may be modeled after infrastructure for other cell based systems such as CAR-T and stem cell transplants. Maintenance of cells long term will be another important design consideration for longer term studies, in the event of cell death, fibrosis, or escape from implantation sites (Haellman 2017).

Theranostic Systems for the Treatment of Metabolic Disease

Cell based theranostics are uniquely suited for the treatment of diseases with complex dynamics or control systems due to their ability to immediately respond to changes in homeostasis (Ye 2017). Metabolic diseases such as diabetes mellitus are a strong initial use case given the fundamental causes of the disease being rooted in dysregulated control systems (Rössger 2013, Krawczyk 2020). Diabetes mellitus is a disease characterized by high blood glucose, caused by dysregulation of insulin production or sensitivity (Kharroubi 2015). In Type 1 diabetes patients, pancreas cells are unable to secrete enough insulin due to β cell dysfunction. In Type 2 diabetes patients, normal cells develop a resistance to insulin, preventing glucose from being absorbed by normal tissue. In each case, there is an opportunity for theranostic devices to synthesize compounds in situ to reverse the effects of disease.

One method for addressing this problem has been the development of theranostics mimicking β cells to alleviate symptoms of β cell dysfunction in diabetic mice. In 2016, Xie and colleagues described a method of pairing glycolysis-mediated calcium entry to an excitation-transcription system controlling transgene expression of therapeutic insulin production (Xie 2016). The authors devised a switch mechanism, transducing an input of high glucose concentration, into production of insulin using a synthetic promoter construct. The mechanism of action took advantage of the native calmodulin calcineurin signaling cascade to transduce surface signaling to the cell nucleus. At the end of the cascade, the authors designed a promoter for insulin production that was activated only by hyperglycemic extracellular conditions. Implanting

these designer cells covered in an immunoprotective covering intraperitoneally into mice, the authors were able to observe a dose and time dependent normalization of insulin in Type 1 diabetic mice comparable to wild type concentration levels.

The same group in 2017 published research describing a designer cell system to combat insulin resistance, the other half of the diabetes problem (Ye 2017). The authors developed a similar system in that they utilized a native intracellular IRS-1–Ras–MAPK signalling cascade and paired it with synthetic transcription factors and promoters designed to produce a desired regulatory output hormone, adiponectin. In addition, they engineered the TetR-ELK1 transcription factor such that it could be diabled by a commercially available antibiotic, doxycycline. The adiponectin producing cellular devices were responsive only to high levels of insulin and were able to produce long lasting insulin sensitization, along with reduced caloric intake, weight loss, lowered cholesterol, and reduced serum insulin in treated obese mice. Though the final device was developed in HEK-293 cell lines, the circuit was expressed and could successfully discriminate high versus low insulin in CHO, HeLa, hMSC, hMSC-TERT cell lines as well. Thus, a scalable genetic circuit was developed that is capable of reversing diabetic disease markers in mice.

Theranostic Systems for the Treatment of Inflammatory Diseases

Another ideal systems based target for cell based theranostics are inflammatory diseases. Inflammation is typically useful when mounting an immune response against pathogens and is able to alert and recruit immune system agents to the site of infection. However, dysregulated inflammatory responses, especially those developed against self-antigens can become harmful (Chen 2018). The rapid and excessive production of pro-inflammatory cytokines is characteristic of several autoimmune diseases such as inflammatory bowel disease, rheumatoid arthritis, systemic juvenile idiopathic arthritis, and psoriasis (Smole 2017). Current treatments for inflammatory diseases include non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids or other immune response neutralizers and suppressors (Chen 2018). However, this could lead to non-ideal long term effects such as decreased immune response to infections and cancer (Smole 2017). Due to this, there has been a need for a therapeutic that can be activated only when an inflammatory flare up is detected, and suppress the flare up before they are amplified through positive inflammatory feedback loops and cause damage (Smole 2017).

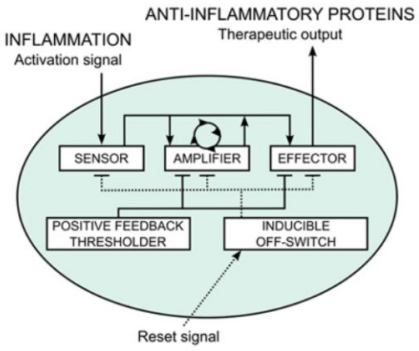

Figure 2: Circuit to sense and suppress inflammation. Developed by Smole et al with a sensor, amplifier, threshold for positive feedback, and an inducible off-switch.

A synthetic cellular device can be constructed to serve as a theranostic system for treating inflammatory diseases. A proposed genetic circuit achieving this goal consists of a sensor (to

detect inflammatory agents), an amplifier, a thresholder (to restrict overactivation of the positive feedback loop), and an effector to produce therapeutic proteins (refer to Fig. 1).

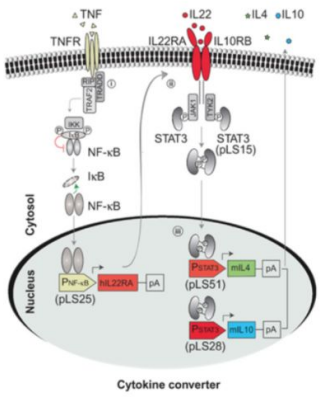

Figure 3: Schematic of the cytokine converted designed by Schuker et al. Two sequentially interconnected cascades quantify TNF and IL22, process their relative presence with AND-gate logic, and program the adjusted production of the anti-inflammatory cytokines IL4 and IL10

Another group developed a synthetic cellular device for the detection and treatment of psoriasis, following a similar network to the graphical representation from Smole et al. Psoriasis is a chronic inflammatory disease affecting the skin, and is characterized by unpredictable flare ups of proinflammatory cytokines such as tumor necrosis factor (TNF) and interleukin 22 (IL22) (Armstrong 2020). There is currently no cure for psoriasis, and treatments remain challenging due to the recurrent nature of the disease. Clinical trials using immunomodulatory cytokines IL4 and IL10 have previously shown rapid patient improvement; however, the short half lives of these immunomodulatory agents require daily injections, which creates a major setback for patient compliance (Armstrong 2020). Therefore, Schuker et al. developed a cellular device capable of diagnosing increased TNF and IL22 levels characteristic of psoriasis (sensor), and producing therapeutic doses of IL4 and IL10 in response (effector).

This theranostic circuit uses AND-gate logic, to ensure that the therapeutic agents IL4 and IL10 are produced only when both increased TNF and IL22 levels are detected. This allows for accurate detection of psoriasis-specific cytokine flare ups, and reduction in production during other inflammatory events that may not be psoriasis related. This is achieved by rewiring the TNF triggered TNF receptor (TNFR) signaling through nuclear factor kB (NFkB) to a synthetic NFkB responsive promoter controlling the expression of human IL22 receptor α (hIL22RA) (Schuker 2015). IL22 activates the hIL22RA complex to start a Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling cascade. Schuker et al. rewired this signaling cascade by connecting ectopically expressed human STAT3 (pLS15), which is a compound produced from the signaling cascade, to a synthetic STAT3-responsive promoter driving expressions of IL4 and IL10 (refer to Fig. 2 for schematic) (Schuker 2015). Chaining the TNF and IL22 activated cascades provides the AND-gate logic required of the circuit to ensure that IL4 and IL10 are produced exclusively at the onset of a psoriatic flare.

To test the circuit, Schuker et al. implemented a secreted embryonic alkaline phosphatase (SEAP) expression vector driven by a synthetic STAT3 promoter (Schuker 2015). SEAP levels were measured with various levels of TNF and IL22. Figure 3 illustrates the effectiveness of the AND gate, while figure 4 demonstrates that the AND gate expression is reversible and can be turned on and off by repeated addition and withdrawal of the TNF/IL22 inducer set (Schuker 2015).

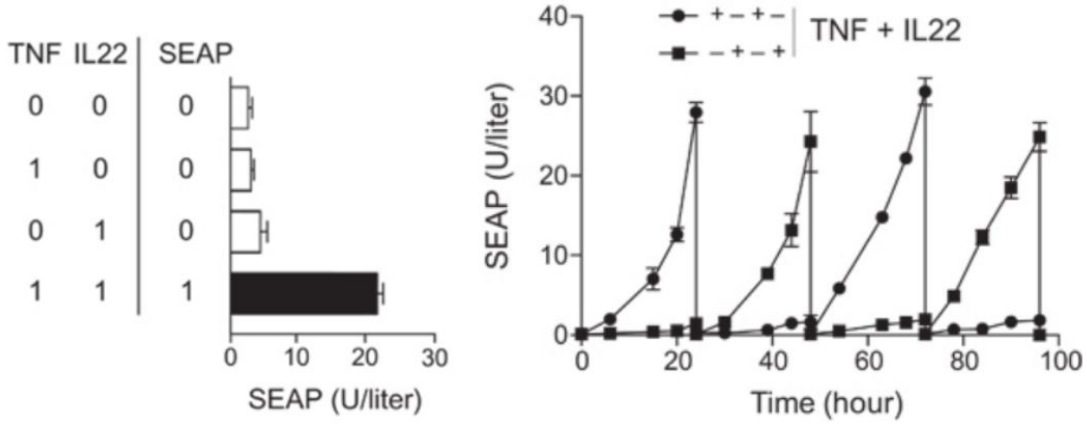

Figure 4: SEAP Production can be tightly controlled with AND gate like behavior. On the left panel, Schuker and colleagues demonstrated SEAP production only in the context of both TNF and IL22. On the right panel, they were also able to demonstrate switch-like behavior in response to on and off signals (withdrawal or exposure to TNF + IL22). Interestingly, off signaling was more sensitive than on signaling.

The synthetic cellular device for the treatment of psoriasis was implemented in a mouse model, with promising therapeutic results. The effects of implementing the cytokine converter gene circuit include preventing psoriasis-like plaque formation and attenuating established psoriasis-like plaques (Schuker 2015). This demonstrates that, at least in a murine model, this synthetic cellular device can detect and reduce the symptoms of psoriasis. In following human experiments, Schuker et al. found that their AND-gate circuit can successfully detect the proinflammatory cytokines TNF and IL22 in blood samples of psoriatic patients (Schuker 2015).

Limitations

Although current cell systems have made significant progress over the past decade, various challenges including sustained delivery, safety, and reliability will have to be addressed before clinical application can take place. As a genetically modified cell based system, cellular theranostics will need to be thoroughly validated and regulated to ensure safety as well as efficacy over long periods of time. Once a therapeutic candidate has been identified and developed, it will need to be approved by a regulatory body. In the U.S., this will involve submitting and getting approved via a biologics license application (BLA) and multiple rounds of clinical trials (Mendicino 2019).

On the technical side, sustained delivery of the cellular devices to sites of disease has emerged as a central design problem. Cells can be encapsulated and implanted surgically via hydrogel (Courbet 2015) or alginate beads (Bai 2016) that are auto-vascularizing and immunoprotective. However, no studies have been conducted yet in humans, leaving significant questions about durability and scalability past murine model systems. The specificity and reliability of these devices are another key consideration that is currently limiting clinical

translation. Specificity towards disease biomarkers especially over time is especially important to monitor considering that treatment with such devices is not transient, but rather sustained over long periods of time. Thus, important long term trials need to be conducted in order to ensure that off-target effects are minimal and that the delivered cells can remain therapeutically beneficial over a treatment period (Kojima 2020).

Another key issue that remains for practical applications of engineered mammalian cells is adequate quality control of the engineered cells to meet standards required for clinical use. Safety is important because these devices will be used within humans, so the engineered cells need to be guarded against unexpected failures such as mutations, circuit malfunctions, or immune rejection. Most of the theranostic agents that have been developed so far are single-input sensor, single-output secretion devices (Haellman 2017). As future theranostic agents become more complex and sophisticated, it is thus critical to carefully monitor the safety profile of such dynamic therapies.

Other than the safety of engineered mammalian cells, another important issue to consider is cost. Cell and gene therapies are already among the most expensive therapies available, potentially costing more than $400,000 for one-time treatments (Lyman 2020). This expense could contribute to long standing health disparities between those who do and those who do not have the means to afford insurance or out of pocket costs for such therapies (Lyman 2020).

Future Directions

Overall, these devices have the potential for great clinical impact. Further research into optimizing these cell based systems and implementation studies in vivo to monitor side effects, complications, and reliability of such devices will be critical to the progress towards the clinic. With rapid advances in gene editing systems, it should become possible to construct engineered mammalian cells more reliably and efficiently, and with higher safety profiles (Dunbar 2018). Supplying theranostic agents with even more sophisticated decision-making capacity so they can simultaneously process multiple disease inputs and provide multiple therapeutic outputs with the highest-possible precision will be part of the next steps in this field technologically.

While such research stage technologies mature, the current state of the art describing the most well defined system types have already begun translation into clinic. Companies like Senti Biosciences are currently conducting preclinical studies for leading candidates and are expected to start clinical trials within the next couple of years. The earliest use cases are expected to be in existing systems such as CAR-T and stem cell transplant therapies. However, Senti Bioscience's lead candidate, SENTI-101, utilizes tumor homing allogeneic cells to achieve localized expression of cytokines to stimulate immune responses against immunologically 'cold' tumors (Gonzales 2020). Regardless, cell based theranostics are at the cusp of clinical translation, providing a promising new therapeutic modality to look forward to in the coming decade.

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