Programmable Bacteria: An Emerging Therapeutic

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Are all bacteria harmful to us? Bacteria are often associated with illness and it is common to find people constantly using hand sanitizer or spraying disinfectants to kill surrounding germs. It was as early as 1673 when Antoine van Leeuwenhoek first discovered bacteria under the microscope, or as he called them “animalcules,” and made these organisms known to the world. For centuries afterwards, the relationship between bacteria and humans was studied extensively, and experimentation focused on the pathogenic properties of bacteria and their role as the source of human disease. However, in recent years the long-held view of bacteria as pathogens has been transformed by microbiome data revealing the prevalence of trillions of functional microbes in the human body. More recently, biologists have adopted the language of computer scientists and applied the same logic to living matter, allowing for the unique manipulation of bacterial behavior for a desired functionality. With the rapid advancement of microbiome research and development of biotechnologies, there is an emerging paradigm to program living matter and activate microorganisms to fight disease and benefit human health.

I. Introduction

It was in 1865 when Gregor Mendel’s experiments on pea plants seeded the beginnings of basic genetic principles, accelerating the field of genetics. In the next one hundred years, scientists would coin the word “gene,” define chromosomes, begin to understand recessive and dominant traits in accordance to Mendelian rules, and eventually discover the structure of the “molecule of life,” or deoxyribonucleic acid (DNA). In just over a century, the scientific world had been transformed by the rapid advancements in not only our understanding of human life at its core, but also by the advancing biotechnologies that allow for such fast-paced innovation.

Equipped with the knowledge about DNA and its replication, structure, and function, researchers began to experiment with cutting, pasting, and rearranging its parts. By modulating its sequence, one can alter the resulting protein and biological function. With this emerging biotechnology, a sub-discipline of genetics was formed – genetic engineering. Defined as the manipulation of an organism’s DNA, genetic engineering serves the purpose of editing genes to produce a desired characteristic. Historically, humans have indirectly modified genomes of animals and plants through domestication and the selection of seeds from high yield produce. Through selection and preference, selective pressures were created such that certain genes remained in the gene pool thus indirectly modulating future genetic code. More recently, however, genetic engineering is known as a fast-paced process resulting in a deliberately altered organism through the use of recombinant DNA, or DNA formed by combining constituents from multiple organisms.

In 1973, Herbert Boyer and Stanley Cohen performed the first recombinant DNA cloning experiment in which a gene conferring antibiotic resistance was cut and pasted into the plasmid, or circular DNA, of bacteria. This plasmid was then transformed back into the bacteria and cloned as the bacteria replicated. Bacteria served as an ideal vehicle for gene cloning due to its short replication rate of 20 minutes, and allowed Boyer and Cohen to confirm the retaining of gene function over several generations of bacterial populations (Cohen, Chang, and Hsu 1972, 2004). Furthermore, their extensively studied genetic system allowed for increased feasibility in programming their behavior. As the idea of modulating bacterial functions for human health emerged, there was a greater need for evolving biotechnologies and understanding of bacterial regulation.

Early studies by Monod and Jacob investigating lactose regulation in bacteria laid the foundation for the development of a quantitative framework of regulatory networks that govern bacterial responses to the environment (Monod and Jacob 1961). With the rapid expansion of automated high throughput sequencing technologies and improved computation in the 1990s, researchers were able to characterize a handful of networks in bacteria that could be deconstructed into simple components (Cameron, Bashor, and Collins 2014). In the year 2000, two seminal papers published in *Nature* forward engineered two modules from a few well-characterized components: a toggle switch, and an oscillator, marking the beginning of the field of synthetic biology (Gardner, Cantor, and Collins 2000, Elowitz and Leibler 2000). Soon thereafter, multiple modules, termed genetic circuits, had been designed allowing bacteria to sense light, invade cancer cells, and alter metabolic processes (Levskaya et al. 2005, Anderson et al. 2006, Dueber et al. 2009, Tabor et al. 2009). Applying these same principles to other microorganisms, synthetic biology has contributed to the development of anti-tuberculosis compounds, conversion of biomass into biofuels, environmental and biosensors, and the larger scale production of therapeutics (Khalil and Collins 2010, Weber et al. 2008, Ro et al. 2006, Fortman et al. 2008). Currently, scientists are designing and synthesizing entire genomes of bacteria by building minimal cells (Hutchison et al. 2016). In just over a decade, humans have learned how to rewrite, rewire, and reprogram genomic code to control living organisms for a myriad of applications. Below we will discuss the core principles molecular biology and technologies of genetic engineering, how to engineer bacteria, and emerging therapeutic applications for diseases, including cancer.

II. Molecular Biology of the Cell

Cells contain numerous molecules that orchestrate with one another to sustain life. On the scale of nanometers, molecular machinery called proteins carry out specific tasks within a cell. All of the tasks are processed with surprising precision and efficiency, ranging from simply capturing and transporting molecules to synthesizing complex structures and generating energy. A single micron-sized bacterium such as *Escherichia coli* (*E. coli)* contains over 4,000 types of discrete proteins that constantly work to keep the cell functioning (Keseler et al. 2005). Not only do proteins process their own tasks, but their dynamic interactions are what enables a cell - and hence a whole living organism - to adapt to a multitude of situations. In fact, proteins are constantly made and degraded to respond to a changing environment in a matter of seconds. For example, when a cell senses sugar in the environment (e.g. lactose), it can produce proteins to transport additional sugar into the cell and degrade proteins not necessary at the moment.

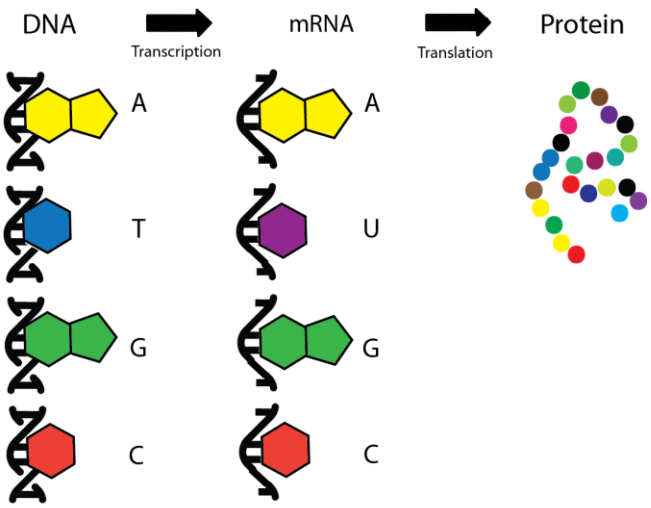

Figure 1. Central dogma of molecular biology. Double helix DNA with nucleotides A, T, C, and G, is transcribed into a single-stranded mRNA with nucleotides A, U, C, and G. The sequence is then translated into a string of amino acids that folds into a protein.

Information encoded in the cell flows from gene to protein, a process referred to as the central dogma of molecular biology (Fig.1). A cell regulates intracellular protein concentrations by reading the code of DNA and transcribing it to ribonucleic acid (RNA), which is eventually translated to protein. Simply put, all of the information necessary to perform molecular tasks is stored in the form of DNA that is copied to a temporary information storage called RNA. Proteins are made based on the RNA sequence where their parts fit together, move, and interact to perform essential cellular functions.

DNA is a genetic material that stores all the information necessary for a cell to thrive and is encoded by a sequence of four different nucleotides; Adenine (A), Thymine (T), Cytosine (C), and Guanine (G). Two strands of DNA are paired (A to T, C to G), forming a double helix. Within the entire sequence of nucleotides, or genome, there exists functional units called genes that provide instructions for each life-sustaining

task. When a cell needs to make a protein, a gene that codes for a specific protein is first “transcribed” into a single-stranded messenger RNA (mRNA). The level of gene activation, termed “expression”, is initiated by regulatory regions of DNA preceding the gene called promoters. When a cell senses changes in its environment, a complex called RNA polymerase recognizes a specific promoter and transcribes the gene by producing complementary base pairs to the provided DNA code. Chemically, mRNA slightly differs from DNA in that it contains a uracil (U) nucleotide instead of a thymine (T). However, its function differs greatly in that unlike DNA, mRNA serves as a temporary information cassette that includes additional levels of regulation as it is transported, edited and eventually discarded. Once a gene is copied from DNA to mRNA, the nucleotide sequence is “translated” via the ribosome into a protein in which every three nucleotides form a codon that corresponds to a distinct amino acid. In human cells, the protein size can range from a mere 29 amino acids like that of hormones to 34,000 amino acids like that of muscle (Bromer et al. 1956, Labeit and Kolmerer 1995). It is the diverse characteristics of amino acids that can produce a wide variety of protein functions.

III. Sequencing, Amplification, and Assembly of DNA

To engineer complex genetic circuits in bacteria, one must (1) know the DNA sequences, (2) produce enough DNA to manipulate the DNA pieces, and (3) assemble DNA together into the desired location (Fig. 2). The first step in this process is DNA sequencing. In 1977, a team led by Drs. Frederick Sanger and Walter Gilbert developed Sanger Sequencing, a then novel DNA sequencing technique that proceeded to be the most widely used sequencing technology for the next 40 years (Sanger, Nicklen, and Coulson 1977). Sanger sequencing uses a system where DNA is replicated with a portion of fluorescently labelled nucleotides that terminate replication and allow for specific size fragments to be read one by one. While Sanger sequencing became the standard method in laboratories around the world, it was slow and costly to read an organism’s entire genome. Recently, high-throughput sequencing methods termed Next-Generation Sequencing (NGS) have been employed more commonly for large-scale synthesis (Illumina 2016, Goodwin, McPherson, and McCombie 2016). In particular, Illumina Inc. developed HiSeqX™Ten, which parallelizes the sequencing process and assembles multiple sequencing results computationally. One NGS machine can read over 45 human genomes in a day, costing approximately $1,000 for each sequenced genome, making it both a rapid and cost-efficient technology (Illumina 2016).

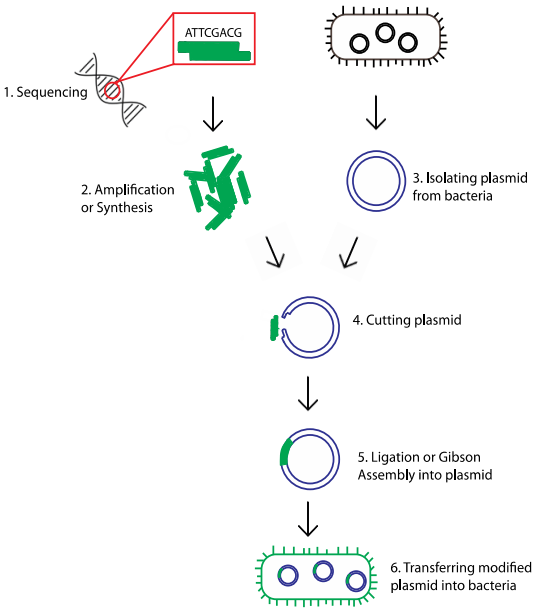

Figure 2. Overview of molecular cloning. (1) Gene of interest is sequenced or identified, **(2)** and amplified or synthesized using PCR. **(3)** Plasmid isolated from bacteria, **(4)** and cut using restriction enzymes. **(5)** Gene of interest is ligated or assembled into the plasmid, and **(6)** plasmid with genetic insert is transformed back into the bacteria. As the bacteria replicates, the gene of interest is cloned.

Amplifying DNA is a necessary step to create a sufficient amount of DNA for assembly and reinsertion into the host organism. In 1983, Dr. Kary Mullis utilized thermostable DNA replication machinery called DNA polymerase to amplify desired DNA segments (Saiki et al. 1988). The method, Polymerase Chain Reaction (PCR), separates double-stranded DNA and initiates replication using short primers of DNA that uniquely bind to a given position. With multiple reaction cycles, larger amounts of a specific DNA can be produced. A disadvantage of PCR, however, is that this method requires template DNA, and causes errors in each cycle, hence making construction of a large, reliable, novel DNA sequence challenging. As technology evolved, methods to chemically synthesize DNA sequences from constituent molecules improved exponentially, allowing for the creation of new sequences for which no template exists. By 2010, the entire genome of a bacterium *Mycoplasma mycoides* (*M. mycoides)* was designed and chemically

synthesized by a team led by Dr. J Craig Venter. By replacing the genome of another bacteria *Mycoplasma capricolum* with synthesized *M. mycoides* genome, they were able to demonstrate that DNA can be designed and printed to construct life from scratch (Gibson et al. 2010). With increasing capacity and lowering costs of DNA synthesis, it is now possible to order DNA sequences from commercial companies around the world.

With either amplified or synthesized DNA in hand, assembly of DNA into functional pieces is the next step. There are many approaches to assembling DNA. Traditionally, restriction enzymes that recognize specific sequences and cut DNA are used, and subsequently, a different DNA fragment cut with the same restriction enzyme can be joined together with a DNA ligase (Morrow and Berg 1972, Jackson, Symons, Berg 1972, Cohen, Chang, and Hsu 1972). To date, over 3,000 restriction enzymes has been discovered and many of them are commercially available, allowing researchers to cut and paste DNA into a variety of sites. While restriction enzymes have served as a standard technique for gene editing, new tools have been recently invented for faster and more accurate genetic engineering. For example, Gibson Assembly, named after Dr. Daniel Gibson at the J. Craig Venter Institute, was introduced in 2009 (Gibson et al. 2009). This method allows for an assembly of multiple DNA fragments with a single experimental step, making insertion of multiple genes more efficient. More recently, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system is emerging as a precise, effective and flexible method. CRISPR consists of two components: a "guide" that recognizes a specific sequence of DNA and an enzyme (Cas9) that cuts at the recognized site (Mali et al. 2013, Cong et al. 2013, Jinek et al. 2012). CRISPR is not limited by the sequence and therefore cut sites can be tailored to a specific gene with precision. As the tools for engineering genetic materials emerged, these successes stimulated interest in reprogramming organisms.

IV. Engineering Gene Circuits

Analogous to programming software or engineering electrical circuits, DNA sequences can be designed to introduce genes and novel regulatory networks into a cell. At the foundation of synthetic biology design is the building of genetic circuits that perform logic functions, created with two essential components: a promoter that senses an input, and a gene that produces an output. For multiple inputs, these are typically described as Boolean logic functions like AND (all inputs satisfied) and OR gates (some inputs satisfied). One of the earliest works in genetic circuit design incorporating logic functions was published in 2000 when Dr. James Collins and colleagues produced a genetic toggle switch circuit. In their work, *E. coli* was programmed to toggle between two stable states based on external cues. To do so, two genes (*lacI* and *cI*) were designed to repress the expression of each other, but either gene could be disengaged by an external stimulus, letting the unrepressed gene dominate the state of the cell (Gardner, Cantor, and Collins 2000). This allowed the cells to maintain a stable ON or OFF state after a transient induction.

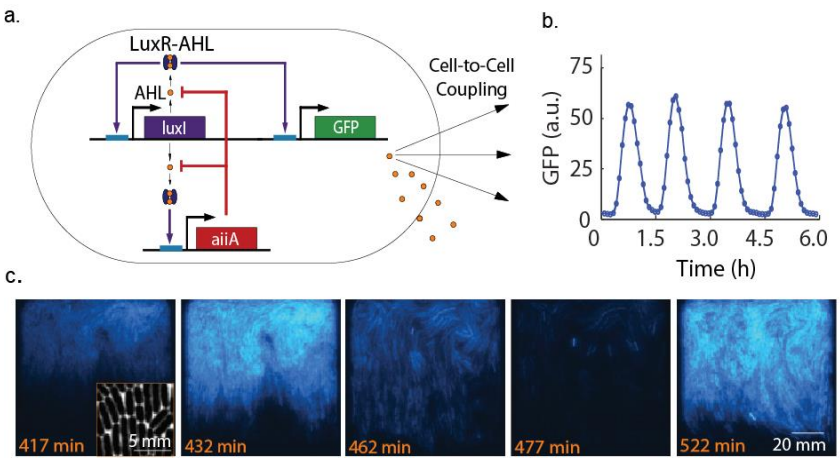

Figure 3. Synchronized genetic clocks. (a) Network diagram. The luxI promoter drives production of the three molecules (luxI, aiiA and yemGFP) in three identical transcriptional modules. LuxI produces a small molecule AHL, which can diffuse outside of the cell membrane and into neighboring cells, activating the luxI promoter. AiiA negatively regulates the circuit by acting as effective degradation machinery called protease for AHL. **(b)** Bacteria population (measured by fluorescence) as a function of time. **(c)** Fluorescence slices of a typical experimental run demonstrate synchronization of oscillations in a population of E. coli. Inset in the first snapshot is a 3100 magnification of cells.

Since then, more complex circuitry has been created. Dr. Chris Voigt has used multiple logic gates to create gene circuits that

detect light-dark edges (Tabor et al. 2009). In this edge detection circuit, unilluminated bacteria are programmed to sense the absence of light and secrete a signaling molecule, N-Acyl Homoserine Lactone (AHL), whereas illuminated bacteria receive AHL as an "input" and produce a black pigment. Because the AHL receiver cells cannot produce AHL by themselves and can only receive it from neighboring cells, a black outline is formed at the light-dark interface. Another example of a recent logic gate, is a safeguard gene circuit, known as the "kill switch" (Chan et al. 2016). In this system, the bacterial gene is designed to produce toxins to self-destruct in the absence of the molecule Anhydrotetracycline (ATc). Since ATc is rarely found in nature, this switch ensures that the engineered bacteria can only survive if provided with the supplement.

Oscillatory circuits are another type of genetic circuit that has attracted synthetic biologist's attention. In 2000, Drs. Michael Elowitz and Stanislas Leibler engineered the first oscillatory circuit termed the 'repressilator' (Elowitz and Leibler 2000). Their genetic circuits are composed of three promoters driving three repressors that cyclically repress one another. Due to the interaction between the three repressors and delay in protein production, the genetic circuit produces oscillatory dynamics, observed by fluorescent protein expression on a microscope. Since then, several other oscillators have been constructed by teams including ones led by Drs. Alexander J. Ninfa, James C. Liao, and Jeff Hasty (Atkinson et al. 2003, Fung et al. 2005, Stricker et al. 2008).

Since these oscillators functioned only within a single cell, we engineered a synthetic clock circuit that is capable of generating synchronized oscillations in a growing population of bacteria (Danino et al. 2010) (Fig. 3). In this circuit, a combination of positive and negative feedback produces and degrades AHL which can freely traverse among the neighboring bacteria and binds to the promoter to express a green fluorescent protein (GFP). This mechanism established the communication between bacteria to synchronize these oscillations across a population along with spatiotemporal waves occurring at millimeter scales.

The aforementioned works demonstrate the multiple layers of genetic circuits that can enable diverse and complex bacterial responses to environmental cues. Utilizing this system, synthetic biologists are now engineering bacteria that utilize logic circuits and sensing in therapeutic contexts. Below, we will discuss recent works in cancer as a primary example.

V. Cancer Therapy

Cancer is a heterogeneous disease caused by the uncontrolled and abnormal division of cells. It can originate anywhere in the human body and may metastasize if left untreated. The National Cancer Institute estimates that in 2016 there will be about 1.6 million new cases of cancer diagnosed in the United States and that almost 600,000 people will die from the disease (NIH 2015). With the growing number of cancer incidents per year, there is a pressing need for a clinically effective therapy. Current cancer treatments often include surgery, radiation therapy, or chemotherapy. A major limitation of these options is the treatment's inability to discriminate between benign and malignant cells thus causing further damage and side effects for patients. Thus, there is a need for a targeted therapeutic that homes to tumor cells, adequately penetrates through the tumor mass and is cytotoxic to all of the cancer cells.

Over the past century, several bacteria have been explored as a potential cancer therapy. Studies have specifically documented bacterial colonization and growth within the necrotic cores of tumors, microenvironments previously thought to be sterile (Forbes 2010). Bacteria's role as a cancer therapeutic was first noticed in 1868 by physicians W. Busch and F. Fehleisen, who observed regressions of tumors in cancer patients with bacterial infections, showing minimal harm to the patient's overall health (Oelschlaeger 2010). Then in 1891, bone surgeon, William Coley intentionally infected his cancer patients with *Streptococcus* bacteria and reproduced similar results seen in 1868 (McCarthy 2006). He hypothesized that the induced bacterial infection shrunk the malignancy by provoking an immune response. This method was initially controversial because of inconsistent results and the use of toxic bacterial strains, however, the idea later resurfaced when more was known about the tumor microenvironment and attenuated bacterial strains were available. Later studies demonstrated that attenuated *Clostridium novyi* spores showed colonization and regression of tumors over time in mice, but the treatment was difficult to standardize with effectiveness being highly dependent on tumor size

and spore dose (Dang et al. 2001, Malmgren and Flanigan 1955, Leschner et al. 2009). With less toxic bacterial strains available, bacteria was seen as an optimal therapeutic with its natural selectivity to tumors, motility to penetrate into the tumor mass, and cytotoxicity.

With the emergence of genetic engineering, there has been a shift towards bacterial hosts such as *S. typhimurium* and *E. coli* primarily due to their ease in genetic modification, allowing for strains with attenuated virulence properties such as the ability to infect macrophages, and reduced endotoxicity (Leschner et al. 2009, Zhao et al. 2005, Zhang, Gao, et al. 2007, Zhang, Yong, et al. 2007). In particular, a 2002 study demonstrated that chromosomal deletions of genes, *purl and msbB*, in *S. typhimurium* VNP20009 had the potential to facilitate bacterial targeting and colonization of tumors in patients with metastatic melanoma cancer. The modified strain was taken as far as Phase I human clinical trials showing that 10^8 bacteria can be injected safely in humans, though no efficacy was observed (Toso et al. 2002, Heimann and Rosenberg 2003). Subsequently, a more efficacious *S. typhimurium* A1-R strain was tested in animal models and showed increased tumor targeting and virulence with attenuated growth in normal tissue. Furthermore, the mice tolerance of the A1-R strain at higher doses than with VNP20009, suggests that A1-R has greater clinical potential (Zhang et al. 2015, Zhao et al. 2005, Zhao et al. 2006, Zhao et al. 2007).

In addition to engineering bacteria to more safely colonize tumors, anticancer agents have been engineered into bacterial genomes to increase their therapeutic effectiveness (Forbes 2010). Two major mechanisms studied include the direct expression of proteins with antitumor activity and the transferring of expression vectors to infected cancer cells. In both mechanisms, bacteria express an anticancer agent that is either cytotoxic or provokes an immune response. Bacterial toxins, such as Cytosolin A (ClyA) were early cytotoxic agents used because their genes are native to bacterial physiology (Forbes 2010). In particular, ClyA functions by forming pores in cell membranes and inducing apoptosis (Jiang et al. 2010, Ryan et al. 2009, Nguyen et al. 2010). Other cytotoxic agents induce apoptosis through mediators such as Caspase 8 and 3(Ganai, Arenas, and Forbes 2009, Loeffler, Le'Negrate, et al. 2008). Additionally, bacteria can be engineered to deliver cytokines which stimulate immune cell activation, proliferation, and migration to the tumor site (Forbes 2010). Examples of cytokines recombined into the bacterial genome include IL-2, which promotes lymphocyte proliferation and IL-18, which suppresses angiogenesis thereby cutting off the blood supply to the tumor (Loeffler, Le'Negrate, et al. 2008, Micallef et al. 1997, Barbé et al. 2005). Alternatively, bacteria can also be engineered to express markers upregulated by tumor cells, thus sensitizing the immune cells and destructing tumors presenting those markers (Forbes 2010). Recent studies show that the production of Raf1, an upregulated transcription factor in tumor cells, by attenuated *S. typhimurium* using the *E. coli* hemolysin secretion system led to a regression of tumors in Raf-1 induced lung adenomas (Gentschev et al. 2005). Utilizing bacteria as vehicles for therapeutics is beneficial in that it allows for the localized production of therapeutics at the tumor site, but the control of therapy often stops once the bacteria is injected resulting in an "always on" cargo production leading to high dosages, off target effects, and potential development of host resistance.

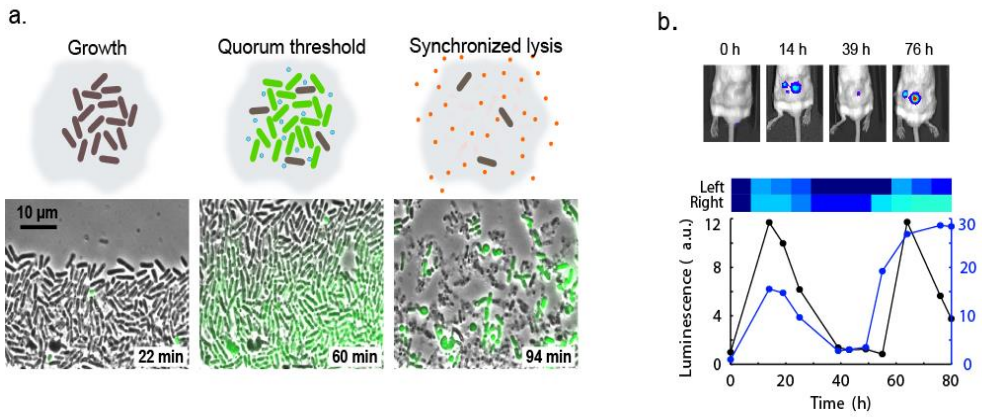

Figure 4. Bacterial dynamics of the synchronized lysis circuit (SLC). (a) The main stages of each lysis cycle from seeding to quorum 'firing'. The schematic depictions are typical time series images of the circuit-harboring cells undergoing the three main stages of quorum firing. Shown below is a fluorescence profile of the bacteria in a chamber. **(b)** *In vivo* bacterial dynamics. Pictures shown above are *In vivo* imaging over time of a mouse bearing tumors injected with the bacteria. Below graph is a bacteria population over time tracked by luminescence. Data for each line represents tumor mass on the right (blue) and left (black) side of a mouse.

With rapid advances in the field of synthetic biology, gene circuits and logic gates have been incorporated into bacterial genomes to provide more control over these bacterial therapeutics. Early examples include regulatory circuits that allow *E. coli* to invade cancer cells in low oxygen conditions, characteristic of tumor microenvironments (Anderson et al. 2006). Recently, we have expanded upon these initial circuits and explored the use of bacteria for cyclic drug release (Din et al. 2016). We constructed a genetic circuit to mediate drug production and release in a synchronized mechanism (Fig. 4). The synchronized lysis circuit (SLC) relies on quorum sensing to identify a critical density and threshold concentration of a signal molecule. When the threshold concentration is reached, the circuit is activated and the cells lyse in synchrony, releasing the therapeutic. The few bacteria that survive lysis repopulate, feeding the next cycle. This circuit not only allows temporal control of drug release, but also serves as a safety mechanism to ensure a controlled growth of the bacterial population. In a separate experiment, we engineered three different therapeutics into the SLC, allowing for a multi combination therapy in a single delivery vehicle. Haemolysin (hylE), immune cell recruitment therapeutic (CCL21) and a trigger for tumor cell apoptosis (CDD-iRGD) was combined into an SLC triple strain (SLC-3) and transfected into *S. typhimurium*. Upon oral delivery of the SLC-3 combined with a common clinical chemotherapy, 5-FU, to colorectal tumor-bearing mice, 50% increase in mean survival time for the animals was observed. We demonstrated that SLC could produce a significant therapeutic effect both on its own, and in combination with chemotherapy in subcutaneous and liver metastasis models.

One challenge in exploiting synthetic biology for translational applications is to develop robust systems that function in *in vivo* contexts. One major hurdle is that culture-based selective pressures such as antibiotic resistance are absent *in vivo* leading to strain dependent instability of plasmid-based networks over time (Danino et al. 2012). To minimize the extent of plasmid loss in the absence of antibiotic selection, stabilizing elements for plasmid retention can be incorporated into the strain (Danino et al. 2015, Darwin 1872). For example, a toxin-antitoxin system can be employed where the toxin and antitoxin are produced simultaneously and in the event of plasmid loss, the cell will be killed by the long-lived toxin (Wood, Kuhn, and Peretti 1990, Gerdes 1988). Furthermore, *alp*, originating from the *Bacillus subtils* plasmid, ensures equal segregation of plasmids during cell division by producing filaments that push plasmids to the poles of cells (Danino et al. 2015). Additionally, genes of interest can be integrated into bacterial genomic DNA, which differs from plasmid DNA in that it cannot be lost to the environment or transferred to other bacteria (St-Pierre et al. 2013, Haldimann and Wanner 2001, Sibley and Raleigh 2012), could produce less therapeutic, or mutate with higher frequency.

While plasmid stability strategies allow for the bacterial circuits to be more viable in animal models, they also may allow for transmission and survival of the engineered plasmids at low frequency posing a biosafety issue. Moving forward, it will be critical to ensure that these engineered bacteria or their engineered plasmids do not escape from and survive outside of the laboratory environment. This can be done by attenuations that limit a bacteria's growth on a synthetic amino acid (Mandell et al. 2015) or other nutrient not present in the environment. Alternatively, biocontainment circuits, like the previously

mentioned, “kill switch,” have been developed to couple biosensing with circuit-control of cell viability (Chan et al. 2016). These circuits specify environmental “input” signals that lead to cellular destruction, ensuring the controlled growth of bacterial populations in the intended microenvironments. The challenge with these circuits will be to have them maintain functionality in the environment, given their strong selective pressure with expressed toxins.

VI. Future Directions

The treatment standards for cancer are being revolutionized by reprogramming biology. Today, the tools to engineer regulatory circuits and kill switches to control bacterial cancer therapeutic production and release are rapidly evolving. As safety mechanisms develop, it will be possible in the future to incorporate therapeutic bacteria into our daily diets and have them detect, target, and treat tumors unbeknownst to us.

Cancer therapy is just one of the many applications for programmable bacteria. The ability to engineer one of life’s oldest forms as smart therapeutic agents has the potential to transform our current therapeutic capabilities across a range of diseases. Since bacteria are found almost everywhere in our bodies, this presents an opportunity to engineer bacteria for many applications such as prevention of infections in the upper respiratory of cystic fibrosis patients and engineering of skin microbes for UV protection to name a few.

Over the past century, research has accelerated from the naming of “hereditary units” as “genes” to the intentional reprogramming of life. Parallel to the field of biology, we have seen the rapid advancement of biotechnologies such as improved computation, high throughput assays, and clinically relevant animal models that have made these scientific strides possible. Despite the progress, synthetic biology has several challenges ahead before reaching its full clinical potential. With issues like public perception and integration into standard treatments, there is still room for the field to grow. Even so, with the fast-paced design of novel bacterial circuits and the concurrent development of innovative technologies, the diverse capabilities of engineered bacteria will soon be realized.

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