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# The New Role of the Microchemostat in the Bioengineering Revolution

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*Abstract*— **Since the inception of synthetic biology as a discipline, bioengineers have used the electronic circuit paradigm to analyze, model, simulate and interpret the behavior of genetic circuits. In this paper, we elaborate upon the effect of evolution as an overriding attribute of the biological systems, which makes genetic circuits inherently fickle compared to their electronic counterparts. Shrinking the volume of programmed microbial population reduces the effects of evolution. This concept was demonstrated by characterizing the dynamics of *Escherichia coli* cells carrying a synthetic "population control" circuit, which regulates cell density through a feedback mechanism based on quorum sensing. The microchemostat prolonged the lifetime of the programmed circuit by at least an order of magnitude compared macro-scale characterization schemes.**

## I. Introduction

Synthetic biology – the synthesis and insertion of complex networks of genes into living cells to program novel and non-native biological functions [1, 2], is an emerging theme in the bioengineering revolution. In the same way that electrical engineers wire together transistors – the basic on-off switches of Integrated Circuits (IC), synthetic biology researchers string together genes in novel combinations to build living circuits inside cells. Synthetic gene circuits equivalent to the fundamental building blocks of any logical operation (the '*inverter*' and '*and*' logic gates) have been comprehensively demonstrated [3]. Indeed, this approach has allowed the creation of synthetic ecosystems, pattern-forming systems, genetic clocks, organisms endowed with programmed toggle switches, oscillators, diverse gene regulation patterns of every shape and size [4-8]. Key concerns that have been voiced in the research community as circuits increase in complexity include: the laborious and time consuming process of building and optimizing circuits, function-voiding evolution [9] as well as internal and external random stimuli (noise) [10].

The design of synthetic circuits, which dwell in the biological substrate (cells), has drawn inspiration from electronic integrated circuits built in the silicon substrate [3, 11]. Yet, the overriding difference between the two contexts (biological and silicon) [12] invariably implies that the synthetic circuits built on an IC concept chassis – as most unfortunately are – operate by luck and are ultimately succumb to premature failure. Here, we will qualitatively explore evolution as the overriding attribute that distinguishes synthetic circuits from their electronic counterparts. We will also discus the new role that only microbial micro-bioreactors [4, 9] can fill to mitigate the effects of evolution. For simplicity, we will limit the scope of this manuscript to prokaryotic synthetic circuits although the arguments put forth may be recast to fit the eukaryotic synthetic gene circuit context.

### A. The Electronic vs. Synthetic Circuit

An electronic circuit, which consists of a network of electronic components, typically works as a stand-alone unit that solely performs a specific function. Electronic devices therefore need to be 100% error free to be declared functional. In addition each component of an electronic device is designed to work in a specified way each time it runs and for its components to remain unaltered in the process. On the contrary, synthetic gene circuits are artificial networks of transcriptional control elements inserted into living cells in order to 'program' cellular behavior. As each cell reproduces by duplicating its DNA and then dividing into two, it passes a copy of the genetic instructions encoded in the DNA to each of its daughter cells. The daughter cells themselves resemble but do not necessarily match their parent given that the DNA copying is not always perfect and the instructions are occasionally corrupted. Mutations – that is, changes in the DNA – can result in a daughter that is (i) changed for the worse, in the sense that it is less able to survive and reproduce; or (ii) changed for the better, in that it is better able to survive and reproduce; or (iii) changed neutrally, being different but equally viable. The struggle for survival (competition for limited resources) eliminates the first, favors the second, and tolerates the third. Whichever descendants survive in the next generation will inherit the altered instructions that brought them into being [13].

Thus every large population of bacteria contains a pool of mutants that provides a generous supply of genetic variants. The genetic variant best-suited for the prevailing environmental conditions proliferates with the highest efficiency and becomes dominant. If environmental conditions to change, a different genetic flavor could gain a selective advantage and become predominant. Thus the fact that DNA replication is not perfectly accurate all the time allows a population of bacterial cells to adapt genetically to changes in its surroundings. This simple principles of change and selection applied repeatedly over many cell generations is the basis of evolution, the process according to which species become gradually modified and adapted to their environment in more and more sophisticated ways [13].

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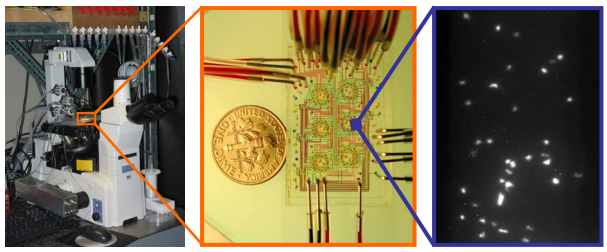
### B. *Evolution in Synthetic Circuits*

The novel traits that a recombinant circuit bestows upon its host cell are typically nonessential to the host's viability yet maintenance, propagation and expression of the synthetic non-native genes taxes the host's energy [14]. From this perspective, a genetic alteration that leads to loss of circuit function becomes a positive career move for the host cell. Thus, given the nature of the biological substrate, unless the expression of the synthetic circuit is engineered to contribute to the host cell's viability, programmed bacterial populations will always gravitate to a dominant genotype with dysfunctional synthetic circuits. For this reason, evolution becomes the force that mediates the observed fickleness and ultimately, the premature demise of synthetic gene circuits operated over sufficiently long periods of time [9, 15].

## II. Microbial Micro Bioreactors

A parallel but critical development in this field is the miniaturization of the culturing process of microbial systems through microfluidics—the science and technology of systems that manipulate small amounts of fluids ($10^{-9}$–$10^{-18}$ liters), using micro-sized channels [16]. Particularly exciting is Microfluidics Large Scale Integration (MLSI) [17], which facilitates the integration of biological procedures such as continuous culturing into a single monolithic process that is faster, more precise and more reproducible than the sum of its stand-alone components [18]. In addition, MLSI metering enables ultra-low consumption of biological samples and reagents, allowing high-throughput research at low cost with short analysis time.



(A) (B) (C)

**Figure 1: (A)** The microchemostat platform consists of a microchemostat chip positioned for imaging on an inverted microscope. **(B)** Optical micrograph showing six microchemostats that operate in parallel on a single chip. Various inputs have been loaded with food dyes to visualize channels and sub-elements of the microchemostats. The coin is 18 mm in diameter. **(C)** Micrographic image of GFP fluorescent *E. coli* bacteria in the microchemostat. *In situ*, single-cell resolved, real-time micrographic images of the chemostat micro-cultures, obtained non-invasively, reveal information pertaining to cell density, growth rate, motility and gene expression (reported by fluorescence markers).

The considerable challenges of characterizing synthetic circuits prompted Balagaddé and colleagues to devise and implement a miniaturized 16 nanoliter microchemostat [4, 9] (Figure 1). The entire on-chip bacterial culturing system is computer controlled and enables automated, continuous culturing and single-cell resolved monitoring (cell density, morphology and fluorescence) of small populations ($10^2$ – $10^4$ bacteria, compared with $>10^9$ in conventional settings). Such micro-sized populations undergo proportionately fewer cell division events per hour, which effectively insulates the micro-cultures from evolution and promotes genetic stability for prolonged circuit monitoring [19] over five hundred hours (at least 10 times longer than circuit lifetimes in conventional macro-sized populations for the calibrated circuits). Its unique design also allows multiple experiments to be run in parallel on the same chip (Figure 1) under a matrix of well controlled growth conditions, which speeds up the synthetic circuit characterization process. The miniaturized format greatly reduces reagent costs, and allows single cells (Figure 1C) to be tracked through time as gene expression occurs.

## III. Slowing Down Evolution

Previous research [9] has used the microchemostat device to monitor the dynamics of cell populations containing a synthetic 'population control' circuit [15], which autonomously regulates the cell density via a quorum-sensing [20] based negative feedback system. With the circuit ON, the cell density was broadcasted and detected via the synthesis and sensing of a signaling molecule (acyl-homoserine lactone, or AHL), which in turn modulated the expression of a killer gene (lacZα-ccdB). The killer gene regulated cell density by controlling the cell death rate. The circuit, under control of a synthetic promoter, was inducible with isopropyl-β-Dthiogalactopyranoside (IPTG). In one of the runs, we performed six experiments simultaneously on a single microchemostat chip using *E. coli* MC4100Z1 cells and a dilution rate of 0.16 $hr^{-1}$ (Figure 2). Cultures in reactors 1-3 with circuit-bearing cells were induced with IPTG (circuit ON), while those in 5 & 6 were not induced (circuit OFF). Reactor 4 contained a circuit-free population with IPTG. Circuit-free and circuit OFF cultures (4, 5 & 6) grew exponentially to a steady-state density of ~3.5 cells/pL. In contrast, circuit ON populations (1, 2 & 3) exhibited oscillatory dynamics before reaching a lower steady-state population density after ~125 hrs. Using the ability to monitor the microchemostat with single cell resolution, we also observed that the oscillations in cell density correlated with specific cell morphologies (Figure 2).

The microchemostat enabled the monitoring of programmed behavior of bacterial populations for hundreds of hours despite strong selection pressure to evade population control (expression of the circuit at moderately high cell densities resulted expression of a Killer gene and ultimately suicide for the host cells). This feat was not achievable in macroscopic reactors: during macro-scale experiments with reaction volumes of 3 to 50 ml, circuit-ON cultures lost regulation within ~70 hours. Population control in the microchemostat, in contrast, was routinely maintained for more than 200 hours and sometimes more than 500 hours.

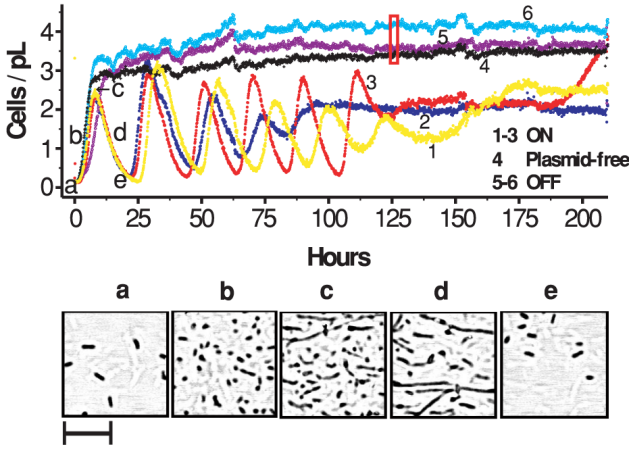



**Figure 2:** (A) Growth of MC4100Z1 cells with the population-control circuit ON (reactors 1 to 3), OFF (reactors 5 to 6), or absent (reactor 4) on a single chip. Bottom panels (a to e) show micrographs of the culture in reactor 3 at the corresponding points during the first oscillation (scale bar, 25 mm). Cells were grown at 32°C in LBK medium [9], buffered at pH 7.6, at a dilution rate of 0.16 hour$^{-1}$.

## IV. Conclusion

In this paper, we explored the effect of evolution on synthetic circuits and argued as to why modeling them after electronic circuits is bound to disappoint. The evolution-mediated premature loss of programmed function makes it virtually impossible to project, characterize, ensure or even observe long-term programmed circuit behavior, which conditions are requisite for any circuit to be reliably deployed. Ergo, from a programming standpoint, the only reliable way to ensure the long-term integrity of a synthetic system is to couple the survival of the host organism to the circuit's function, to make each programmed trait necessary for survival. In the meantime, we have demonstrated that culturing programmed cells in micro-bioreactors stabilizes programmed function over a longer period of time and facilitates long-term characterization.

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