5-D(b) Emerging Fields Catalyzed by ERCs

i.

Role of NSF Engineering Research Centers in Development of the Field of Bioengineering

Lynn Preston and Randolph Hatch[1]

This history provides an overview of the early development of the field of biochemical engineering in the 1940s and 1950s through to the later impact of a series of National Science Foundation (NSF) Engineering Research Centers (ERCs) funded between 1985 and 2008, which contributed to building the interdisciplinary platforms underlying the emerging discipline of bioengineering.

These efforts were motivated by the engineering mindset: namely, how do I understand biological components and systems in order to control or manipulate them to produce new pharmaceuticals, cure diseases, and/or build new constructs as substitutes for failing or diseased body parts? The early biochemical engineering ERCs initially integrated biology and chemical engineering to advance *biochemical engineering* and begin to develop the field of *bioengineering*, which reflected a deeper intersection of biology and engineering. Later ERCs built platforms that integrated bioengineers, biologists, chemists, materials scientists, and clinicians to explore new opportunities to engineer substitutes for failing organs and tissues or substitutes for petroleum-based chemicals.

One of the most important byproducts of these ERCs was the education of new generations of engineers who understood biology and the interface of biology and engineering, new generations of biologists who learned from engineers how to go beyond discovery to the use of their knowledge to advance new technologies, and new generations of physicians who could work in partnership with engineers to advance medicine and clinical practice. The ability to build these ERC platforms rested on earlier investments by NSF and industry in faculty research at the interface of biology and chemical engineering.

From Chemical Engineering to Biochemical Engineering to Bioengineering: Pathway to Improved Processing and Manufacture of Drugs and Other Products

The foundation of the field of bioengineering is in the early work at the interface of biology and chemical engineering that began in the 1940s and 1950s, as engineers like Elmer Gaden (Columbia University) began to apply chemical engineering fundamentals to the production of a biologically-derived pharmaceutical: penicillin. Others were Bob Finn (Merck and Cornell), and Charlie Wilke (UC-Berkeley). They and other early pioneers of the field worked on fermentation of pure cultures of molds and yeasts, cell damage caused by aeration, isolation of enzymes to produce new metabolites, and early bioprocess engineering technologies.

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[1] Ms. Preston is retired from the National Science Foundation (2014) as one of the founders and the long-time Leader of the ERC Program. Dr. Hatch is a biochemical engineer and President of Cerex, Inc. who has served as a reviewer of most of the bioengineering ERCs discussed here.

These professors have been called the "fathers of biochemical engineering" for pioneering the engineering and commercialization of biological systems for large-scale manufacturing of antibiotics and other drugs. One of Gaden's graduates, Art Humphrey, began to further explore the field by working with enzymes at the University of Pennsylvania in the late 1950s. At the same time, soon after NSF was established, Lewis G. "Pete" Mayfield—later one of the two founders of the ERC Program—came to NSF. By the early 1960s he saw an opportunity to explore how to use chemical engineering skills to process media with biological origins. Mayfield later told Lynn Preston, the other founder of the ERC Program, that he wondered at the time whether it was possible to engineer enzymes to carry out functions that had not been developed through evolution. Motivated by this question, he championed the enzyme engineering program and supported investigators like Art Humphry. An important contribution in 1965 was the textbook that Humphry and two colleagues authored that integrated chemical engineering fundamentals with bioprocess technology for students for the first time.[2] This was the foundational work for new young entrants to the field, like Daniel (Danny) I.C. Wang, who continued the exploration of the interface of biology and engineering as a faculty member at MIT.[3] Two of Wang's students have characterized these early efforts as a precursor to the ERC research model: "These investigators invented the model of a successful, multidisciplinary collaboration toward a systems goal."[4]

Mayfield transitioned into other roles at NSF and in the late 1970s, Oscar Zaborsky and Tapan Mukherjee (later an ERC PD) joined NSF to continue Mayfield's work in supporting enzyme engineering and bioprocess engineering, expanding the support for early work in recombinant *E. coli* fermentation as a bioprocess tool. Figure 1 shows the evolution of the field under NSF support and the leaders in three generations of biochemical engineers by the late 1970s and early 1980s in academe and NSF.[5]

[2] Aiba, S, A.E. Humphry, and N.F Millis (1965). *Biochemical Engineering* New York, NY: Academic Press.

[3] Preston, Lynn (2014). *NSF: The Catalyst that Sparked the Field of Biochemical Engineering*. Plenary Remarks on NSF's Role in the Development of the Field of Biochemical Engineering upon receipt of the D.I.C. Wang Award for Excellence in Biochemical Engineering, March 19, 2014.

[4] Croughan, Matthew S., Wei-Shou Hu (2006). *From Microcarriers to Hydrodynamics: Introducing Engineering Science into Animal Cell Culture.* Published online 24 August 2006 in *Wiley InterScience* . https://onlinelibrary.wiley.com/doi/pdf/10.1002/bit.21088

[5] Preston, op. cit., slide 5.

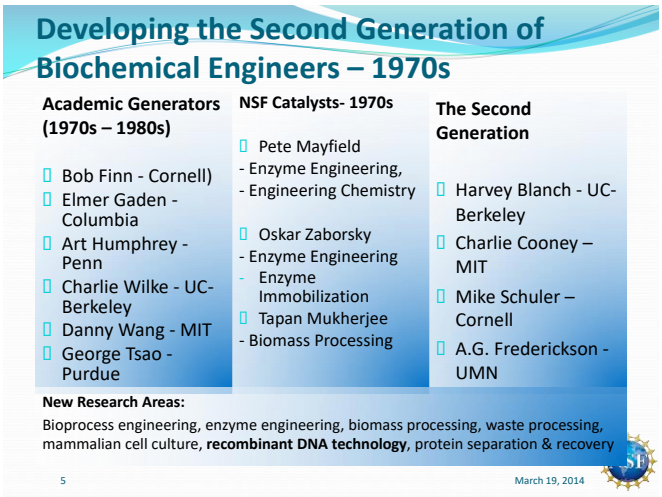

***Figure 1: Development of Leaders in Biochemical Engineering in the 1970s* (Credit: Lynn Preston)**

During the 1970s industry relied on *E. coli* fermentation to produce insulin, growth hormones, and other proteins. However, the "game changer" was the invention of gene splicing technologies to create recombinant DNA (rDNA). Suddenly, it was possible for human genes to be inserted into bacteria, yeast, and other animal cells to produce human proteins. Patients who lacked a certain protein or had a defective protein could be given the correct human protein produced by recombinant DNA technology as a therapy. Before the development of rDNA technology, some of those proteins (e.g., insulin for diabetes, human growth hormone for dwarfism, and Factor VIII for hemophiliac patients) were obtained from human or animal tissues or cadavers; they were often in short supply and there was a high risk of viral contamination being passed from contaminated source tissues."[6] To combat these risks, commercialization of new pharmaceuticals employing these new rDNA technologies was pursued by the then-nascent biotechnology industry.

These were the challenges that motivated Carl Hall, the Deputy Assistant Director of Engineering at NSF, to begin to organize NSF to deal with the research opportunities in support of the emerging field of biotechnology. He asked Lynn Preston, by then leading the Office of Interdisciplinary Research at NSF, to develop collaborations across the NSF research directorates with research program directors who supported fundamental research underpinning biotechnology, instrumentation, and industry/ university cooperation in projects and centers. In 1983, she established the NSF Program Committee on Biotechnology to bring Program Directors from biology, chemistry, materials science, and engineering together to support interdisciplinary fundamental research on the scientific and engineering principles underlying biotechnology, educate the technical personnel needed for this new field, encourage university/industry interaction, and analyze the role of the government and private sector in biotechnology. She issued the first Program Announcement in the field: "Biotechnology and NSF." The announcement targeted opportunities for research to advance the field, which included:

[6] Hu, Wei-Shu (2013). Biochemical engineering over half a century. *SBE Supplement, Biochemical Engineering*, November 2013, p 4.

- Stabilization of enzymes for use in industrial processes and products
- Organisms adapted to extreme environments—deep ocean hydrothermal vents, etc.—to yield alternative processes in industry or alternative materials
- Plant and animal cell culture for diagnostic and therapeutic biologics
- Bioreactors containing microbes, enzymes, or other biocatalysts
- Sensors to monitor biological reactions
- Low-cost raw materials substrates
- Separation and purification of biological molecules
- Microbial processing, such as leaching of ores degradation of inorganic and organic wastes.

The announcement coordinated funding from 32 programs in science and engineering, for a total of $53M.[7]

While this team worked from the inside of NSF, also in 1983 the Office of Science and Technology Policy in the White House elevated the visibility and importance of the field of Biotechnology throughout the government through a series of studies and workshops. One, the Working Group on Competitive and Transfer Aspects of Biotechnology, led by William J. Walsh III, the OSTP Coordinator for Biomedical Research and Health Affairs, from the Department of State, recommended:

- Increased support for effective education, research and development in biotechnology
- Specific measures to improve the commercial climate for biotechnology
- Specific improvements in the regulatory processes—especially regarding pharmaceuticals
- Better communication, coordination, and cooperation among government, academia, and the biotechnology industry to simplify government contracting procedures.[8]

On the academic research front, Croughan and Hu point out that by the early 1980s it was becoming apparent that *E. coli* could not be used to produce all recombinant proteins of interest because molecules such as recombinant human tPA and monoclonal antibodies required substantial post-translational modifications for full bioactivity and acceptable pharmacokinetics.

These were the challenges that stimulated Danny Wang and his faculty colleagues at MIT to develop a proposal for the first ERC solicitation in 1984, in partnership with their industrial supporters. They understood that another medium would be necessary to advance the emerging biopharmaceutical industry. The proposal was successful and the Bioprocess Engineering Center (BPEC) was established in 1985 as one of the first six ERCs. The focus was on how to employ an interdisciplinary skill base in biology and biochemical engineering, built up over time at MIT, to address a major drawback of mammalian cell-

[7] NSF (1983). *Biotechnology and NSF: Opportunities for Support of Biotechnology Research and Related Activities*. National Science Foundation, NSF 83-90.

[8] Walsh, William J. III (1983). *Report of a Working Group on Competitive and Transfer Aspects of Biotechnology.* Washington, DC: Office of Science and Technology Policy, May 27, 1983. p. 2.

based processing: its relatively low cell concentration in a stir-tank bioreactor that had become the prevailing method for culturing mammalian cells.

BPEC investigated the effect of hydrodynamic stress on cells and explored different reactor configurations, process optimization, and the recovery of protein products. As Figure 2 shows, the systems challenges that motivated the ERC were large-scale, stirred-tank mammalian cell cultures—i.e., large-scale microcarriers cultures and large-scale suspension cultures. These systems could only be realized through enabling bioreactor technology. To enable these technology goals, a body of fundamental research was focused, for example on: stoichiometric and metabolic analysis, which enabled medium optimization protocols for the reactor and systems; carrier charge density and inoculation, which enabled optimal microcarriers and seeding of the reactors; hydrodynamic effects on cell growth and death for bioreactor design and operation; and flocculation dynamics and charge balance to enable reversible flocculation and settling for the reactors.[9]

Over the course of its first ten years of operation, the ERC produced the following types of breakthroughs in fundamental knowledge, with corresponding impacts on technology and industry:

- Developed the SV40 expression vector for protein expression in mammalian cell systems
- Provided a fundamental understanding of the fluid mechanics in animal cell bioreactors leading to the criteria for design of industrial bioreactors
- Devised an intracellular redox probe based on molecular biology and biochemistry to assess the importance of redox state as related to disulfide oxidation
- Controlled product secretion in animal cell cultures to enhance downstream processing
- Developed metabolic flux analysis of mammalian cell metabolism that allowed the assessment of energy and mass fluxes to increase cell productivity
- Delineated the mechanism of cell death due to gas sparging and thus allow better methods for bioreactor control

Provided a fundamental understanding of *in vivo* protein folding and devised co-solvent assisted *in vitro* protein refolding to increase process efficiency.

[9] This paragraph represents a synthesis of pages 221–223, Croughan and Hu, op. cit.

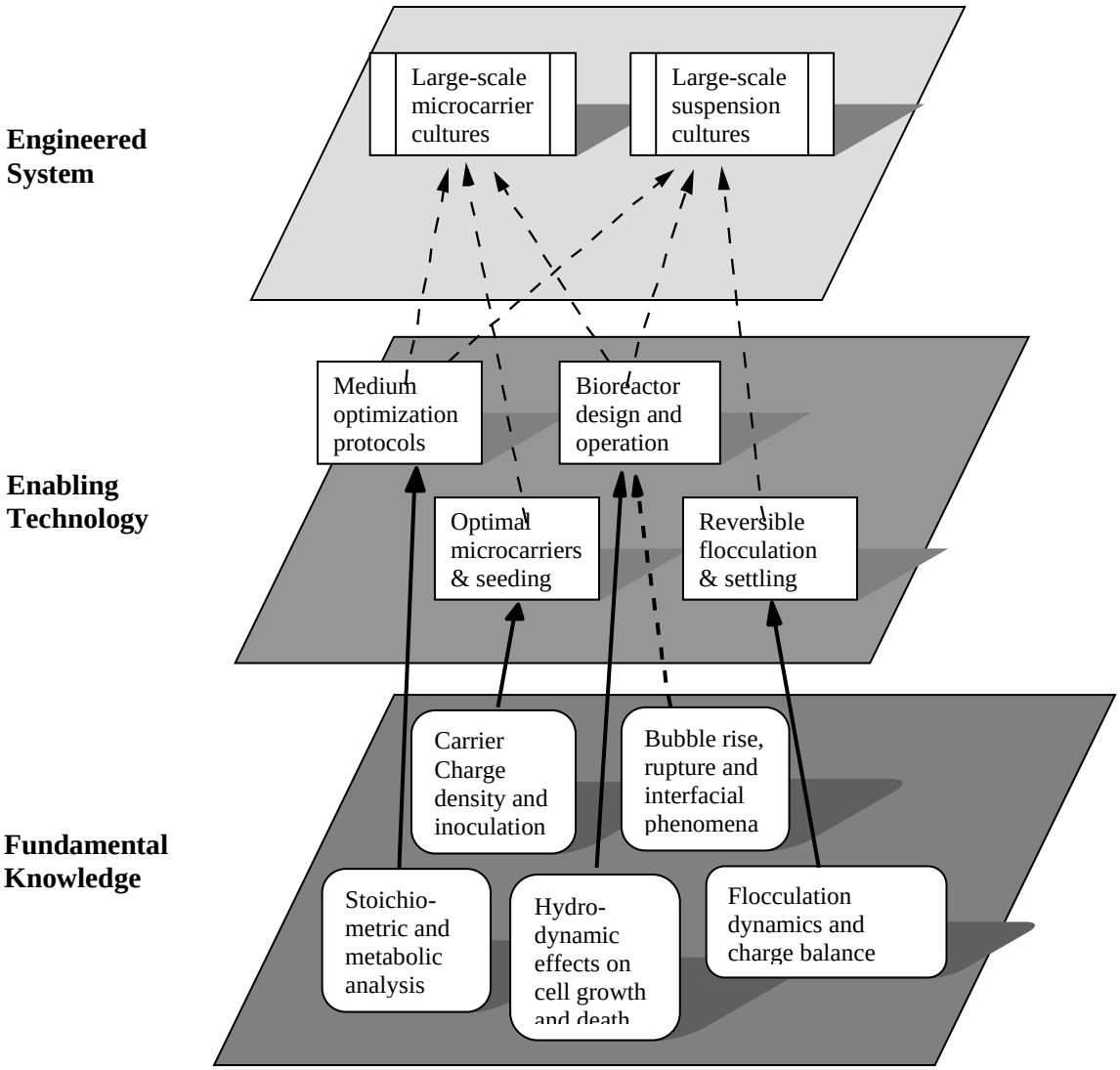

Figure 2: Three-plane illustration of Professor Wang's research program in large-scale, stirred-tank mammalian cell culture **(Credit: Daniel Wang)**

- BPEC's research supported the emerging biopharmaceutical industry by solving bioprocess challenges to the proof-of-concept phase, to allow industry to take over and explore issues in scale-up. When these new processes faced barriers, these were often fed back to BPEC as new research challenges.

BPEC also laid the foundation for the progress of the industry by training hundreds of biochemical engineers who were well grounded in biology and chemical engineering. See Section 10-C(e) for a list of exemplary BPEC graduates and their contributions to industry.

BPEC and its partnership with the emerging biotechnology industry helped to "establish" the emerging field of biochemical engineering in academia and its follow-on field, bioengineering. Several Chemical Engineering departments around the country changed their names to Chemical and Biochemical Engineering or various combinations of the fields in order to "voice" the new focus on the interface of biology and chemical engineering.

During the years that BPEC I was funded,[10] Frederick Heineken, an NSF Program Director hired in 1986, was providing a broad base of support to small research groups exploring issues needed to develop the nascent field of bioengineering. More specifically, by the mid-1990s this funding was playing an important role in the development of the field of metabolic engineering.

According to Gregory Stephanopoulos, from the Chemical Engineering faculty at MIT and a member of the BPEC I team, metabolic engineering was then emerging as a new discipline that was occupied with the directed modification of metabolic pathways for the microbial synthesis of various products and the design, construction, and optimization of native as well as non-natural routes of product synthesis, aided by the availability of synthetic DNA. He points out that metabolic engineering came to be defined formally as the directed modulation of metabolic pathways using methods of recombinant technology for the purpose of producing fuels and chemical and pharmaceutical products. This was achieved by focusing on integrated pathways and genetic regulatory networks—as opposed to individual genes and enzymes, which was the subject of molecular biological research at that time. Through this work, metabolic engineering preceded systems biology by championing the need for a systemic view of metabolic pathways and approaches for their optimal functioning and was also distinct from the field of synthetic biology, which emerged at the turn of the 21th Century.[11]

1. Synthetic Biology

Around the turn of the 21st Century, synthetic biology was emerging as a new field of bioengineering resting on the foundation of knowledge that had been laid over the prior 20 years to support the assertion that engineering principles could be used to understand biological and, therefore, cellular systems—and, perhaps, to manipulate their functions to ends not foreseen in nature. Steps in this direction were taken as early as the 1980's. The control of metabolic pathways was being manipulated to allow bacterial cells to over produce small molecules in commercially viable fermentation processes. Examples of this came from BioTechnica International, which was able to use advances in molecular biology and couple with biochemical engineering to produce amino acids (phenylalanine and tryptophan) and an important vitamin, riboflavin. These processes were then commercialized by DSM and Hoffman La Roche.

While cell and pathway performance was the goal of metabolic engineering, synthetic biology has been defined as the "use of molecular biology tools and techniques to forward-engineer cellular behavior…. Gradually it was recognized that the rational manipulation of biological systems, either by systematically tuning or rearranging their modular molecular constituents, could form the basis of a formal biological engineering discipline."[12] More specifically, it was envisioned that molecular parts could be drawn upon to forward-

[10] BPEC is the only ERC ever to successfully complete two full life-cycles as an ERC. BPEC I operated from 1985 through 1996.

[11] Stephanopoulos, Gregory (2012). Synthetic biology and metabolic engineering. American Chemical Society. *ACS Synthetic Biology*, *1*: 514-525 (p. 514).

[12] Cameron, D. Ewen, Caleb J. Bashor, and James J. Collins (2014). Timeline. A brief history of synthetic biology. *Nature Reviews/Microbiology*. Vol. 12, May 2014: 381-390 (p. 381).

engineer regulatory networks. The initial intellectual framework mimicked the circuits and networks constructs of electrical engineering, so as to understand natural systems which might eventually lead to artificial regulatory networks that underlie biotechnology and health applications. In 1999, Adam Arkin and Andrew (Drew) Endy, "realized that the heterogeneous elements that made up a genetic circuit were lacking standards, and they proposed a list of standard biological parts."[13,14]

From these insights a number of breakthroughs emerged between 2000 and 2003. The first reported genetic circuits that had been engineered to carry out designed functions—a constructed genetic toggle switch (Gardner et al., 2000)—demonstrated that it was possible to control expression of mutually inhibitory transcriptional repressors in response to external signals.[15] This was followed by research (Elowitz and Leibler, 2000) that demonstrated that activation of an oscillatory circuit resulted in ordered, periodic oscillation of repressor protein expression.[16] Another groundbreaking study established methods for engineering transcription-based logic gates and contributed to formalization of the language and practice of circuit engineering in biology. Through this work, the authors (Weiss and Basu) noted that: "Biochemical logic circuits that precisely control gene activity in cells are useful for creating novel living organisms with well-defined purposes and behaviors. An important element in designing and implementing these circuits is matching logic gates such that the couplings produce the correct behavior. ...(We are reporting) in-vivo experimental results that examine and optimize the steady state behavior of cellular logic gates and genetic circuits synthesized in our lab. The optimized gates have the desired input/output characteristics for constructing robust genetic logic circuits of significant complexity."[17] These breakthroughs and others established the foundation for the development of synthetic biology as a field.

To support the need for standardized parts, *BioBricks* were described and introduced by Tom Knight at MIT in 2003. "The lack of standardization in assembly techniques for DNA sequences forces each DNA assembly reaction to be both an experimental tool for addressing the current research topic and an experiment in and of itself. One of our goals is to replace this ad hoc experimental design with a set of standard and reliable engineering mechanisms to remove much of the tedium and surprise during assembly of genetic components into larger systems."[18]

Between 2004 and 2007, Cameron's timeline points to expansion of the field and the formation of a community of investigators. The first international synthetic biology conference was held at MIT in 2004, helping to galvanize this young and growing

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[13] https://en.wikipedia.org/wiki/BioBrick

[14] http://dspace.mit.edu/bitstream/handle/1721.1/29794/Arkin.Endy.DARPA.pdf?sequence=1

[15] Gardner, T.S., C.R. Cantor, and J.J. Collins (2000). Construction of a genetic toggle switch in Escherichia coli. *Nature 403*: 339-342.

[16] Elowitz, M.B. and S. A. Leibler (2000). A synthetic oscillatory network of transcriptional regulators. *Nature 403*: 335-338.

[17] Weissand, R. and S. Basu (2002). The device physics of cellular logic gates. *First Workshop on Non-Silicon Computing (online) http://www.cs.cmu.edu/~phoenix/nsc1/paper/3-2.pdf*

[18] http://dspace.mit.edu/handle/1721.1/21168

community. Cameron points out that Drew Endy, an investigator at MIT, created collections of modular parts and developed methods to construct and tune particular circuit design.[19]

Crucially, demonstrating the potential for synthetic biology to produce useful biotechnology, Keasling and his team announced in 2003[20] and later in 2006[21] that they had forward-engineered the production of artemisinic acid, the precursor to artemisinin. Artmesinin is an active agent derived from the sweet wormwood plant, *artemesin annua*, which is used to control the malaria parasite, multi-drug resistant *Plasmodium falciparum*, but it is very expensive to produce from its natural source. Keasling and his team engineered artemisinin production by constructing a twelve-gene biosynthetic pathway using genes from *Artemisia annua*, *Saccharomyces cerevisiae (yeast)*, and *E. coli* (bacterium) to transform a simple and renewable sugar, like glucose, into the complicated chemical structure of the anti-malarial drug artemisinin. The engineered yeast was capable of producing artemisinic acid much more efficiently than can be done through natural sources. But the process needed to be optimized and scaled up for industrial production to reduce the cost of therapies significantly below their current prices. The discovery was licensed to Keasling's spinoff company, Amyris Biotechnologies, and to facilitate scale-up and commercialization, in 2008 it was transferred to global pharmaceutical firm Sanofi-Aventis, for large-scale development of the product.

According to Wikipedia, "Commercial production of semisynthetic artemisinin is now underway at Sanofi's site in Garessio, Italy. This second source of artemisinin is poised to enable a more stable flow of key antimalarial treatments to those who need them most. The production goal is set at 35 tonnes for 2013. It was expected to increase to 50–60 tons per year in 2014, supplying approximately one third of the global annual need for artemisinin. May 8, 2013, WHO's Prequalification of Medicines Programme announced the acceptability of semisynthetic artemisinin for use in the manufacture of active pharmaceutical ingredients submitted to WHO for prequalification, or that have already been qualified by WHO. Sanofi's API, produced from semisynthetic artemisinin (artesunate), was also prequalified by WHO on May 8, 2013, making it the first semisynthetic artemisinin derivative prequalified."[22]

In the education arena of synthetic biology, in 2003, Randy Rettberg, an MIT graduate and engineer who had worked for technology companies including BBN (on the ARPAnet in 1972), Apple, and Sun retired from industry and returned to MIT where he founded the International Genetically Engineered Machines (iGEM) competition with his collaborator, Tom Knight. When Randy was at MIT, Tom was a student there while still in high school, who stayed on at MIT and became a highly innovative research scientist at the MIT Computer Science and Artificial Intelligence Laboratory and a part of the MIT School of

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[19] Cameron et al., op. cit., p. 383 and Endy, Andrew (2005). Foundations for engineering biology. *Nature 438*:449-453.

[20] Martin, V.J., D.J. Pitera, S. T. Withers, J.D. Newman, J.D. Keasling (2003). Engineering a mevalonate pathway in Escherichia coli for production of terpenoids. *Nature Biotech*. *21*: 796-802.

[21] Ro, D.K. et al. (2006). Production of the antimalarial drug precursor artemisinic acid in engineered yeast. *Nature 440*: 940-945.

[22] https://en.wikipedia.org/wiki/Artemisinin#Synthesis_in_engineered_organisms

Engineering. Later, he devoted part of his career to carrying engineering design principles to biology. Together, in 2003, Randy and Tom challenged MIT undergraduate students to develop projects using synthesized DNA. By 2004, a competition with five teams from various schools nearby to MIT was held and in 2005 teams from outside the United States took part for the first time. IGEM addressed the question, "Can simple biological systems be built from standard, interchangeable parts and operated in living cells? or Is biology simply too complicated to be engineered in this way?" iGEM has since grown into a major international competition for high school students, undergraduates, and beyond, which Rettberg continues to lead, even as it was spun out of MIT as an independent organization. In 2016, 300 teams from all over the world spent the summer conceiving of, designing and building a system using synthetic biology. In the fall, they came together to share their work with other teams at an annual jamboree where 37% received a gold medal, 25% a silver medal, 26% a bronze medal and 12% were not awarded a medal.[23]

In 2005, Andrew (Drew) Endy, a collaborator with Rettberg in the iGEM competitions, continuing to pursue his goal of standardized biological parts to enable reliable circuit design, established BioBricks to achieve it.[24] Drew became a proponent for this new field of bioengineering and led many efforts to standardize biological parts and promote synthetic biology education. For example, to support the iGEM students, parts are made available from BioBricks, and each team is required to characterize their parts and submit them to BioBricks at the end of the competition. The teams are free to choose a project, which can build on previous projects or be new to iGEM. Successful projects produce cells that exhibit new and unusual properties by engineering sets of multiple genes together with mechanisms to regulate their expression. At the end of the summer the teams add their new BioBricks to the Parts Registry, which the scientific community can build upon through the expanded set of BioBricks over the next year.[25] However, the parts may lack important characterization data and metadata that would be essential when it comes to designing and modeling the functional components.

In 2006, the field received a significant boost in recognition through the award of the Synthetic Biology ERC (Synberc) to the University of California, Berkeley (UCB). The Center Director was Jay Keasling. He brought together a team of innovators in synthetic biology research and education that included Adam Arkin (UCB), George Church (Harvard Medical School), Drew Endy (MIT and later Stanford), Wendell Lim (UC-San Francisco), Kristala Jones Prather (MIT), Randy Rettburg (MIT), Pam Silver (Harvard Medical School), Christopher Voight (then UC-SF and later MIT), and Ron Weiss (MIT), among others.

The vision of the Synthetic Biology Engineering Research Center was to develop the foundational understanding and technologies needed to build biological components and assemble them into integrated systems to accomplish prescribed tasks. The novel aspect of the research was the application of the engineering paradigm for product development—

[23] Synthesized from: https://vimeo.com/194410756 and https://en.wikipedia.org/wiki/International_Genetically_Engineered_Machine

[24] Endy, Andrew (2005). Foundations for engineering biology. *Nature 438*: 449–453.

[25] https://en.wikipedia.org/wiki/International_Genetically_Engineered_Machine

the assembly of standard parts into devices, which would be integrated into systems (chassis) to provide desired functions. To accomplish this vision, Synberc would need to develop a means to express the design specifications of biological components, standardize their functionality, and characterize them for future use in the development of engineered organisms designed to deliver a desired task. Synberc proposed do this at three levels—parts, devices, and chassis—each representing an increasing level of complexity. The end product, a synthetic microorganism, was to be constructed from computationally designed cellular parts (e.g., ribosome binding sites, enzymes for metabolic pathways, and gene regulatory proteins) that can be assembled into devices (e.g., gene expression, metabolic, and sensing/signaling/actuating devices) that will perform well-characterized functions under specific conditions. They envisioned that they would develop the transformative knowledge needed to allow the engineering of biological components to move from time-consuming, one-of-a-kind development efforts to the rapid development of new products from standardized components seen in other technology domains (e.g., electronics). The plan was to focus on synthetic microorganisms constructed from *E. coli* DNA to serve as proof-of-concept testbeds for a microbial drug factory and for microbial agents that could seek and destroy tumors. Through an open source registry that would define, describe, and store basic parts already in use or newly designed, Synberc's proposal promised the availability of these off-the-shelf biological components through a process of standardization of physical connections, functional connections, and performance.

In addition to these proposed goals, Lynn Preston, by then the Leader of the ERC Program, required Keasling to develop a new thrust focused on the integration of ethics and risk in the synthetic biology design process, because synthetic biology inherently entails high biological risks. She added funds to the award to support that newly required thrust, which was initially called Human Practices led by Paul Rabinow (UCB) and then Policy and Practices led by Kenneth Oye (MIT). Staff were added to the team with policy, ethics, and anthropology backgrounds. This was the first time that an NSF award was made that would include the integration of biological risks into the synthetic biology design and manufacturing process. (For a full discussion of the impact of this requirement on Synberc and the broader synthetic biology community, see Chapter 5, Section 5-D(a)i, ERCs and the Interface with Society, Synthetic Biology and Risk.)

During the course of development of the research program, it became apparent that designing and crafting the parts required to carry out the research was not an appropriate task for graduate students. In addition, as noted above, the parts designed by iGEM students lacked characterization and metadata. Keasling and Preston had a discussion about what to do and she suggested that they needed to add another plane to their three-plane strategic research plan that included a "manufacturing facility," where technicians would assemble the parts. She suggested he seek funding from the industrial community. However, an opportunity came up for support to be provided by the ERC Program through the 2009 ERC Innovation awards (see Section 9-E(d) of Chapter 9.) Adam Arkin, Drew Endy (then at Stanford), and Jay Keasling submitted a proposal, which successfully passed the review process and was funded. BioFAB started up in 2009 with matching support from

NSF, the Lawrence Berkeley National Laboratory (LBNL), and the BioBricks Foundation (BBF), a non-profit organization that supports and promotes the use of synthetic biology.[26]

As stated in the award abstract, the primary purpose of BioFab is to ensure that all of the driving testbed applications of Synberc, both existing and future, have rapid access to high-quality standard biological parts as well as DNA synthesis and part assembly services. Two critical secondary purposes were: (1) the development and testing of the best organizational architecture for operating and scaling a production facility that is capable of producing many standard biological parts and that supports broader community participation across both academia and industry; and (2) ensuring that all foundational Synberc-developed technologies, from rapid genome-scale reengineering of chassis to function composition standards for devices are broadly accessible and rapidly translated into Synberc testbeds and to industry. The facility pursued three specific projects at launch. Specifically, the BioFab staff (1) rapidly prototyped Synberc-specified engineered genetic systems to achieve testbed-driven objectives; (2) design, construct, and test a collection of 6,000 new BioBrick parts for controlling replication, transcription, RNA processing and degradation, translation, and protein degradation in *E.coli* and *S. cerevisiae*; and (3) work in partnership with industrial and academic partners to develop improved tools for supporting the design, construction, and characterization/testing of engineered genetic systems assembled from standard biological parts.

Synthetic Biology Tools and Technologies[27]

MAGE (MicroArray and Gene Expression)

MAGE was developed by George Church and his colleagues at the Harvard Medical School to help to overcome one of the bottlenecks in the construction of complicated genetic systems through synthetic biology. MAGE allows researchers to simultaneously and specifically edit multiple target sites in bacterial genomes using short DNA fragments called oligonucleotides, or oligos for short. In a single application, the automated MAGE device creates a large bacterial library that the researchers can then screen and subsequently optimize for various purposes. The lab used MAGE to produce high levels of the antioxidant lycopene, commonly found in tomatoes, in just three days. Thus, "MAGE allows the synthesis of oligos at a fraction of the cost (>1000×) compared to traditional column-based oligo synthesis to make large-scale genome engineering accessible to most laboratories. At a price of 36 USD per column-based oligo, MO-MAGE (2800 USD) is currently cost competitive when using more than 78 oligos. This method could provide a paradigm shift by making large-scale genome engineering of many thousands of targets available as a

[26] https://www.nsf.gov/awardsearch/showAward?AWD_ID=0946510

[27] Keasling, Jay (2015). *Synberc: Building the Future with Biology – Ten Years at the Genesis of Synthetic Biology*. University of California, Berkeley. These tools and technologies are synthesized from this book that was produced by Synberc to celebrate its achievements under NSF/ERC Program support.

standard tool for strain optimization and other projects where large-scale targeted mutagenesis searches are needed."[28]

D-Glucaric Acid Production

D-Glucaric acid is derived from the sugar glucose and has many important uses in the production of a wide range of chemical products. It can be extracted from biomass but the extraction process is slow and expensive, so industry processes it from sugar, using nitric acid, which is also expensive and not environmentally benign. Kristala Prather (MIT—see sidebar) and her colleagues produced glucaric acid from glucose using synthetic biology methods, enabling both lower costs and higher purity. Using myo-inositol as a substrate for production in *E. coli*, they reported two methods for improving D-glucaric acid production that resulted in a 75% production increase for one, and for the other, a 65% increase. However, combining the two methods, the productivity was lost due to the impact of the pH of the culture. They noted that "Further work such as scale-up and process engineering will be necessary to continue to increase productivity towards titers relevant for commercialization of D-glucaric acid. However, this successful application of two parallel methods towards improved D-glucaric acid production bodes well for continued engineering efforts, as this expanded toolset provides a much greater potential for synergistic improvements to D-glucaric acid titers in the future."[29] A Synberc start-up, Kalion, performed scale-up and process engineering in order to achieve significantly higher titers. They are currently working to improve production from glucose, and went into manufacturing in 2018.[30]

Scaffolds

Two Synberc researchers (John Dueber, UCB, and Kristala Prather, MIT) and their colleagues developed scaffolds to hold enzymes in place in a configuration that would produce optimal functioning of the desired metabolic pathway. The scaffold is an engineered protein consisting of multiple domains that co-target enzymes that have been tagged to be recognized by these domains, resulting in co-assembly on the scaffold. These scaffolds enable living cells to function like a self-replicating factory to inexpensively produce desirable compounds. They tested the construct to increase the efficiency of D-glucaric acid production with a five-fold improvement in product titers over the non-

[28] Bonde, Mads T., Sriam Kosuri, Hans J. Genee, Kira Sarup-Lytzen, George M. Church, Morten O.A. Sommer, and Harris H. Wang (2015). Direct mutagenesis of thousands of genomic targets using microarray-derived oligonucleotides. *ACS Synth. Biol. 4*(1):17-22 (Jan. 16, 2015).

[29] Shiue, Eric and Kristala L.J. Prather (2014). Improving D-glucaric acid production from myo-inositol in E. coli by increasing MIOX stability and myo-inositol transport. *Metabolic Engineering 22*:22-31.

[30] Kalion was one of four 2019 EPA Green Chemistry Challenge Award winners. Receiving the sole 2019 Small Business Award, Kalion was honored "…for commercializing the first microbial fermentation process to produce glucaric acid, which offers the possibility of replacing environmentally polluting chemicals with a biodegradable, non-toxic, sugar-derived product. Kalion is initially using it as a corrosion inhibitor for water treatment plants." See https://www.epa.gov/greenchemistry/green-chemistry-challenge-2019-small-business-award

scaffold control.[31] In addition, Dueber and Keasling produced an intermediate, mevalonate, important in the production of artemisinin and some biofuels. One of these scaffolds resulted in a 77-fold increase in the mevalonate production.[32]

Computer-Aided Design for Biology

Clotho, a CAD tool for synthetic biology, was developed by J. Christopher Anderson (UCB) and Douglas Sensmore (Boston U.). It enables users to create modular apps which share common data, to ease standardizing and managing biological designs among labs.

Programmable Organoids

Ron Weiss and his lab, in collaboration with Linda Griffith (BMES II) at MIT, developed programmable organoids created from mammalian stem cells grown to mimic tissue of a human organ. These organoids can be used to test candidate drugs, thereby reducing drug testing and optimization. They can be genetically programed to contain sensors for various biological molecules that provide a detailed view of the organ system and how it is impacted by a testing compound[33]

Nitrogen Fixation

Chris Voight and his lab developed a synthetic nitrogen fixation pathway that can be transferred to soil microbes that associate with cereal crops. The potential use would be in reducing the costs for fertilizers by enabling plants to fix nitrogen themselves. The lab reports that: "We have developed a systematic approach to completely specify the genetics of a gene cluster by rebuilding it from the bottom up using only synthetic, well-characterized parts. This process removes all native regulation, including that which is undiscovered. First, all noncoding DNA, regulatory proteins, and nonessential genes are removed. The codons of essential genes are changed to create a DNA sequence as divergent as possible from the wild-type (WT) gene. Recoded genes are computationally scanned to eliminate internal regulation. They are then organized into operons and placed under the control of synthetic parts (promoters, ribosome binding sites, and terminators) that are functionally separated by spacer parts. Finally, a controller consisting of genetic sensors and circuits regulates the conditions and dynamics of gene expression. We applied this

[31] Moon, Tae Seok, John E. Dueber, Eric Chun-Jen Shiue, and Kristala Jones Prather (2010). Use of modular, synthetic scaffolds for improved production of gluceric acid in engineered E. coli. *Metabolic Engineering, 12*(3):298-305.

[32] Dueber, John E., Jay D. Keasling, Reza Malmirchigini, Tae Seok Moon, Christopher J. Petzold, Kristala L.J. Prather, Adeeti V. Ullal, Gabriel C. Wu (2009). Synthetic protein scaffolds provide modular control over metabolic flux. *Nature Biotechnology 27*:153-759 and Keasling et. al. (2015), op. cit.

[33] Keasling, op. cit. and Guyre, Patrick, Mohammad R. Ebrahimkhani, Nathan Kipniss, Jeremy J. Velaques, Eldi Schoenfeld, Samira Kiani, Linda G. Griffith, and Ron Weiss (2016). Genetically engineering self-organization of human pluripotent stem cells into a liver bud-like tissue using Gata6. *Nature Communications, 7*:10243.

approach to an agriculturally relevant gene cluster from *Klebsiella oxytoca,* encoding the nitrogen fixation pathway for converting atmospheric N_2 to ammonia."[34]

CASE STUDY

Kristala L. Jones Prather's pathway into engineering and throughout her higher career is an example of the crucial role good mentors can play in a person's life, especially for her as a young African-American woman. While in high school in Longview, Texas, she showed a talent in mathematics and an interest in chemistry. A family friend who is a chemist and an MIT graduate encouraged the chemistry interests and her high school counselor recognized her talents in math and listened to her interests in using science and math to make things, not just for theory. She suggested to Kristala that she apply to MIT and major in engineering, which she did. Her comments on the mentoring she received during her engineering education reflect the commitment of many male professors to her success: "For my BS degree from MIT in Chemical Engineering, I have both academic and non-academic mentors. In the former category, Charles (Charlie) Cooney was very influential in my navigation of a path to graduate studies in biochemical engineering. (Charlie, as you know, was a huge part of BPEC.) He taught my kinetics class junior year at MIT, and he offered me a UROP (undergraduate research opportunity) in his lab that I conducted in my senior year."

"Truthfully, I was not a very good UROP student! I didn't have the proper background and really didn't understand what I was supposed to be doing until after my first year in graduate school. I actually used my UROP data for a kinetics project at Berkeley, after I had finally figured out what it meant! Charlie gave me exceptional advice for graduate school, including giving me a run-down of all of the places where great bioengineering research was underway in Chemical Engineering departments around the country. I remember him mentioning Mike Shuler at Cornell, Chaitan Khosla at Stanford, and Doug Clark and Harvey Blanch at Berkeley, among others. (Jay Keasling was a "young nobody" back then :-).) It may seem trivial by today's standards, but in the pre-internet age, having a respected faculty member provide that kind of insight was invaluable. And he offered to write me a letter of recommendation, to boot!"

"I had wonderful support as a PhD student at Berkeley. Jay Keasling served as my research advisor and was a superb mentor, incredibly supportive and encouraging throughout my time at Berkeley and beyond. I TA'd twice with Doug Clark, who was also a good mentor, and Harvey Blanch was chair of my thesis committee. "Uncle Harvey" was fantastic. He gave me spot-on advice about my desire to work in industry first before pursuing an academic career. It took me a while to understand why his advice was so perfect, but it definitely was. And here I am today—a member of the faculty of the MIT Department of Chemical Engineering, with research and industrial interests, and a mentor myself."

2. Metabolic Pathways Engineering

In 2008, the ERC for Biorenewable Chemicals (CBiRC) was established at Iowa State University and led by Professor Brent Shanks, who has a background that integrates industrial and academic experience in chemical engineering. The goal of CBiRC was to create a broad-based technological framework that could be used to generate a flexible system for producing a large number of biorenewable chemicals, which can be further converted to existing and novel chemical products. This approach envisioned a framework that would be in contrast to the then-current efforts in biorenewable chemical development, which targeted one chemical product at a time. The original basis for the framework was to exploit the polyketide/fatty acid biosynthetic pathway starting from glucose to generate an array of chemical intermediates that can be subsequently converted to industrial chemical products using chemical catalysts. The ERC generated key biocatalysts/enzymes from this pathway that were incorporated into microbial host systems to produce a range of polyketide/fatty acid-based platform chemicals. These were then converted to final chemical products using chemical catalysts specifically designed for their selective conversion. By integrating biological catalysis and chemical catalysis, CBiRC worked on creating a consolidated technological framework that can be used to produce a broad array of biorenewable chemicals such as alpha-olefins, alcohols, diacids, and diols.

An example focused on the intermediate compound triacetic acid lactone (TAL). In 2012, CBiRC rationally designed 2-pyrone synthase incorporated into engineered yeast for a large increase in TAL production. By 2014, a TAL purification approach was developed and demonstrated. By 2017, they had demonstrated that TAL derivatives were promising insecticides and entered a corporate licensing arrangement for the technology.

The original CBiRC vision rests on the assumption that industry would turn to bio-based chemicals as an alternative to high-price petrochemical-based chemicals because producing the same molecule from a biomass source rather than a fossil carbon source would be price-competitive. However, at the time the ERC was funded crude oil prices were over $100/barrel and there was a looming prospect of a carbon tax, both of which would stimulate the introduction of renewable carbon into the chemical enterprise. As crude oil prices began to decline and there were no policies put in place to favor renewable carbon over fossil carbon, the ERC shifted its focus to novel chemical compounds that result in improved performance properties in their end use application. The platform framework of CBiRC did not change, but the emphasis shifted from replacement chemicals to novel chemicals. These trends also shifted the composition of the industrial membership, reducing the number of petrochemical producers and increasing the membership of end-use firms who look to novel chemicals to impart improved performance properties to their products.

Center Director Shanks noted in a conversation with Preston that: "In the new context, the CBiRC pyrone testbed has been particularly powerful since the biologically produced TAL allows access to promising novel molecules as well as direct placement molecules. This shift can be seen from our movement from sorbic acid as the benchmark for the pyrone testbed to novel biobased chemicals that show efficacy as antimicrobials and insecticides. The novel biobased chemicals are the ones receiving commercial interest." As an example of the type of flexibility needed in an ERC, Shanks pointed out that: "The research plan for

CBiRC moving forward is the systematic identification of intermediate molecules such as TAL that can be leveraged for both novel biobased chemicals and direct replacement chemicals. We have called the intermediate molecules having this attribute '*bioprivileged molecules*.'"

As in the other ERCs working in the bioengineering domain and in other fields, while industry was actively engaged in CBiRC, early on they signaled that they would not be carrying out translation of research-based innovations into production at a viable scale because of the high cost and high risks involved. Spin-off start-up firms were created to fill that gap. Targeting novel biobased chemicals rather than direct replacements stimulated several spin-off firms to "de-risk" the novel biobased chemicals and their production.

By its ninth year, Shanks and his colleagues were evolving their vision to sustain the CBiRC once NSF ERC support ceased at the end of the tenth year. The idea was to focus on *bioprivileged* molecules to create new products and opportunities. Shanks and Peter Keeling, the ERC Industrial Collaboration and Innovation Director, noted that the "petrochemical industry hasn't produced new commercial molecules in two decades. And that has created what could be an opening for valuable biobased chemicals. The question you have to ask is, 'Do we have all the molecules we need? Are we done?'"

Shanks said CBiRC researchers have been asking scientists and engineers in the consumer products industry if they had all the molecules they needed. The resounding answer we get is "no," he said. "They say, 'We need new innovation, new products, new molecules.' So where are those molecules going to come from?" Shanks and Keeling point to biomass from plants as a source of new intermediate molecules that can be leveraged to produce novel molecules as well as direct replacements for petrochemicals. One such example is muconic acid. Last year, two researchers affiliated with CBiRC—Zengyi Shao and Jean-Philippe Tessonnier, Iowa State assistant professors of chemical and biological engineering at the time—reported how they produced a biorenewable-enhanced nylon. They started with genetically engineered yeast—"a microbial factory," Shao termed it—that ferments glucose into muconic acid. Then, using a metal catalyst and a little electricity, the researchers produced 3-hexenedioic acid. With some simple separation and polymerization steps, they ended up with biobased, unsaturated nylon-6,6 that can be subsequently modified for a range of application properties. 'There is no good way to get to this molecule from petrochemicals," Shanks said. "But biology can do so many things."'[35] By 2018, DOE had awarded CBiRC with $2.5M to advance the CBiRC bioprivileged molecule concept, and technology translation efforts began for bioadvantaged nylon utilizing muconic acid as a bioprivileged molecule.

This demonstrated capacity for learning and progressing over time also impacted the ERC's educational efforts. As the ERC became more engaged with spinning out start-up firms, they developed a course in entrepreneurships for graduate students and postdoctoral scholars with an emphasis on novel biobased chemicals. That emphasis had other impacts on education at Iowa State and beyond:

- The undergraduate organic synthesis laboratory at Iowa State University began examining the synthesis of compounds using TAL as the starting reactant. The

[35] https://www.news.iastate.edu/news/2017/08/25/bioprivileged

synthesized compounds were then sent to an undergraduate microbiology laboratory course for testing of their antimicrobial activity.

- The synthesis of TAL derivatives was carried out at a partner university, Tuskegee University, to strengthen the research aspects of that collaboration.
- The interaction of biological and chemical catalysis researchers, which is still unique to CBiRC, created an environment in which researchers educated in their areas of expertise were exposed to new interdisciplinary ideas.
- The Center partnered with the Des Moines Public Schools to support teachers in making engineering and science exciting to their students through placing graduate students one day per week in elementary, middle school, and high school science classrooms as well as leading summer enrichment programs for middle and high school science and technology teachers. From those interactions, top underserved high school students were identified to participate in the ERC's Young Engineers and Scientists program.
- The Center improved its Research Experiences for Undergraduates program to increase the role of mentoring by graduate students, supported by training programs, and expanded outreach to students underrepresented in engineering.

3. Bioengineering and Medicine

Starting in the 1990s, it was becoming clear that ERC Program recognition and support for the emerging field of bioengineering was serving as a proving ground for new ideas at the interface of engineering and medicine. The role of bioengineering in medicine—as opposed to biomedical engineering, with its device focus—was not fully recognized at the National Institutes of Health and support for high-risk fundamental bioengineering research with potential medical applications was not readily forthcoming. It wasn't until 2000 that the National Institute of Biomedical Imaging and Bioengineering (NIBIB) was established, as the potential for this new field to contribute to clinical medicine was then recognized.

The sections below provide detail on the roles of several ERCs to advance bioengineering at the interface with medicine. The work at BPEC I on therapeutic proteins and biopharmaceuticals in the 1980s and, especially through the support of BPEC II, advanced the role bioengineering played in biopharmaceuticals and medicine. The Montana State University ERC for Interfacial Microbial Process Engineering funded in 1990 explored the biological foundations of biofilms and their role in disease. Three other ERCs also were proving grounds for new high-risk roles for bioengineering in medicine: the University of Washington Engineered Biomaterials ERC (UWEB, Class of 1996), the Georgia Tech/Emory Center for Tissue Engineering (GTEC, Class of 1988), and the North Carolina A&T State University ERC for Revolutionizing Metallic Biomaterials (RMB, Class of 2008).

The original BPEC (BPEC I) was funded by NSF for 10 years and upon recompetition in 1995, it was reestablished with a new focus in the field and, later, new leadership. It is the only ERC to be reestablished for a new 10-year period. The second BPEC, or BPEC II, was initially led by Professor Daniel Wang, who transitioned leadership to Professor Douglas Lauffenburger in 1998, when DuPont provided a significant grant to Wang to continue to pursue the bioprocess goals of the original BPEC. The focus of BPEC II was initially on therapeutic protein quantity, quality, aggregation stability, formulation, and delivery,

following on from the research of the original BPEC. By 1998, in response to input from site visit teams and advisory committees, those areas were phased out of NSF support and transitioned to the now DuPont-supported BPEC I. In their place, BPEC II leaders shifted the vision to therapeutic gene biotechnology through stem cells and vectors for *in vivo* and *ex vivo* approaches for transgene delivery and regulation, signaling the rapidly changing capacity and challenges in bioengineering.

Another significant achievement of BPEC II was the broad-based impact of the ERC on the culture of research and education at MIT. The collaboration between engineers and biologists that underlies the whole history of BPEC I and BPEC II, began haltingly before BPEC I was funded, and strengthened in depth and breadth through the life span of BPEC I and BPEC II. It resulted in the creation in 1998 of a new department – Biological Engineering Division – that was built on the foundation of interdisciplinary collaboration established by BPEC I and fueled by joint appointments and joint, cross-disciplinary teaming in education and research.

Professor Linda Griffith joined BPEC II in 1998 and began an innovative research career and a campaign to strengthen the educational underpinnings for the field of biological engineering at MIT. This effort led to a new Bioengineering Ph.D. program, established in 1999. In 2003, BPEC II's leadership transitioned to Linda Griffith after Lauffenburger assumed the role of leading the new interdisciplinary Biological Engineering Division, with 20 faculty and 125 Ph.D. students.

In addition to her highly interdisciplinary and exemplary research, Griffith led an interdisciplinary team of professors who convinced MIT to establish the first new undergraduate major in 29 years at MIT, the Biological Engineering "Course 20" which began operation in 2005.[36] At the establishment of Course 20, MIT announced that it "is the first university in the nation to take the step of fusing molecular and cellular bioscience with engineering to create a new biological engineering discipline. Many other universities and medical schools offer biomedical engineering (or bioengineering) programs aimed at applying engineering to medicine, and there are biological engineering programs that have an entirely different focus--generally mainly on agriculture. But an engineering discipline grounded in molecular and cellular biology, enabling a broad spectrum of applications, including but not focused on medicine, has not been established before now. Other universities are expected to be influenced by MIT's approach."[37]

The research achievements of BPEC II rest on its "commitment to bring analysis, design and synthesis into the mainstream thinking of molecular cell biologists and clinicians developing molecular and cell therapeutics—to change the mindset, at least in a part of the community, toward quantitative analysis that can predict cell, tissue, and organism behavior in ways not previously possible."[38] Initially, BPEC II focused its research efforts on two classes of engineered gene delivery systems: (1) an *ex vivo* approach employing

[36]http://news.mit.edu/2005/major

[37] Ibid.

[38] Griffith, Linda G. (2005). *Biotechnology Process Engineering Center, an Engineering Research Center, Final Report*. Boston, MA: Massachusetts Institute of Technology, p. 29.

genetically engineered stem cells, and (2) an *in vivo* approach employing targeted viral or synthetic vectors. This research led to advances in fundamental knowledge, such as:

- New protein cues to regulate stem cell behavior;
- New ways to parse cue-signal response relationships of stem cells by applying engineering analysis and computational tools;
- New genetic mechanisms of stem cell regulation, and cell regulation generally, through identification of new types of RNA and through the methylation of genes, enabled by predictive models of cell behavior;
- Advances in understanding of how molecules are moved through cells to carry out cell functions, enabled by advances in synthesizing new molecules with specific properties to make them sensitive to particular nodes in intracellular trafficking patterns;
- Improved tissue mimics—models that are more like real tissue than cell culture models, cheaper, and can be made from human cells; and
- A microscale perfusion reactor to support 3D liver culture, including support of delicate liver sinusoidal endothelial cells, applying the liver system to a variety of important physiology and drug development problems, including liver inflammation and drug toxicity, and as a model of single-cell cancer metastasis growth and response to chemotherapy.

While the ability to impact gene therapy vectors and stem cell therapy based on these advances was slowed by clinical setbacks in the field, industry used these advances to advance molecular therapeutics.[39] In addition, the profound shift in emphasis from bioprocess engineering in BPEC I to the more fundamental biological engineering vision of BPEC II enriched the research environment at the interface of biology, engineering, and medicine. This culture produced a whole new generation of biological engineers who would go on to fuel more advances in biotechnology in industry and more frontiers in research at the interface of biology and engineering, such as synthetic biology, in academe.

4. <u>From Slime to Biofilms</u>

While chemical engineers and cell biologists were collaborating to improve the knowledge of how to control cellular mechanisms and solutions of cells to enable efficient processing of mammalian cells for new pharmaceuticals, a few chemical engineers and microbiologists, initially independently, began to explore bacterial growth in aquatic systems/environments.[40] In the 1960s, microbiologist J. Van Houte explored clusters of bacteria in dental caries and plaque. In the 1970s, other microbiologists, led by J. William (Bill) Costerton at the University of Calgary, were observing clumps of bacteria in environmental and medical contexts, which were heterogeneous distributions of physiologically distinct populations of bacterial cells encased in "slime." In 1978, Costerton was working on the predominance of aggregations of bacteria in aquatic environments in

[39] Synthesized from Ibid., pp. 17-19.

[40] The following material is synthesized from a tribute to Bill Costerton on YouTube entitled, "Dr. Bill Costerton, the 'Father' of Biofilms" (https://www.youtube.com/watch?v=M_DWNFFgHbE; and from material prepared and submitted in annual reports by the Center for Biofilm Engineering, Montana State University.

the form of a slime. Costerton's early observations of biofilms indicated that the bacteria grew and thrived as colonies in water encased in slime or in other protective material inside a body. He observed that the bacteria functioned like colonies, creating microhabitats, sticking to surfaces and covering themselves with a slimy layer of protective molecules, which he named biofilms. Prior to these discoveries it was assumed that bacteria were "free agents" in these environments, like plankton, and as such they could be easily killed with doses of antibiotics or, in non-human environments, chemicals.

Costerton and his microbiology colleagues in Canada demonstrated the existence of biofilms and went on to show that biofilms are the dominant mode of growth for bacteria. The elaboration of an extensive sugar network, an exopolysaccharide, that adheres bacteria to surfaces and subsequently buries them was revealed. Research over a decade demonstrated the importance of this exopolysaccharide in enabling the bacteria to survive doses of antibacterial agents, including antibiotics that readily killed bacteria grown in conventional lab cultures. This research was so convincing that an initially skeptical scientific community became convinced of the importance and widespread nature of biofilms.[41]

By the 1970s, engineers also were observing interfacial microbial processes in the form of slime in wastewater treatment facilities and the fouling of industrial systems. The engineering leader in these investigations was William Characklis, who moved from Rice University to Montana State University (MSU) in 1979. During the 1970s and 1980s, Characklis and other chemical engineers were applying engineering techniques to better understand the nature and impact of bacterial slime/biofilms in industrial/environmental settings and to explore chemical methods to control or obliterate it.

After Characklis moved to MSU, he formed an interdisciplinary institute, the Institute for Biological and Chemical Process Analysis (IPA), where he joined chemical engineers and microbiologists to explore the nature of biofilms and how to control and destroy them. Characklis cross-trained in microbiology so he could serve as the bridge between the two disciplines. At the same time, in Canada, Costerton and his student, Gil Geesey, were exploring biofilms and established "interspecies cooperation between bacteria within biofilms and discovered that bacteria within these sessile populations are inherently resistant to biocides and antibiotics at concentrations as much as 1,500 times those that kill floating (planktonic) cells of the same species."[42]

Fate would have it that these two groups would eventually merge due to a triumph and a tragedy. The triumph was the awarding in 1990 of an Engineering Research Center to Montana State University, with Characklis serving as the Director of the new ERC. He and his MSU team named the ERC the Center for Interfacial Microbial Process Engineering—an interesting title, given that the focus of the ERC was on slimes that fouled industrial and wastewater treatment processes, which had already been named *biofilms* by Costerton. Characklis expanded the team to bring in a broader range of faculty from engineering,

[41] Ibid.

[42] Center for Biofilm Engineering (1996). *Year Six Report and Renewal Proposal.* Boseman, MT: Montana State University, p. 2.

science, and mathematics. At the same time, he began more formal collaborations with the Costerton group.

Given the engineering motivation to not only understand the nature of a biofilm but also to control and destroy it, the ERC team expanded the biofilm research tools to include the new confocal scanning laser microscopy (CSLM) which enabled real-time scanning of successive planes in living biofilms in real time. The important discovery was that biofilms have an architectural structure, functioning as complex microcolonies of bacteria with channels that allow the passage of water and nutrients to the microcolonies. This discovery led the team to try to understand how this structure was formed and maintained. However, before they could complete this inquiry, fate intervened and Characklis met an untimely death from lymphoma at the age of 50 in 1992.[43]

NSF was concerned about the leadership of the ERC and considered winding down funding, since there had been difficulties at start-up in integrating the chemical engineers and the microbiologists at MSU into a fully functioning team. However, MSU leadership came up with a creative solution. They proposed to hire Costerton as the Center Director to lead the center and to bring a bioengineer from Duke, James Bryers, on board as the Director of Research.[44] Bryers and his collaborator, Dawn Applegate (a BPEC graduate), had been working in the field and discovered the effects of nutrient limitations on biofilm removal.[45] Once NSF approved the change, Costerton transferred to MSU and brought members of his biofilm group, Gil Geesey and David Davies, to the ERC.

With the new leadership on board, the name of the ERC was changed to the Center for Biofilm Engineering (CBE). Costerton and his ERC colleagues were determined to understand the biological nature of these films so they could engineer them in order to control their formation and use. They continued to use confocal microscopy and physical probes to examine the structure of the films, continuing to examine their complex, sophisticated architectures. The biofilms were seen to form slimy tower- and mushroom-shaped structures, with water channels that carried nutrients to all parts of the community. The outcome of the impact of knowledge gained from exploring biofilms with an interdisciplinary perspective and examining the films through confocal microscopy was a major discovery by a team of researchers from MSU (David G. Davies), the University of Iowa (Matthew R. Parsek and E.P. Greenberg), and the University of Rochester (James P. Pearson and Barbara H. Inglewski). They found that the microcolonies set up communication channels using cell-to-cell signals, or quorum sensing. They published a seminal article in *Science* that established this wholly new perspective on biofilms and presented pathways for possible means to control and destroy biofilms in industrial and wastewater processes and within the human body.[46]

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[43] http://www.biofilm.montana.edu/people/william-g-characklis.html

[44] See Section 4-D(e)iii, in Chapter 4 for a first-hand story of how MSU officials solved the problem.

[45]Applegate, Dawn H. and James D. Bryers (1991). Effects of carbon and oxygen limitations and calcium concentrations on biofilm removal processes. *Biotechnology and Bioengineering,* Jan. 5, 1991, *37*(1):17-25.

[46] Davies, David G., Matthew R. Parsek, James P. Pearson, Barbara H. Inglewski, J.W. Costerton, and E.P. Greenberg (1998). The involvement of cell-to-cell signal in the development of a bacterial biofilm. *Science, 280*, 10 April 1998.

The paper's lead author, David Davies, explained that bacteria have a chemical language that governs the structure of a biofilm. The bacteria are excreting homoserine lactones all the time, but when enough bacteria gather, something called quorum sensing occurs. The homoserine lactones start to diffuse back into the cells and trigger genetic changes within the bacterium. The bacterium starts making slime and a biofilm is the result. The signaling process takes about 15 minutes, according to Davies. When working with a strain of, *Pseudomonas aeruginosa*, that could not make the primary lactone quorum-sensing chemical, Davies found they could grow only "wimpy" biofilms that formed in sheets, lacking the complex and resilient slime structure that characterizes a healthy bacterial community. "This shows that the biofilms we all know and love are determined by the presence of these molecules," Davies said. "If we can knock out the ability to communicate, we can disperse the biofilm."[47] Figure 2 is a visualization of the biofilm and quorum sensing developed by the ERC.

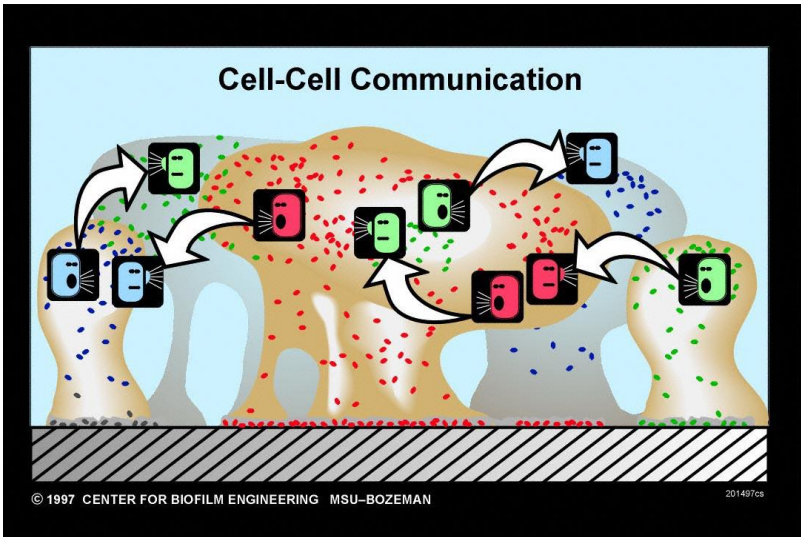

Figure 2: The CBE's elucidation of the mechanisms by which cells in a biofilm communicate was a much-heralded discovery. **(Source: CBE)**

This discovery put the ERC on the map of innovative bioengineering research centers and helped to more fully establish biofilms as a new field in bioengineering with profound impacts in medicine, dentistry, and environmental engineering. Industry support continued to grow and because of these new directions a broader array of firms became members of the ERC.

Without the signaling molecule, the cells cannot make a biofilm and the bacteria that are "locked" in the floating planktonic mode-of-growth are readily killed by host defenses and by antibiotics or chemicals. This interdisciplinary team of engineers and biologists sought to discover a class of compounds that could prevent biofilm formation. They saw that the applications in industry and medicine were legion, including the protection of patients from device-related biofilm infections, cystic fibrosis, prostatitis, etc. They postulated that if they could manipulate at least one and probably many more behaviors of bacterial cells,

[47] https://www.sciencedaily.com/releases/1998/04/980413000709.htm

instead of simply killing them with toxic agents that harm the environment or the host, they could use these simple nontoxic molecules—active blocking analogues—for biofilm control in industry and in medicine and dentistry. They envisioned a new era in which chemical manipulation would replace indiscriminate killing with toxic agents. Researchers and clinicians have since come to understand that a wide range of infections within the body are caused by the adhesion of bacteria to tissue and their formation of biofilms—e.g., cystic fibrosis, prostatitis, and bladder infections—as well as to implants within the body, such as catheters and pacemakers. In addition, plaque that adheres to teeth is started by biofilm, which calcifies to form tartar.

These challenges inspired a new generation of bioengineers and microbiologists to continue exploring the fundamental nature of biofilms and learn how to control or eradicate them. However, biofilms proved harder to kill—especially within the body—than they envisioned. In contrast to their free-floating "relatives," biofilms resist treatment with antibiotics for several reasons including:

Restricted Penetration: Restricted penetration of antimicrobials through the biofilm matrix can, in some cases, contribute to the antimicrobial tolerance that biofilms display. Although biofilm matrices do not inhibit diffusion of antibiotics in general, restricted penetration of antibiotics through biofilms may occur in cases where the antibiotics bind to components of the biofilm matrix or the bacterial membranes such as extracellular DNA, or when the antibiotics are inactivated by enzymes present in the matrix, such as beta-lactam inactivation by beta-lactamases.[48]

Differential Physiological Activity: Differential physiological activity of the bacteria in biofilms is another underlying cause of biofilm-associated antimicrobial tolerance. Studies of *Pseudomonas aeruginosa* biofilms have provided evidence that the metabolic activity of the bacteria is high in the outer part of the biofilm whereas it is low in the inner part of the biofilm. The available evidence suggests that the differential physiological activity seen in biofilms is caused by limited oxygen and nutrient penetration through the biofilm due to bacterial consumption. Because many antibiotics target processes that occur in growing bacteria (e.g. replication, cell wall synthesis), biofilm bacteria with low metabolic activity display increased antimicrobial tolerance to these kinds of antibiotics.[49]

Survival Mechanisms: Under stressful conditions—such as the presence of antibiotics—quorum sensing in oral *Streptococcus mutans* leads to a dramatic increase in what are known as "persister" cells—members of the bacterial colony that lie in a dormant state. Because they are not actively growing, these cells are not susceptible to antibiotics. Even if the rest of the colony is wiped out, once the course of antibiotics is finished the persister cells can awaken to re-establish the biofilm. Persister cells are now thought likely to be the

[48] Ciofu, Oana, Estrella Rojo-Molinero, Maria D. Macia, and Antonio Oliver (2017). Antibiotic treatment of biofilm infections. Review Article. *Wiley Online Library* (https://doi.org/10.111/apm.12673. p. 2.

[49] Ibid.

main cause of recurrent, chronic infections throughout the human body, even though they do not display standard antibiotic resistance mechanisms.[50]

Thus, Phil Stewart, a former Director of the ERC, noted in correspondence with Preston in 2019 that "No quorum sensing inhibitor has yet been commercialized for medical application, though research and interest in the potential of such drugs remains intense. New inhibitors continue to be discovered and synthesized and tests of the anti-virulence properties of these molecules have been demonstrated in the lab and in animals. The only quorum sensing inhibitor we are aware of that is being actively developed by a company at this time is a sulfur-containing compound, ajoene, that occurs naturally in garlic. This compound attenuates the virulence of the biofilm-forming bacterium *Pseudomonas aeruginosa,* allowing the immune system to more effectively clear infections. Ajoene targets the same microorganism and same quorum-sensing circuitry described in the seminal paper from CBE in 1998."[51]

However, researchers have found that the cell-to-cell signaling that is crucial to biofilm formation and stability is interrupted by electrical currents and ultrasound. Shock wave therapy has been used to treat chronic wounds. A team in India "demonstrated the use of two different shock wave generators for the disruption of biofilms formed *in vitro* as well as *in vivo* conditions. The biofilms formed by *Salmonella*, *Pseudomonas,* and *Staphylococcus* on urinary catheter surfaces have been exposed to shock waves produced by a hand-held device. A diaphragm-less shock tube has been used for *in vivo* treatment of *Pseudomonas* lung infection and *Staphylococcus* skin suture infection in mice. This is first time that an *in vivo* study of shock wave treatment of biofilms has been presented. The shock waves generated for the *in vivo* study are low amplitude and repeatable. The studies show that, in combination with antibiotic therapy, shock waves have the potential to treat lung and skin infections caused by bacteria."[52]

5. Bioengineered Materials

Buddy Ratner, the Director of the University of Washington Engineered Biomaterials (UWEB) ERC envisioned implants that would function inside the human body without triggering inflammatory response that eventually interrupts their functionality or renders them useless or even harmful. The goal of the ERC, he said, was "to engineer materials that on a molecular level look like normal body parts and induce a natural healing response."[53] Normally, when foreign materials such as an implant are introduced into the body, the natural inflammatory response triggers cells to produce fibrous collagen, which forms scar

[50] Levesque, Celine (2018). Bacterial Biofilm, the New Super Organism. https://researchfeatures.com/2018/03/07/bacterial-biofilm-new-superorganism/

[51] Stewart, Philip (2019). Personal correspondence via email with Lynn Preston.

[52] Divya Prakash Gnanadhas, Monalisha Elango, S. Janardhanraj, C. S. Srinandan, Akshay Datey, Richard A. Strugnell, Jagadeesh Gopalan, and Dipshikha Chakravortty (2015). Successful treatment of biofilm infections using shock waves combined with antibiotic therapy. *Sci. Rep*. 2015; *5*:17440. Published online Dec 10 2015. doi: 10.1038/srep17440

[53] Feder, Toni and Jean Kumagal (1996). New NSF research centers will focus on biomaterials and semiconductors. *Physics Today 49*:7, 54 https://doi.org/10.1063/1.2807687

tissue around the implant, disrupting its functionality, a process termed the foreign body response (FBR). The Center was organized to bring together an interdisciplinary team that Ratner had been building at the university with expertise in bioengineering, biomaterials, biophysics, cell biology, chemistry, and medicine. They were joined by industrial members from firms specializing in medical devices, implants, and other treatment modes. Their ambitious vision was at that point outside the realm of NIH support, which was why NSF would invest in the fundamental research and technology exploration needed to advance biocompatible materials. The goal was to use an engineered-system construct to understand the foreign body response and the interplay of non-fouling and porous materials to minimize that response.

The ambitious goal was not only to understand the FBR process but to control it. Most importantly, UWEB was the first group to establish a systematic approach that relies on the use of (1) genetic knockouts (gene-specific deficient mice) to study the role of influential signaling proteins, (2) *in vivo* implantation, and (3) local gene delivery to elucidate the chief biological processes of the FBR. "These critical systems have been used successfully to dissect the complex biology of implant healing. More importantly, UWEB has demonstrated, as proof of principal, that many contributory factors to the foreign body reaction and other pathological responses can be controlled favorably by the presentation of the correct biological signal. These fundamental genetic methods have not been applied to the biomaterials problem outside of UWEB. Examples of knockout mice and the signaling discoveries made with them include the following proteins: osteopontin, which controls ectopic calcification; thrombospondin-2, which controls angiogenesis; SPARC, which controls the behavior of collagen fibers; and MCP-1, which controls the migration of macrophages and possibly the formation of foreign body giant cells. Studying and controlling the biomaterial-tissue interface in this fashion was not possible without these knockout mice providing critical infrastructure to the research program."[54]

Through this work the UWEB team demonstrated that many FBR and other pathological responses can be controlled by: (1) producing the specifically correct biological signals to block the function of TSP-2 (thrombospondin-2), which regulates the growth of blood vessels as well as the formation of the collagen matrix, or scar tissue, around the implant; (2) blocking the function of SPARC, which plays a role in collagen formation; and (3) blocking the chemokine MCP-1 to prevent the formation of foreign body giant cells surrounding the implant. The team showed that osteopontin (OPN) can control the deposition of hydroxyapatite that is responsible for bioprosthetic heart valve failure. They created a group of biomaterials based on their chemical composition and porous architecture that can promote angiogenesis upon implantation and a special porous biomaterial through unique templating, which was optimized and substantiated the predictive model that can be used to improve vascularization.[55]

Given the 10-year time frame of ERC support, the long-term fundamental nature of these findings and the need for clinical trials before implementation, UWEB's major impact on

[54] This section is synthesized from the following document: Ratner, Buddy, UWEB Research Program, Strategic Vision and Accomplishments (2007). Seattle, WA: University of Washington. pp. 1-8.

[55] Ibid.

the medical device industry was not possible in that time-frame. Because the medical device industry does not readily engage in the early-stage research needed to move breakthroughs in knowledge into technology (translational research), UWEB's solution at the advice of industry was to focus on spin-off firms. "The industry prefers that small firms assume the risk of early stage technology development and to become involved (via merger/acquisition or in-licensing) when the technology has been sufficiently advanced in terms of proof of principle, manufacturability, and market assessment. In other words, the vast majority of UWEB's industrial sponsors generally engage in product development and not technology development."[56] As a consequence, in 2006 Ratner formed the Ratner Biomedical Group (RBG) as an incubator of companies. RBG deploys its licensed intellectual properties that are focused on medical devices, biocompatible materials, tissue engineering, and drug delivery. RBG provides early-stage funding for startups in its portfolio and helps them to develop the management teams and the corporate partnerships that are necessary for sustained growth and capitalization.

One firm, Healionics, was the first firm spun out of UWEB by RBG in 2007 and continues to function today. Healionics is commercializing a synthetic vascular graft (STARgraft) with superior patency (ability to maintain blood flow), based on its platform STAR biomaterial technology, invented at UWEB by Buddy Ratner and Andrew Marshall. The graft addresses the urgent need for improved vascular access for hemodialysis patients, with subsequent applications to peripheral artery disease and potentially coronary artery bypass. They are also developing a device to enable needle-free hemodialysis (*STARport*).[57] In 2017 Dr. Marshall and Healionics received a $1.7M grant from NIH in support of regulatory approval and clinical studies for STARgraft. The clinical study is evaluating STARgraft as a vascular access graft for dialysis patients and is conducted in collaboration with University of Washington Medicine.[58]

6. Explorations in Tissue Engineering

As defined by the National Institute of Biomedical Imaging and Bioengineering, "*Tissue engineering* evolved from the field of biomaterials development and refers to the practice of combining scaffolds, cells, and biologically active molecules into functional tissues. The goal of tissue engineering is to assemble functional constructs that restore, maintain, or improve damaged tissues or whole organs. Artificial skin and cartilage are examples of engineered tissues that have been approved by the FDA; however, currently they have limited use in human patients. *Regenerative medicine* is a broad field that includes tissue engineering but also incorporates research on self-healing—where the body uses its own systems, sometimes with help from foreign biological material, to recreate cells and rebuild tissues and organs. The terms "tissue engineering" and "regenerative medicine" have become largely interchangeable, as the field hopes to focus on cures instead of treatments for complex, often chronic, diseases. This field continues to evolve. In addition to medical

[56] Ibid. p. 10.

[57] http://healionics.com/applications/

[58] http://healionics.com/wp-content/uploads/2017/11/Healionics-Press-Release-171116-SBIR-Clinical-Fast-Track-v0.6.pdf

applications, non-therapeutic applications include using tissues as biosensors to detect biological or chemical threat agents, and tissue chips that can be used to test the toxicity of an experimental medication."[59]

That is a relatively optimistic definition of tissue engineering and regenerative medicine, given the complexities of developing viable tissue substitutes that can survive in a body and restore the desired function. A cautionary view is provided by an analysis of the history of tissues engineering research and applications in medicine by Martin Yarmush and his colleagues:

"Tissue engineering and regenerative medicine is an extremely interdisciplinary and complex field that requires a deep understanding of the effect of a myriad of factors on the development and sustainability of tissues and organs. The early successes with skin and cartilage encouraged many to posit that they could place any cell type in a matrix and then implant the resulting construct into the body with the hope of success. These simplistic and fanciful notions were a major factor in the so-called crash of the tissue engineering field in the 1990s, which was fueled by hype, overpromising, gullible early investors, and bad science. What worked with tissues that are relatively avascular and have low metabolic rates was doomed to fail when applied to more complex tissues. Furthermore, function of complex tissues is also dependent on proper homotypic and heterotypic cell-cell interactions, which requires spatial control of the various components (i.e., parenchymal cells, blood vessels, and nonparenchymal cells) at the micrometer scale."[60]

From these definitions of tissue engineering and regenerative medicine, it is clear that the explorations of the interface of biology and engineering in medicine at BPEC II and UWEB have contributed to the field and it is also clear why these advances were slow to impact medicine. Another ERC, funded in 1998, with equally ambitious goals in the then-nascent field of tissue engineering, was the Georgia Tech/Emory Center for Engineering of Living Tissue (GTEC), which used the ERC systems construct to explore how to develop and "manufacture" cardiovascular tissues, metabolic/secretory cell systems, neural tissues, and orthopedic tissues. These were ambitious goals, which as Yarmush and his colleagues cautioned above, proved to be a bit too ambitious, given the realities of introducing new constructs into the body to replace tissue. As a consequence, the weight of the Center's research vision and funding shifted toward an increased level of fundamental inquiries as it transitioned its vision over time to regenerative medicine. Nevertheless, this ERC served as a platform to develop new capacity to work at the interface of bioengineering and medicine and its Director, Robert Nerem, served as a leader of tissue engineering and regenerative medicine, working at the interface of engineering and medicine through the NIBIB (2003–2006) and serving as the Founding President (1992–1994) of the American Institute of Medical and Biological Engineering.

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[59] https://www.nibib.nih.gov/science-education/science-topics/tissue-engineering-and-regenerative-medicine

[60] Berthiaume, Francois, Timothy J. Maguire, and Martin L. Yarmush (2011). Tissue engineering and regenerative medicine: History, progress, and challenges. *Annual Review of Chemical and Biomolecular Engineering 2011*(2):403-30. p. 403.

7. Biodegradable Materials

Another ERC was funded in 2008 to explore frontiers in biodegradable materials that could be introduced into the body, for example as stents or in the form of screws, etc. to fix broken bones, which would degrade over time as the bone healed. An example of a critical need is in treating patients with coronary artery blockages. Balloon angioplasty is widely used when coronary bypass surgical procedures can be avoided. Without further treatment, about 30% re-stenose (become blocked again); hence, metallic wire mesh stents are also inserted, which reduce the restenosis rate to about 10%. However, the stent induces a foreign body response. This may cause scar tissue (cell proliferation) to rapidly grow over the stent. In addition, there is a strong tendency for clots to form at the site where the stent damages the arterial wall. Unfortunately, if arterial restenosis occurs, the stents are difficult to remove, so additional stents are instead inserted in adjacent areas. This increases the probability of further complications, possibly requiring major surgery. Resorbable stents would solve this problem and insertion would be a relatively minimally invasive procedure.

Research at the time the ERC started had shown the promise of magnesium (Mg) alloys as a new class of biodegradable metals for use in stents as well as for orthopedic applications. Mg is exceptionally lightweight and about five times less dense than aluminum and steel. Mg also has greater fracture toughness than ceramic biomaterials such as hydroxyapatite (HAp), while its elastic modulus and compressive yield strength are closer to those of natural bone than other commonly used metallic implants. These attributes make it an ideal candidate for scaffolds, fixation plates, and implant applications.

To address these opportunities, the ERC for Revolutionizing Metallic Biomaterials (RMB) was established as a partnership between the North Carolina Agricultural and Technical (NCAT) State University (an HBCU[61]), the University of Pittsburg (Pitt), and the University of Cincinnati (UC), The Director of the ERC is Jagannathan (Jag) Sankar (NCAT) and the two deputies are William Wagner (Pitt) and Mark Schulz (UC). In the process of competing for the award, the team developed a relationship with Professor Frank Witte, MD, Ph.D., then from the University of Hannover, Germany.

When the award was made, Witte joined the team and brought to the ERC first-hand clinical trial experience in Germany with biodegradable stents made from Mg alloys. This partnership grew over time, even as Witte later moved to Berlin, where he serves (at time of writing) as Professor for Bioactive Implants, Charité–Universitätsmedizin. He travelled back and forth to the ERC, especially to the ERC partner, the McGowan Institute for Regenerative Medicine at the University of Pittsburg Medical School, where he became an Adjunct Professor.

The RMB ERC has been quite successful, for such a high-risk area in bioengineering. Examples are:

- In 2013, a study of the corrosion of RMB's new magnesium alloys using nanotomography revealed deeper insights into their *in vitro* and *in vivo* behavior in terms of types and mechanisms of corrosion product formation. This investigation

[61] Historically Black Colleges and Universities.

for the first time helped bridge the gap between static and dynamic environments. The knowledge gained allowed the team to establish protocols for the corrosion testing of biodegradable metals, with an emphasis on experimental design. Iterative application of these protocols informs the judicious selection of alloying elements that lead to a better Mg system with smaller and more uniform grain boundaries and smaller cathodic areas.

- In 2014, with support from the NSF Small Business Innovation Research Program, nanoMAG LLC, a medical device company headquartered in Ann Arbor, Michigan, developed a bioabsorbable alloy called BioMg 250 that has twice the strength of polymers used in commercial bioabsorbable implants. The material is made of magnesium alloyed with small amounts of a ternary combination of elements that are naturally found in the body—zinc, calcium, and manganese—which stimulate new bone growth. Microalloying enables superior strength and bioabsorption and prevents the formation of large intermetallic particles that can accelerate corrosion. nanoMAG is continuing the development of the material in partnership with the University of Michigan, Pitt, and NCAT. Researchers at NCAT conducted *in vivo* studies to measure the interaction of BioMg 250 screws with bones and tissue and to assure nontoxicity. Histology studies revealed no sign of cell toxicity.[62]
- In 2015, InoVasc, an ERC start-up, developed the trade name, Flo-Fluent, for a biodegradable drug-eluting (releasing) stent that can be placed into narrowed, diseased peripheral or coronary arteries that slowly releases a drug to block cell proliferation. The new stent is the culmination of the ERC's research and successful testing. In a month-long *in vivo* testing in pigs, the biodegradable stent demonstrated the ability to dilate and increase blood flow in an arteriovenous fistula (AVF), with no observed negative response. Collaborative effort within RMB on the new stent included: incorporation of an anti-proliferation polymer coating and a novel magnesium (Mg) alloy; the experiment model; and design, simulation, and manufacturing technology. During testing, hydrogen sensors were used to measure degradation of the stent. The RMB has established intellectual property rights to most of the technologies involved in the new stent and increased industrial interest in the technology is leading to new collaborations.[63]

8. Conclusions

A number of conclusions can be drawn from the history of the bioengineering ERCs, which are somewhat different from other, more mainstream engineering ERCs that depend less on science.

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[62] NSF (2014). Bioabsorbable magnesium alloy doubles the strength of orthopedic implants. *Chemical Engineering Progress*, American Institute of Chemical Engineers, December 2014. https://www.nanomag.us/pdfs/Bioabsorbable_Mg_Alloy_Doubles_Strength_of_Orthopedic_Implants_2014_12_14.pdf

[63] RMB (2015). From Highlights, 2015 annual report. "Advanced Stent Offers Better Care for Kidney Patients" (file in Sharepoint, file RMB2-T..) http://erc-assoc.org/content/advanced-stent-offers-better-care-kidney-patients

Bioengineering ERC's provide an excellent example of how engineers form cross-disciplinary efforts, reaching into fundamental science, to develop transformative technologies and make major impacts on society. The disciplines range from biosciences (molecular biology, biochemisty, microbiology), environmental sciences, and medical disciplines to all of the engineering disciplines and even including social sciences to build a strong ethics component. This has allowed bioengineering ERCs to be an excellent platform for development of the next generation of engineers, particularly as these centers emphasize cross-disciplinary efforts and team research—often more broadly than other ERCs, which can be more focused on engineering and physical sciences challenges.

The ambitious goals of bioengineering ERCs, however, are often not achievable in the 10-year timeframe of NSF funding due to the need for advances in fundamental science and engineering before applications can be established. Bioengineering ERCs, starting with BPEC I in 1984, have consistently been based upon strong fundamental science. When more fundamental scientific understanding was required to achieve the bio ERCs' goals, however, the development of applications supported by testbeds could be delayed. This would in turn delay strong interest by potential industrial partners, particularly when potential applications would require FDA approvals for commercialization. This situation led to a need for follow-on funding and efforts to de-risk identified applications of the technologies. The result was a new emphasis on the spinning-off of startup companies from ERCs, beginning with Gen 3.

Bioengineering ERCs often struggle to build a strong base of industrial support due to the long lead times for potential applications; therefore, up front they need to focus their efforts on potential applications that would attract industrial partners, in addition to the inherently needed fundamental research efforts.

For the first time in the ERC Program, bioengineering ERCs led to a clear need to incorporate ethics into the fabric of the ERC research program. This has subsequently led to an ethics component being considered for other areas of research in ERCs, as well as stimulated the consideration of an ethics component across NSF for other funded research and in the broader biological research community.