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Building Better Bacteriophage with Biofoundries to Combat Antibiotic-Resistant Bacteria

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Abstract

Resistance to antibiotics is an escalating global crisis, presenting a major health, social, and economic burden. An underexplored alternative to antibiotic treatment is phage therapy whereby bacteriophages are used to infect and kill pathogenic multidrug-resistant (MDR) bacteria. A primary challenge is the highly specific infectivity range of phages that can limit their ability to infect across different bacterial strains. Synthetic biology can enable the design, modification, and synthesis of phages with improved antimicrobial performance and efficacy to help realize novel strategies to study and treat bacterial infectious diseases, including those caused by MDR pathogens. In this perspective article, we discuss the potential for an innovative synthetic biology approach to enhance phage therapeutics and the role a biofoundry can play in bringing phage therapy to fruition.

Keywords: bacteriophage, biofoundry, antimicrobial resistance, laboratory automation, multidrug-resistant bacteria

The Emerging Antibiotic Resistance Crisis

BEFORE THE MASS INTRODUCTION of antibiotics in the 1940s, common infections now routinely treated with antibiotics often proved fatal. The advent of antibiotics resulted in a powerful tool to address communicable diseases caused by microbes and helped to displace it as the leading cause of human death. Today, antimicrobial resistance (AMR) of pathogenic bacteria is an escalating global problem that represents a large clinical and public health burden, as the number of emerging bacterial pathogens resistant to available antibiotics rapidly increases. The World Health Organization has forecast that, by 2050, if no alternatives to current antibiotics are found, AMR may represent a global cost of USD$100 trillion and up to 10 million deaths per year, outnumbering cancer and heart disease.[1]

The Challenge of Biofilms in Infectious Diseases

Multidrug-resistant (MDR) bacteria protect themselves from antibiotics by several resistance and tolerance mechanisms that can be synergistically enhanced when the microbes are within biofilms.[2] Biofilms consist of bacterial consortia embedded in an extracellular matrix secreted by the microbes. Pathogenic bacterial biofilms form a stable three-dimensional structure conferring protection from the host immune system and antibiotics. Moreover, biofilms enhance the spread of antibiotic resistance genes through horizontal gene transfer. Thus, biofilms present a major therapeutic challenge in the clinical setting and are a critical blind spot in current antibiotic treatment.

Phage Therapy: An Alternative to Antibiotics

The global emergence of MDR pathogenic bacteria is driving the need for research into effective therapeutic antimicrobial alternatives. Phage therapy uses bacteriophages to infect and kill pathogenic bacteria (reviewed in[3]) and until recently has been an overlooked alternative to antibiotic treatment in Western medicine. A renaissance in phage-based therapies is now highly anticipated, with the safety and efficacy of phage therapy having been demonstrated in recent compassionate cases that have attracted a flurry of popular media interest. However, there is an urgent need for further research and rigorous clinical trials before wider clinical acceptance.

Phage therapy is a radically different treatment to antibiotics due to the mechanism and dynamics of bacterial lysis. Phage naturally self-amplify, producing phage progeny during infection that go on to bind and lyse target cells. Phage combine high target specificity and lower endotoxin production after cell lysis, coupled with an ability to penetrate biofilms, that unlike broad-spectrum antibiotics, is less likely to lead to adverse side effects such as disruption of the gut microbiome. Many phages have evolved biofilm-degrading

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enzymes enabling enhanced infiltration and access to bacterial hosts within biofilms. Moreover, bacteria that gain resistance to phage that target antibiotic resistance mechanisms (e.g. efflux pumps), can become re-sensitised to antibiotics due to an evolutionary trade-off[4,5] between phage and antibiotic resistance, thus presenting exciting possibilities for synergistic therapeutics.

Barriers to Development of Phage Therapy

One of the major difficulties to developing phage therapies as effective medicine is that many phages are very specific in their targeting, with a narrower target range than narrow-spectrum antibiotics. This narrow range can be problematic as the bacterial species or strain need to be cultured and identified before a therapeutic phage against it can be selected. Time has been shown to be of the essence in phage therapy, as administration earlier in infection rather than later increases positive outcomes. Furthermore, bacterial cells have myriad ways to become resistant to phage, including, but not limited to, mutation of the phage receptor, activation of the CRISPR/Cas and restriction modification systems, and exopolysaccharide production.[6,7] As a result of these considerations, effective phage therapy is likely to require a cocktail of more than one phage.

Searching for a suite of phage that is effective against a range of similar but different strains of pathogenic bacteria, and that works well together without interference, may prove to be a difficult task. Many phage with otherwise desirable targeting characteristics may also be disqualified from use as phage therapy due to being lysogenic rather than purely lytic phage. Temperate phages are generally disqualified from phage therapy because their infection cycle may unpredictably result in lysis or integration into the host genome, they can encode virulence factors (e.g., toxins), they can proffer protection to their host from infection by other phages, and their lifecycle predisposes them to being agents of horizontal gene transfer.

A further barrier to widespread development of phage therapy drugs is the issue of intellectual property (IP) protection. Since phage identical to those isolated from nature are not patentable material, other methods of safeguarding the therapy's IP must be devised, or else funding to bring it through the regulatory process will never materialize.

Could Engineered Phage Be the Answer?

Creating novel phage through bioengineering methods has the potential to solve many of the previously described problems. For example, lysogenic phage can be engineered to be strictly lytic.[8] The targeting specificity of phage can also be altered by modifying their tail fibers.[9] In addition, phage can be loaded with enzymes that enhance biofilm degradation.[8] Lastly, engineered phages are generally patentable in most jurisdictions because they involve artificial or synthetic sequences and their design and creation involves an inventive step. For example, patent US 10,174,295 B1 ''Composition of matter: Engineering of *Escherichia coli* phage K1E'' was recently granted in the United States. This patent protects several different phage nucleotide sequences that can be used as a diagnostic to determine antibiotic susceptibility within a sample by identifying whether the strains have the K1 capsule. It is generally believed that ''composition of matter'' patents are one of the strongest patent types.

The downside to developing engineered phage for phage therapy is that few engineered phages of any type have been made, much less for introduction into humans or other animals. What is needed now is a way to make and test engineered phage in a high-throughput manner to see if their great promise can be realized.

Manufacturing Mix n' Match Phage

Phage genomes are mosaic structures with interchangeable modules, making them ideal targets for redesign to improve antimicrobial performance. Drawing inspiration from their natural mosaic nature, phage genomes can be assembled from different parts sourced from a diverse group of phage and then assembled on a genomic scaffold *in vitro*, before ''rebooting'' the synthetic genome within a well-defined bacterial host.[9] All of the necessary phage-engineering methods are only just becoming available to attempt the large-scale development of a rapid, reliable, and high-throughput phage engineering platform.[8]

Specific functions, such as biofilm exopolysaccharide degradation, can be engineered into designer phage to target specific aspects of biofilms. For example, dispersin B (*dspB*) and amylovoran depolymerase (*dpoL1*) encode enzymes that degrade exopolysaccharides and have been engineered into T7 and Y2 phage. The resulting phage show enhanced cell lysis within biofilms due to their ability to disrupt the biofilm matrix and presumably expose buried cells to infection by their progeny.[6] Importantly, phage receptor-binding genes can be swapped, enabling the creation of engineered phages with increased host range, including across genera.[9]

One major hurdle toward deploying engineered phage at scale is the design, building, and testing bottleneck as currently, compared with screening natural phage against susceptible hosts, it is very labor intensive and low throughput. A new paradigm for phage bioengineering is needed that focuses on increased throughput and standardization.

Biofoundries and Bioengineered Phage Therapy

One route to solving the current low-throughput design-build-test-learn (DBTL) cycle bottleneck for producing engineered phage may be a new cutting-edge facility called a biofoundry (Fig. 1). A biofoundry is a core facility housing fully automated equipment, coupled with software, workflows, and automation specialists, which aims to enable standardized laboratory operations to be performed by robotics programmed by human operators for the production of genetically reprogrammed organisms.

A biofoundry houses cutting-edge technologies, including sophisticated genetic design software and automated liquid-handling and colony (or plaque)-picking robotics, to help accelerate the bioengineering process. In addition, a biofoundry enables automated high-throughput laboratory methods with increased efficiency and reproducibility, allowing researchers to focus on tasks that cannot be automated such as experimental design and interpretation of acquired data. A key component of the DBTL cycle is the iterative process that incorporates machine learning to improve through each cycle without user intervention. Globally, there are currently 16 biofoundries, most located in the United Kingdom and United States, with two currently under development in Australia at Macquarie University and the Commonwealth

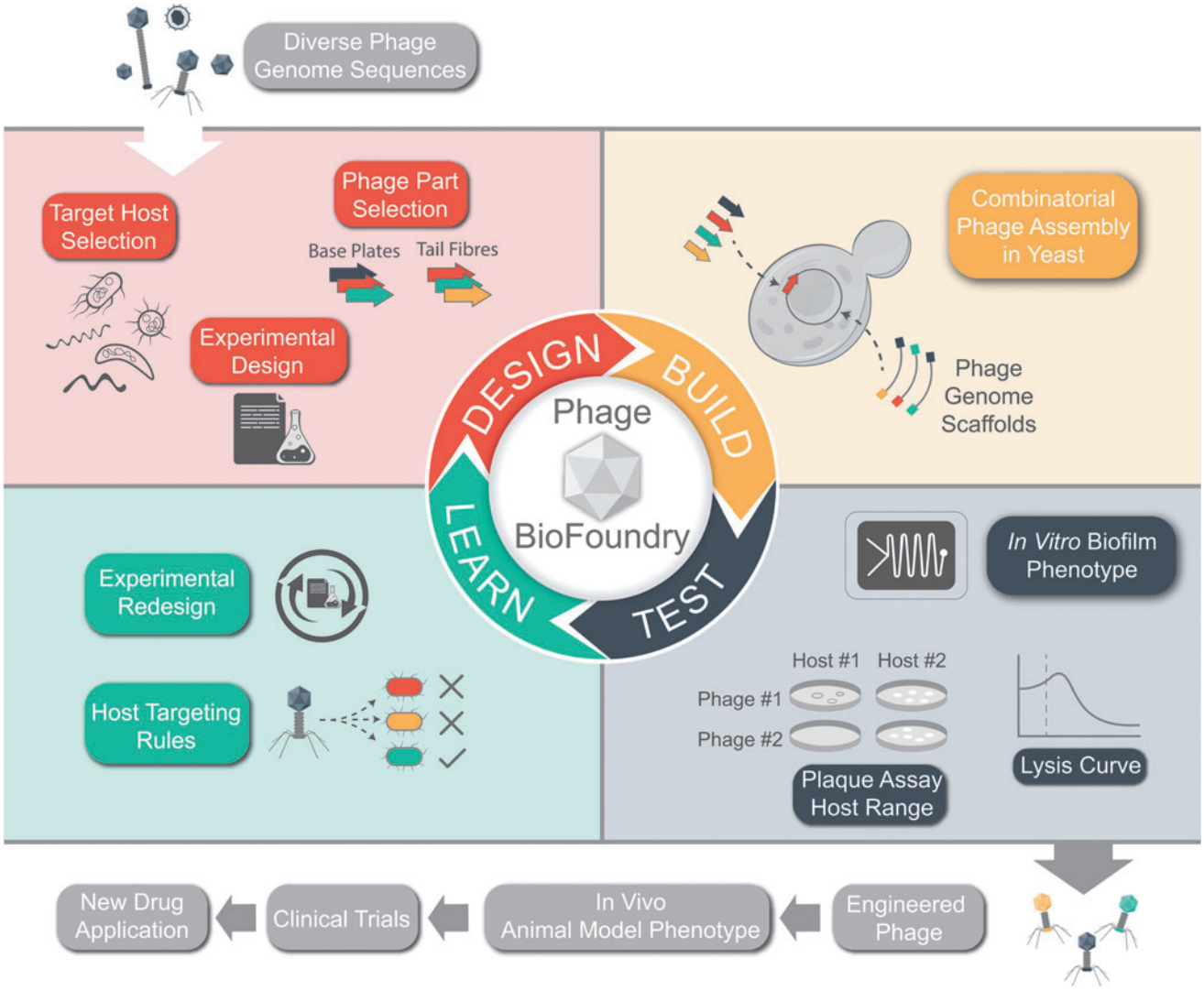

FIG. 1. The design-build-test-learn cycle of phage bioengineering highlighting key steps in each stage of the process. Phage genomes are designed *in silico* and built using a yeast recombineering approach within a biofoundry. Testing of engineered phages is performed *in vitro*, for example, using microfluidics, and the acquired data are used to perform iterative learning cycles for redesign and improvements. The resultant phages can then be tested further *in vivo* using animal models and clinical trials.

Scientific and Industrial Research Organisation (CSIRO) in conjunction with the University of Queensland. Recently, a global alliance of biofoundries was formed to coordinate their activities.[10]

To our knowledge, biofoundries have not been used to design and test engineered bacteriophage before, despite the obvious advantages gained from leveraging this cutting-edge technology. Benefits of using a biofoundry for phage work include assembling diverse phage libraries at the nanoliter scale, testing phage efficacy against different bacterial hosts, and using high-throughput antimicrobial assays performed within the biofoundry. The standardized results of this study can be collected and the genetic basis for phage host-targeting identified, thus completing the DBTL synthetic biology engineering cycle. Throughout the entire DBTL journey, each engineered phage variant would be tracked and traceable, enabling good laboratory practice/good manufacturing practice (GLP/GMP) regulatory compliance (Fig. 1). Further *in vitro* testing within the biofoundry, such as with microfluidic models of the human gut, would speed the identification of phage to be used for *in vivo* models of infection outside the foundry (Fig. 1).

Conclusion

MDR pathogenic bacteria are an emerging threat to human life globally. Engineered phage therapy could play an important future role treating highly resistant pathogens, due to the orthogonal route of attack by phage compared with traditional small molecule therapies. Building phage with enhanced infectivity characteristics is currently too labor intensive and costly to make a significant clinical impact. High-throughput phage engineering facilitated by biofoundries is needed to create repositories of clinical-grade phage to treat the most serious AMR cases across the globe.

Author Disclosure Statement

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