MANUSCRIPT

Synthetic Microbial Therapeutics for the Targeted Elimination of Antibiotic-Resistant Bacteria

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Antimicrobial resistance (AMR) is a growing worldwide concern because it threatens global health. The increase in antimicrobial resistance is attributed to the rapid evolution of bacteria, the ability of bacteria to transfer genes among themselves, and the ongoing selective pressure from the continued use of antimicrobials. New antibiotic development and/or combination therapy approaches are no longer adequate due to the increasing prevalence of multiple drug-resistant organisms. I propose that there is a way to produce engineered microbial systems that can serve as programmable therapeutic agents and can detect AMR bacteria and kill them without damaging healthy commensal microbes in our body. The engineered microbes could have pathogen detection capabilities through pathogen-sensing modules that respond to quorum-sensing signals and genetic markers indicating AMR and have the ability to produce bacteriocins, disrupt quorum sensing, and use CRISPR for the degradation of resistance genes through effector modules. These engineered microbes would act as autonomous and self-regulating therapeutic agents, enabling the targeted inhibition of AMR pathogens while avoiding any significant disruption of the normal microbiome. Thus, these systems could represent a shift from traditional antimicrobial drug development to adaptive and biologically adaptive antimicrobial systems.

Antimicrobial resistance (AMR)
Synthetic Biology
Engineered microbial therapeutics
CRISPR-Cas gene editing
Microbiome preservation

INTRODUCTION

Antibiotic resistance usually arises by 3 main pathways: spontaneous mutation, horizontal gene transfer (HGT), and the selective pressures placed on bacteria from the vast overuse of antimicrobials. Multidrug-resistant pathogens (MDR), such as carbapenem-resistant Enterobacteriaceae (CRE), methicillin-resistant Staphylococcus aureus (MRSA), and vancomycin-resistant Enterococci (VRE), are more challenging than ever to treat due to rapid evolutionary adaptation and the acquisition of resistance mechanisms. Combination therapies and new antibiotics have historically provided effective responses to emerging resistance, however, each of these responses is usually a temporary solution as their characteristic nature revolves around the fact that bacteria are constantly evolving new ways to survive.

With the recent advancements in synthetic biology, it has become feasible to engineer microorganisms for the purpose of therapy. Genetically engineered bacteria have been shown to have the ability to: (i) perceive pathogen-associated signals; (ii) produce antimicrobial compounds; and (iii) use CRISPR-Cas systems for the selective inactivation of resistance genes. However, most of the therapeutic systems used today focus on singular functions of the microorganism rather than integrating the four necessary components of an effective therapeutic system: sensing, response, containment, and ecological compatibility.

Another challenge would be to maintain the stability of the microbiome of the host. Broad-spectrum antibiotics often disrupt the homeostasis of beneficial microbial communities causing dysbiosis and an increased risk of secondary infections. If a therapeutic system could selectively target resistant pathogens while preserving microbial diversity, this would help address one of the biggest limitations of current antimicrobial therapies. This hypothesis investigates if synthetic antimicrobial therapeutic microbes can be developed as self-sustaining biological systems that constantly sense microbial environments, detect resistant pathogens, and exert specific antimicrobial responses while having minimal impact on other ecological systems and providing biosafety.

THE HYPOTHESIS

Engineered Microbial Therapeutics can be thought of as advanced biological systems that integrate environmental sensing with targeted responses to eliminate harmful microbes. The detection module may respond to various types of signals including quorum sensing molecules or genetic characteristics associated with resistance to antibiotics, thus triggering a response to these harmful microorganisms through the activation of effector pathways.

Effector mechanisms could act as bacteria-inhibiting substances (bacteriocins), by producing enzymes that inhibit growth or formation of biofilms, through the destruction of resistance genes (CRISPR gene editing), and by disrupting quorum-sensing pathways and other signaling mechanisms involved in bacterial virulence and pathogenicity. By engaging multiple different approaches at fighting microbes will minimize the chances of any pathogenic microorganism developing a resistance to the engineered microbe itself.

Biosafety systems (e.g., use of kill switches, nutritional dependencies, and environmental containment pathways) would be incorporated into engineered microorganisms to prevent unlimited growth and environmental persistence. These systems would be designed to prevent these microorganisms from surviving outside their designated environment and also from inadvertently spreading to new environments via interactions with other organisms. If engineered microbial therapeutics are programmed to detect antibiotic-resistant pathogens through quorum-sensing and resistance-associated genetic markers, then they will selectively eliminate resistant bacterial populations while preserving commensal microbiota and maintaining ecological stability.

EVOLUTION OF HYPOTHESIS

The potential utility of using microbes in place of antibiotics is evaluated through a series of studies that examine functionality, effectiveness, specificity for the environment, and safety to humans or animals. To evaluate the capacity of engineered microbes to selectively identify the antibiotic-resistant organism, inhibit the growth of these organisms while maintaining the populations of the non-resistant and normal (i.e., commensal) bacterial species, the first study would be conducted in vitro under simplified laboratory co-culture systems consisting of bacteria. The primary endpoints for the first study would be to demonstrate the selective inhibition of antibiotic-resistant bacterial strains, the reduction of the prevalence of antibiotic resistance genes in the microbial community, and the maintenance of overall stability and function of the microbial community.

Quantitative measurements would include pathogen abundance, resistance gene copy number, microbial diversity indices such as the Shannon Diversity Index, and metabolic activity assays. These metrics would provide objective evidence regarding therapeutic effectiveness and ecological compatibility. The second study would utilize ex vivo human-derived microbiome models (i.e., fecal microbial communities) to evaluate the ecological consequences of the use of engineered microbes on bacterial populations. This would enable researchers to determine whether the use of engineered microbes can reduce the abundance of antibiotic-resistant bacteria without negatively affecting microbial community diversity, metabolic activity, and structure. Because ex vivo systems mimic natural microbial ecosystems, they are useful as a transition from laboratory experiments to animal studies.

The third phase is an in vivo validation study using multiple animal models colonized with antibiotic-resistant bacterial populations. The assessment of therapy will include monitoring the burdens of disease-associated bacteria in the animal, determining the effect on the microbiome community structure and function, measuring host physiological responses, and determining the activity of the containment mechanisms. Additional outcome measures may include survival rates, inflammatory biomarker concentrations, histological evidence of tissue damage, and longitudinal monitoring of engineered microbial persistence. These measurements would provide a comprehensive evaluation of therapeutic efficacy and host safety. An important aspect of developing hypotheses is the consideration of the modularity of engineered therapeutic systems. Detection circuits, antimicrobial effectors, and containment mechanisms should undergo testing independently and together to determine which components are most relevant to achieving therapeutic success and to identify potential interactions among subsystems that could influence their performance or safety.

Additionally, computer simulation will help provide an extra level of validation through computational modeling. By predicting how engineered organisms will behave within a microbial ecosystem when interacting with resistant pathogens in differing environments, mathematical simulations of microbial ecosystems will assist in determining the best circuit architectures, dosing strategies, and containment thresholds for engineered organisms prior to biological implementation, thereby reducing the experimental costs and increasing the efficiency of the design process. With continued accumulation of experimental data, the hypothesis will progress from being a conceptual framework to being a refined therapeutic platform. Improvements in the design of genetic circuits will be guided by the data obtained from the validation stages (of increasing precision) as will improvements in detection specificity and antimicrobial effectiveness; ultimately resulting in a clinically viable system capable of addressing antibiotic-resistant microorganisms across a wide range of environments.

HYPOTHESIS TESTING

The global health crisis caused by antibiotic resistance is due to the increased use of antimicrobials and the fast evolution of bacterial cultures. Development of traditional antibiotics has worked well historically, but the continual rise in multidrug-resistant organisms and the decrease in efficacy of broad-spectrum antibiotics will only be a limiting factor in the long-term success of this method. Programmable microbe therapeutic (PMT) strategy represents a radically different method of interfacing with bacteria via the use of genetically engineered microorganisms capable of detecting and responding to resistant pathogens and responding to them within complex communities of microorganisms.

The effectors (the agents of action) and environmental sensing systems work together to utilize the engineered microbes to selectively suppress pathogenic organisms while sparing the

normal flora of the environmental ecosystem. Compared to traditional antibiotics, this method is a considerable improvement because antibiotics indiscriminately kill both pathogenic and non-pathogenic microorganisms in a person's body. Biologically adaptive treatments provide many advantages over traditional antibiotics. First, they provide significantly higher specificity by killing only targeted pathogenic microorganisms and therefore do not also kill the beneficial microorganisms that reside in our microbiota.

Second, the genetic circuitry can be altered or reprogrammed to respond to new mechanisms of resistance as they emerge. Third, biologically adaptive treatments can respond dynamically to changing environmental conditions in their intended microbial communities, whereas traditional antibiotics do not alter their original actions once administered. Improved microbiome-based therapies offer many advantages, but there are also challenges that need to be addressed before these therapies can be considered safe and effective. One of the most significant challenges is the possibility that engineered organisms will transfer genes horizontally to non-engineered microorganisms in the microbiome, which could lead to unintended consequences and the disruption of synthetic, engineered genetic circuits. Therefore, containment and engineered safeguards must be built into the system during its design stage.

Another challenge is that maintaining specificity in microbiomes can be difficult due to the presence of a multitude of species and the overlapping signals generated by species in the microbiome. As a result, engineered microorganisms must reliably identify and distinguish pathogenic bacteria from beneficial commensals, despite the presence of overlapping signalling molecules in the environment, with reliable discrimination requiring highly sensitive detection systems developed and extensively validated across multiple microbiome populations.

The long-term effectiveness of synthetic engineered organisms will depend on their ability to remain genetically stable and resistant to mutational drift. Since genetic circuits can mutate over time and because of exposure to evolutionary pressures in the environment (e.g, whether beneficially or competitively), synthetic circuits will have decreased functionality or may result in unpredictable behaviour if the circuit genetically mutates.

Apart from these challenges, host immune responses must also be taken into consideration when developing engineered microorganisms for therapeutic use. For engineered microorganisms to achieve the desired outcomes, there must be an appropriate balance between sufficient persistence for the engineered organism's function and adequate stimulation of the host immune system. There may also be significant variability between individual patients (for example, patients with suppressed immune systems or inflammatory diseases) that will need to be taken into consideration when assessing responses to engineered microorganisms.

Whether or not engineered microbes used for therapeutic purposes can reliably decrease population figures of antibiotic-resistant pathogens and still keep the integrity of the microbiome intact, maintain ecological containment while avoiding adverse effects on the host represent the minimum criteria required to support the hypothesis.. Once these objectives are demonstrated to successfully meet all of the objectives will programmable engineered microbial therapeutics be

considered a revolutionary development in the treatment of antimicrobial resistance and will create a new era of biologically adaptive medicine.

IMPLICATIONS

If successful, programmable microbial therapeutics could fundamentally transform the treatment of infectious diseases by shifting antimicrobial medicine from broad-spectrum chemical interventions toward adaptive, biologically responsive therapeutic systems.. Currently, if a bacterium develops a new way to resist an antibiotic, the doctor must develop a new antibiotic to treat the infection. However, by using adaptable living bacteria as a treatment, treatment systems could continue to adapt to the demand created by resistant bacteria. As a consequence, the effectiveness of these anti-microbial tools may be prolonged and their overall reliance on the conventional use of anti-microbials can be decreased. In addition to targeting resistant pathogens, using programmed microbial therapies could also reduce the global spread of antibiotic resistance. Programmed microbial systems can decrease selective pressures that allow for the emergence of new resistant strains by eliminating resistant pathogens while preserving healthy bacteria and their genetic material within the same geographic area. As such, this strategy is consistent with global efforts to promote the longevity of anti-microbial therapy.

In addition to their public health benefits, maintaining the integrity of the microbiome may provide additional clinical benefits. In addition to the excellent health benefits provided by healthy bacteria, numerous studies have shown that healthy microbial communities play important roles in regulating the immune system, aiding in metabolic functions, and providing resistance to secondary infections. As a result, it is expected that the number of complications associated with imbalanced bacteria, such as recurrent infections and gastrointestinal disorders, will also decrease through the development of targeted programmed microbial therapies.

The proposed technology framework represents an excellent opportunity to establish a platform for future application of synthetic biology, in addition to treating infectious diseases. The same or similar sensing and response systems could potentially be developed to identify cancer-associated biomarkers, inflammatory conditions, or metabolic diseases. Therefore, the creation of programmable microbial therapeutics has the potential to generate a new class of living medicines that can dynamically respond to a variety of diseased states.

There are significant economic implications associated with the development and implementation of recombinant DNA technology. Healthcare costs associated with treating antibiotic-resistant infections are substantial due to the extended length of stay for patients; the increased complexity and cost of treating these infections; and their increased associated mortality rates. By improving the patient outcome and lessening the reliance on expensive last-resort antibiotics, biological therapeutics would reduce these burdens.

In addition to the potential economic benefits, advances in technology would require new regulatory processes. Current drug approval processes are designed to approve static pharmaceutical products and do not adequately address the approval of self-regulating living products. Regulatory authorities will have to develop new evaluation criteria for the approval of self-regulating living products, which includes data on genetic stability; environmental containment; long-term ecological effects; and monitoring of the product after it has been used.

By implementing these technologies, new regulatory frameworks will be needed as current drug approval pathways focus more on static pharmaceutical compounds and less on living systems that self-regulate. Essentially, current regulatory agencies will likely need to develop new evaluation criteria for products by evaluating them for genetic stability, environmental containment, long-term ecological impact and effective post-treatment monitoring. The successful use of engineered microbes as therapeutic agents could significantly alter the relationship between medicine and synthetic biology. To ensure that these technologies are introduced responsibly, it will be essential to consider public acceptance, ethical oversight and transparent risk evaluation methods. Establishing trust with the help of rigorous safety testing and openness in the scientific community will probably be important to determining how these engineered microbes continue to be used in the future.

CONCLUSION

The emergence of resistance to antibiotics presents a significant challenge to modern medicine, necessitating the development of alternative treatment strategies beyond traditional methods of antibiotic discovery and use. Synthetic antimicrobials represent a potential solution by leveraging the principles of environmental sensing, site-specific antimicrobial activity, and programmable control of genetically engineered microorganisms to target and eliminate resistant pathogens while sparing beneficial microbial communities.

The proposed synthesis of engineered microorganisms could accomplish this objective by incorporating functional components for quorum sensing detection, antimicrobial effector function (eg, bactericidal or bacteriostatic), CRISPR-based gene targeting for resistant genes, and ecological containment strategies to enable the use of non-resistant strains of microorganisms. While numerous technical and biosafety obstacles to the use of engineered microorganisms remain, advances in synthetic biology have demonstrated that the development of such systems is rapidly approaching feasibility. Future research focusing on aspects related to microbial engineering, design of genetic circuits, containment technologies, and host-microbe interactions will be critical for translating the concepts of engineered microorganisms into clinical practice.

Beyond addressing antimicrobial resistance, the creation of living, programmable therapeutics could create an entire new paradigm for responsive biological therapy that detects and corrects pathological processes in live animals in real-time. By facilitating the transition

from static drug-based therapies to adaptive therapeutic systems, programmable living therapeutics define a major departure from existing static drug-based therapy models. Future research should include evaluating the stability, reduction of horizontal gene transfer, and better specificity of engineered microorganisms when applied in complex microbial communities. Computing advances in biology, genome engineering, and systems microbiology will enable more rapid development of engineered microorganisms and create additional opportunities for innovative therapies.

Long-term success in developing engineered microbial therapeutics requires collaboration across multiple disciplines involving microbiologists, synthetic biologists, clinicians, bioengineers, regulatory bodies, and experts in public health. The ability to collaborate will help ensure the safe, ethical, and effective development of engineered microbial therapeutics. In conclusion, programmable microbial therapeutics can be the continuation of the vision for the future direction of antimicrobial therapy, exploiting the adaptive nature of living systems to create sustainable and accurate solutions for one of the most urgent issues in contemporary healthcare. If successful, programmable microbial therapeutics could transform antimicrobial medicine from a reactive discipline dependent upon continual antibiotic discovery into a proactive and adaptive system capable of responding dynamically to evolving microbial threats.

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