Transforming the Healthcare Landscape with Synthetic Biology and Living Medicines

An exciting emerging field of technology, living medicine uses living cells to treat disease. Using synthetic biology tools, the field is tackling difficult-to-treat diseases, including those previously thought to be untreatable. The development of increasingly complex and diverse engineering tools will facilitate the progress of living programmable therapeutics, with a range of applications including neurodegenerative disorders, autoimmune diseases, metabolic diseases and gut health, infections and some cancers.

There is a well-recognised global need for therapeutic options for diseases that are deemed difficult to treat, or even currently untreatable. The emergence of synthetic biology tools and technologies has led to a growing awareness of the potential for engineered living organisms to tackle these challenging areas. The field of 'living medicines' is harnessing the power of synthetic biology to design cells (non-pathogenic bacterial, human or fungal cells) or viruses that are able to produce desirable therapeutic effects. For example, cells can elicit an immune response to target cancer cells and infections, regenerate diseased tissue or deliver therapeutic effectors. Living cells can be engineered to sense and respond to environmental signals, to detect harmful compounds and perform a specific function, thereby targeting specific mechanisms of these unmet diseases. The potential applications for living medicines could also open new avenues into personalised medicine.

The discovery of new living medicines has already resulted in major improvements in healthcare and patient outcomes. For example, the field of immuno-oncology has yielded chimeric antigen receptor technology (CAR-T), a cancer cell therapy which engineers a patient's own immune cells to fight cancer and has already shown significant benefits for certain patients[1]. CAR-T cells are not obtainable naturally and have to be engineered. There are currently two CAR-T therapies on the market, which have been available since 2017 (Novartis' Kymriah for the treatment of B-cell precursor acute lymphoblastic leukaemia (ALL) and Gilead's Yescarta for patients with relapsed or refractory large B-cell lymphoma), and there are many more advancing through the clinic as scientists work to overcome the remaining technical and regulatory challenges[2,3].

Stem cell therapies offer the potential to treat a range of diseases, injuries and health conditions. Research efforts to establish cell therapies using stem cells are focused on the sources of precisely reprogrammed stem cells as it is currently limited by the availability of human cells. Today, the only stem cell based treatment that is established and approved is hematopoietic stem cell transplantation for the treatment of patients with cancers and disorders that affect the blood and immune system. Although this approach does not involve reprogramming, the hope is that this will become possible in the future.

Engineered bacterial cells have been shown to respond to the body's cues, to deliver optimal treatment, with the correct doses of enzymes, biologics and small molecules released at the right time, directly where they are needed. In addition, these engineered bacteria can absorb and break down potentially toxic molecules. For example, strains of *E. coli* are currently being developed that can live in the gut and consume molecules such as phenylalanine, for treatment of diseases where patients' bodies are unable to process these waste products.

Within the bacterial therapeutics field, the focus of significant active research is on two main areas: the identification of microbes that naturally produce therapeutic effects and the engineering of bacteria to produce desirable therapeutic effects. The latter involves the design of genes or complex gene circuits to synthesise molecules inside the cell that in turn will perform a specific function, which in a therapeutic context could mean producing a drug or protein. Researchers are able to hijack the complex system of communication within a live cell by encoding RNA or proteins, controlling the way these cells navigate their environment, respond and interact with one another, and build intricate patterns by responding to specific signals that trigger gene expression.

Focus on Applications of Microbial Living Medicines

As increasingly complex synthetic biology tools become available, we are seeing therapies being developed to treat a wider range of health conditions, including infections, metabolic disorders, immunotherapy for cancers and other health conditions, as well as gut-related disorders.

One of the most active application areas for living bacterial medicines currently is gut health. Probiotics have long been used to treat gastrointestinal conditions, including irritable bowel syndrome, due to their ability to restore balance to the intestinal microflora.

Disorders of the gut can be particularly difficult to treat due to the harsh environment of the digestive tract, meaning that therapies delivered orally must withstand exposure to stomach acid, bile salts and digestive enzymes. However, bacteria can be engineered to survive in this challenging environment and deliver therapeutics that can be absorbed by the body in situ or deliver their therapeutic effect in the gut.

Several strains of bacteria have been genetically modified for therapeutic use in the gut, with popular chassis organisms including *E. coli* , *Bacteriodes*, and lactic acid bacteria (LAB), with *E. coli* being the most studied. Research has demonstrated the success of *E. coli* in reducing bacterial infection of *Pseudomonas aeruginosa* and *Salmonella typhimurium* by delivering the anti-biofilm enzyme, dispersin B, to the gut[4,5].

While naturally occurring microbiota-directed therapeutic approaches for digestive disorders, such as faecal transplants, already exist, engineered bacterial approaches possess advantages over these due to their ability to confer functions that are not naturally present in the microbiota. Engineered bacteria can be designed to improve the capabilities of regular microbes in several ways. This includes carrying out natural biological processes at enhanced rates, producing effectors that are not native to bacteria, such as human proteins, or degrading harmful amino acids that are produced by genetic mutations in patients with phenylketonuria, for example[6].

Another exciting research area and potential treatment application for bacterial therapeutics is for solid tumours, which display abnormal blood vessel structure resulting in hypoxic areas, where anaerobic bacteria can survive. The danger of solid tumours is that they can grow freely if undetected by the body's immune system. Treatment can pose a challenge as the dosage of the drug must be balanced to deliver sufficient toxicity to the tumour to kill the target cells, whilst also being a low enough dose so as not to damage healthy cells surrounding the tumour site.

Engineered bacteria could therefore provide a new wave of cancer immunotherapies by triggering a controlled immune response, localised at the tumour site, to target and kill tumour cells without damaging healthy tissue. A study has shown that non-pathogenic *E. coli* can act as a signal to the immune system when engineered to activate STimulator of INterferon Genes (STING) in phagocytic antigen presenting cells (APCs) and trigger immune pathways in tumours. The resulting effect was shown to remain local to the tumour site, significantly delaying tumour growth, and in some cases even stimulated complete tumour rejection. Not only are the results extremely hopeful in terms of immediate action, but there is also evidence of long-term anti-tumour immunity, with animal models showing 40% long-term survival rates and resistance to secondary tumour challenge[7].

Another mechanism for microbial cancer treatment is by engineering bacteria to deliver anti-tumour cargo to tumour sites, which disrupts the tumour microenvironment. The delivery of these payloads can be controlled through the regulation of bacterial gene expression, making it possible to control the timing of delivery as well as limiting further accumulation of payload. The microbes can secrete bacterial toxins to facilitate tumour regression. Toxins from *S. typhimurium, Listeria, Salmonella* and *Clostridium* have been shown to directly kill tumour cells through inducing apoptosis or autophagy. This offers an exciting advancement over traditional methods of dosing patients, as the precision reduces any damage to healthy tissues whilst maintaining optimal dosage at the tumour site. These treatments can also be used in conjunction with immune activation therapy, for a multi-targeted approach for cancer therapy[8].

Antibiotic resistance is recognised as one of the world's greatest public health threats, with bacterial infections being a major cause of morbidity globally. There is growing evidence that engineered probiotics could provide an innovative, alternative treatment for infections through various mechanisms. They have been shown to demonstrate great specificity and efficacy, including the secretion of antibacterial chemicals, stimulation and modulation of the immune response, competition for nutrition and specific adhesion sites, and inhibition of toxic protein expression[9]. For example, many bacterial pathogens' mode of infection involves formation of a biofilm, which can lead to antibiotic and immune system resistance. As mentioned above, engineered bacteria have proved extremely successful for blocking this, with one study showing an *E. coli* variant inhibiting *P. aeruginosa* biofilm formation by 90%[10].

Another approach to combatting infection through microbial therapy is to orally ingest engineered bacteria, which then enter the lymphatic nodules in the small intestine where they are phagocytosed by APCs and express antigen genes. The antigens are processed by the APCs, stimulating mucosal immune response – the body's first defence against infection, for example in the gut or nose. This approach is very similar to a vaccine, offering protection against transmission with little to no side-effects, as it is made by the body's own immune system. With applications ranging from *Salmonella* to Zika, this platform of oral, living vaccines could play a key role in addressing the challenge of distributing vaccines rapidly across the globe[11].

The Importance of Synthetic Biology in the Development of Living Medicines

Synthetic biology plays a crucial role in facilitating the development of living medicines, providing researchers the ability to fine-tune organisms, using precision DNA synthesis or DNA editing to re-engineer desired characteristics onto the framework, to control cellular or viral behaviour and functions. For example, the first step in developing a novel bacteria-based therapeutic requires identification and design of therapeutic genes or pathways, followed by a series of processes taking the idea from initial concept through to prototype generation, strain optimisation, lead and candidate selection, as shown in Figure 1.

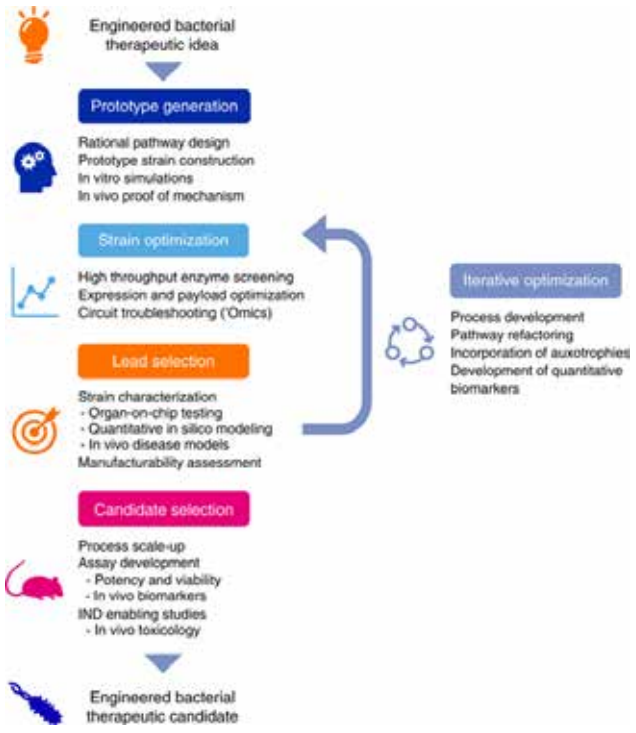

Figure 1: Development of Bacterial Therapeutics: Schematic representation of a workflow for developing clinical candidate-quality engineered strains. The development workflow should incorporate technologies for optimising strain potency, as well as predictive in vitro and in vivo assays, and quantitative pharmacology models, to maximise translational potential for patient populations[6].

Synthetic biology gives researchers a 'genetic toolbox' to enable engineering of more complex functions of organisms and cells. Platforms in development, such as DNA writers, will give researchers access to DNA synthesis, allowing them to identify and test pathways and gene circuits in real time.

CRISPR has played a key part in the development of living medicines, and advancements in this field have been developing rapidly over recent years. There now exists a wide set of genetic tools which give living medicines their desired qualities by controlling input sensing, gene expression control devices, memory, and the production of molecules. There is even the ability to develop biocontainment-enabling tools to address safety concerns, including 'kill-switches', which prevent uncontrolled replication and spread, and protection of therapeutic organisms from antibiotics. Safety measures such as these are vital for the advancement of bacteria-based therapeutics into human trials. Some therapies have shown great promise in animal models but safety regulations mean they are not Generally Recognised As Safe for human use. The development of synthetic biology tools will be key to driving this forward[11].

The ability to control the timing and locality of therapeutics delivery is a huge potential advantage of synthetic biology in the living medicines field. For example, therapies involving bacteria-producing tumour-lethal treatment must only confer toxicity at the target site (i.e. at the tumour mass) to avoid damaging healthy surrounding tissues. In addition, gene expression may be induced through the application of small molecules that 'turn on' molecule production as demonstrated for example by salicylate-inducible gene expression in *Salmonella typhimurium*, which confers the ability to synthesise more salicylate[12].

Future Outlook – Challenges and Opportunities

It remains a challenge to understand the complexity of human diseases and disorders, as well as the relationship with the engineered organisms being used to target them, and this continues to present a major barrier. However, with increasing knowledge of these mechanisms, both the exploitation of living biotherapeutics and development of improved tools will be enabled.

A particular challenge is the development of orthogonal regulators (such as promoters, transcription regulators, and regulatory RNA) to facilitate more complex genetic circuits. This is key to ensuring the safety of therapies and will help them progress through clinical trials. Biocontainment poses a particular challenge in therapies that require repeat dosing, but with more regulators available, it will be easier to incorporate safety features such as biocontainment[12].

Currently, there is no single, broad solution for the manufacture of engineered living biotherapeutics. The scale-up production of engineered cells also presents challenges from a cost and quality perspective.

For bacterial cells, cell viability must be confirmed during and after fermentation, downstream processing, formulation and storage. There is also the challenge of maintaining genetic stability during cell production. In addition, the inclusion of engineered genetic circuits encoding novel effector functions may come at a cost to bacterial fitness and/or growth rates, and this can lead to selective pressure for strain variants that have lost the engineered function. There is therefore a need for tight regulatory control of gene expression and robust assays to assess this.

The expansion of the toolbox for engineering living medicines is expected to address these challenges, facilitated by the fast growth and progression of synthetic biology. Recent years have seen significant progress, and with gene editing technology constantly improving, this is expected to continue apace.

A huge advancement will come when we have reached the milestone of proof of concept and progression of a living therapeutic to clinical trial. Once achieved, living medicines will create a new category of drugs to address significant unmet therapeutic needs and improve outcomes for patients.

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