Postprint: Research Advances in Medical Applications of Synthetic Biology

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Abstract

In the field of medical applications, synthetic biology utilizes artificially designed genetic circuits to engineer human cells themselves, or to engineer artificial living organisms such as bacteria and viruses, which then act indirectly on the human body. These artificially engineered living organisms can sense disease-specific signals or artificial signals, specifically target abnormal cells and lesion areas, express reporter molecules or release therapeutic drugs, thereby enabling the monitoring of human physiological states, as well as the diagnosis and treatment of typical diseases such as tumors, metabolic diseases, and drug-resistant bacterial infections. This article reviews some recent research advances in the medical application fields of synthetic biology.

Full Text

Progress of Synthetic Biology Research in Medical Applications

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Abstract

In medical applications, synthetic biology employs artificially designed genetic circuits to modify human cells or to engineer synthetic organisms such as bacte-

ria and viruses, which then act indirectly on the human body. These engineered organisms can sense disease-specific or artificial signals, specifically target abnormal cells and lesion areas, express reporter molecules or release therapeutic drugs, thereby enabling monitoring of physiological conditions and diagnosis and treatment of typical diseases including tumors, metabolic disorders, and drug-resistant bacterial infections. This article comprehensively reviews recent research progress in the medical applications of synthetic biology.

Keywords: synthetic cell, synthetic bacteria, synthetic virus, synthetic phage

Introduction

Synthetic biology is currently experiencing rapid parallel development in both academic research and industrial applications worldwide. The defining characteristic of this discipline is the systematic use of engineering principles to purposefully design artificial living systems, with broad research scope. Among these, the translational application of synthetic biology technologies in medicine has attracted widespread attention from both scientific and medical communities. In medical applications, synthetic biology employs artificially designed genetic circuits to modify human cells or to engineer synthetic organisms such as bacteria and viruses, which then act indirectly on the human body. These engineered organisms can sense disease-specific or artificial signals, specifically target abnormal cells and lesion areas, express reporter molecules or release therapeutic drugs, thereby enabling monitoring of physiological conditions and diagnosis and treatment of typical diseases including tumors, metabolic disorders, and drug-resistant bacterial infections. Engineered living organisms offer advantages in intelligence, complexity, and safety controllability that will enhance diagnosis, treatment, and prevention of stubborn diseases such as cancer, metabolic disorders, and drug-resistant infections, leveraging the disruptive potential of synthetic biology to usher in a new era of intelligent biological diagnosis and treatment. Recent research progress in these areas is reviewed below.

Engineered Bacteria for Medical Applications

Artificial Bacteria for Tumor Diagnosis and Treatment

Bacteria, the most common and abundant foreign organisms in and on the human body, are intricately linked with human metabolism, immunity, and aging from birth to death. Model microorganisms such as *Escherichia coli* have become ideal chassis for synthetic biology research and development due to their ease of cultivation, relatively simple structure, well-characterized biology, and amenability to genetic editing. In recent years, synthetic biologists have designed and constructed intelligent genetic circuits that enable commensal and pathogenic bacteria to perform computation, sensing, memory, and response functions, which are being applied to medical research and disease diagnosis

and treatment to meet specific medical needs.

Engineering bacteria through synthetic biology technologies provides novel approaches for cancer therapy, with bacterial therapy gaining increasing attention due to its good targeting capability and low toxicity. More than 200 years ago, physicians observed that bacterial infections could sometimes slow tumor growth or even eradicate tumors. Dr. William Coley inactivated bacteria to create "Coley' s toxins," which successfully treated over 1,000 cancer patients with success rates comparable to modern cancer treatments[1]. Various bacteria have been reported for cancer therapy, including pathogenic species such as *Clostridium tetani* and *Clostridium butyricum*, as well as non-pathogenic probiotics like *Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Bifidobacterium*. Salmonella is considered the most ideal vector for tumor therapy due to its facultative anaerobic nature, strong targeting ability, natural toxicity, and ease of modification[2,3].

The team at Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, focuses on employing synthetic biology approaches to reduce bacterial toxicity, enhance targeting capability, and endow bacteria with diverse functions, aiming to transform them into more specific, intelligent, and efficient anti-tumor "weapons." Bacterial therapy in the tumor microenvironment not only exerts strong immunomodulatory effects to reawaken the host immune system' s suppression of tumor cells but also serves as a delivery vehicle for drugs or cytokines to enhance tumor inhibition. Currently, their engineered bacteria have entered preclinical research stages with promising efficacy, potentially becoming the world' s first living biotherapeutic product for solid tumor treatment.

A team from MIT and UC San Diego introduced a lacZ reporter gene into *E. coli* that activates expression upon contact with tumor cells, producing large amounts of LacZ enzyme. The researchers then injected mice with a cross-linked chemiluminescent substrate that is cleaved by LacZ enzyme to release a chemiluminescent signal, which accumulates in mouse urine. Urine samples containing this signal change from yellow to red, allowing researchers to monitor urine color changes to preliminarily assess tumor presence and status in mice. This method was found to be more sensitive than conventional microscopic detection, capable of detecting tumors smaller than 1 cm in diameter[4].

Researchers from UC San Diego and MIT constructed a bacterial system for periodic synchronized drug synthesis and lysis-based release. The team designed a quorum sensing-based genetic circuit that causes bacteria to self-destruct after reaching a certain density threshold in the tumor environment, synchronously releasing anticancer drugs in a burst. This approach maximally maintains low bacterial colonization numbers in vivo while reducing damage and toxic side effects to surrounding tissues[5].

Artificial Bacteria for Metabolic Disease Diagnosis and Treatment

In August 2017, the biopharmaceutical company Synlogic was listed on NASDAQ, focusing on using synthetic biology to genetically engineer probiotics for treating metabolic diseases, inflammation, and cancer. Synlogic' s engineered probiotic product SYNC1618 was designated by the FDA as an orphan drug for phenylketonuria (PKU). The company has also initiated human clinical trials for synthetic biology therapeutics for urea cycle disorders (UCD) to validate the efficacy of artificial bacteria.

Columbia University developed a living bacterial "recorder" for detecting multiple metabolites in the gut. The team engineered a DNA plasmid that creates more copies of itself in gut microbial hosts when responding to external signals, while using another independent plasmid expressing CRISPR-Cas system components to drive the recorder and timestamp events. Without external signals, only the recording plasmid is active, inserting spacer sequence copies into genomic CRISPR loci. When external signals are added, the other plasmid is also activated, and its sequences are inserted into CRISPR loci. The resulting mixed background sequences thus contain rich temporal and signal information. Researchers can read what the bacteria experienced by examining their CRISPR loci using computational tools. The study demonstrated that this system can process at least three simultaneous signals and store data for three days in the host gut[6].

An MIT team developed an ingestible diagnostic tool composed of living cell sensors and ultra-low-power microelectronic devices. Using beneficial *E. coli* expressing specific genetic circuits, the bacteria express luminescent proteins upon encountering heme, enabling detection of gastrointestinal bleeding. The bacteria reside on a customized sensor covered by a semipermeable membrane that allows small molecules to pass through but prevents bacterial leakage. Below the four bacterial chambers is a phototransistor that measures bacterial light production and transmits data to a microprocessor, which then sends it via radio to nearby computers or smartphones. The researchers developed a dedicated Android application to analyze this data. As the sensor passes through the stomach, internal bacteria continuously capture target biomarkers. The sensor measures only about 3.8 cm in length and operates at approximately 13 W; its effectiveness has been demonstrated in large mammals (pigs)[7]. The team has also designed additional genetic circuits responsive to inflammatory markers.

Artificial Bacteria for Malaria Resistance

Malaria is caused by the single-celled parasite *Plasmodium*, which is transmitted to humans through bites from infected female *Anopheles* mosquitoes. Therefore, controlling mosquito populations is considered a crucial strategy for malaria prevention. Researchers from the Shanghai Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, discovered a new *Serratia* strain AS1 that can be transmitted vertically across generations in mosquitoes. They successfully

engineered a strain that simultaneously secretes five anti-malaria genes, effectively reducing *Plasmodium* oocysts by 92-93%. By efficiently driving the rapid spread of anti-malaria effector molecules throughout mosquito populations, this approach can render mosquitoes ineffective malaria vectors, achieving blockade of malaria transmission at its source[8].

Engineered Viruses and Phages for Medical Applications

Artificial Viruses for Attenuated Vaccine Construction

A team from Peking University developed a strategy called "synthetic attenuated virus engineering" for constructing live attenuated vaccines using synthetic biology methods. Leveraging the principle that amber codons (stop codons) can incorporate non-natural amino acids, the researchers mutated several codons in viral replication genes into stop codons, preventing complete protein translation upon infection of human cells, thereby obtaining live virus vaccines with demonstrated safety and efficacy. Further mutation of more than three codons transformed the vaccine from a preventive to a therapeutic agent against viral infections, with efficacy increasing as more codons were mutated. This discovery revolutionizes vaccine development concepts and represents a major breakthrough in live attenuated vaccines. Unlike traditional attenuated viruses that rely on a few amino acid mutations, synthetic attenuated viruses derive their attenuation from cumulative effects of hundreds of codon changes, making reversion to wild-type virtually impossible and substantially improving safety. Additionally, the attenuated strains can be generated rapidly, dramatically shortening vaccine development cycles[9]. A team from the Wuhan Institute of Virology, Chinese Academy of Sciences, successfully applied this technology to develop a novel attenuated Zika virus (ZIKV) vaccine. A single immunization stimulated high-titer neutralizing antibodies in mice, provided complete protection against viral challenge, and prevented vertical ZIKV transmission from mother to offspring. With 2,568 synonymous mutations introduced into its genome, the risk of reversion is extremely low[10].

Artificial Viruses for Tumor Therapy

Engineered oncolytic viruses can selectively replicate in and kill cancer cells without harming healthy tissue through dual mechanisms of selective tumor cell lysis and anti-tumor immunity. The principle relies on tumor-driving mutations that specifically enhance viral replication selectivity in tumor cells, and many tumor cells have defects in antiviral type I interferon signaling, thus supporting selective viral replication. Viral replication in the tumor microenvironment can overcome tumor immunosuppression and promote anti-tumor immunity. For example, OrienX010, developed by Beijing OriGene Biotechnology Co., Ltd., is a recombinant human GM-CSF herpes simplex virus injection with pathogenic genes of HSV-1 deleted and a DNA fragment encoding human granulocyte-macrophage colony-stimulating factor (GM-CSF) inserted. This product specifically replicates in tumor cells, causing tumor cell lysis and death while releas-

ing tumor antigens and activating systemic anti-tumor antigen-specific immune responses through GM-CSF protein expressed by the vector. OrienX010 has entered the national priority review drug catalog and is expected to be approved for marketing in 2019 for intralesional injection treatment of malignant melanoma. Currently marketed oncolytic virus products include Shanghai Sunway Biotech' s Oncorine (adenovirus) for head and neck cancer treatment and Amgen' s T-VEC (herpes simplex virus) for melanoma treatment. In China, other products in clinical trials include Sun Yat-sen University' s alphavirus M1 for efficacy and safety evaluation in 13 high-incidence tumors, and Beijing Neurosurgical Institute's oncolytic virus ON-01 for treatment evaluation of recurrent glioblastoma[11].

Artificial Phages for Drug-Resistant Bacteria Treatment

Sample6 Technologies uses synthetic biology to engineer phages that can directly identify and kill bacteria or produce specific enzymes to destroy bacterial protective membranes, making bacteria susceptible to antibiotics or host immune system elimination.

In August 2018, the Shanghai Phage and Antibiotic Resistance Institute successfully cured a patient with superbug infection. The patient had complex, recurrent urinary tract infections following bladder tumor surgery. Since 2014, multidrug-resistant *Klebsiella pneumoniae* had established infections in the left and right renal pelvis and bladder, with interconnected yet distinct bacterial populations at the three sites, making treatment extremely challenging. The treatment team finalized a protocol: after nephrostomy, phage irrigation was administered through the nephrostomy tube combined with intravenous antibiotics for one week, followed by discontinuation of antibiotics while continuing phage therapy for another week, and finally complete drug cessation with one month of continuous observation. Ultimately, the *K. pneumoniae* in the patient' s urinary system was completely eradicated, urinary irritation symptoms significantly improved, and quality of life markedly enhanced[12].

Human Cell Design and Modification

Artificial Cells for Genetic Disease Therapy

Several paradigm-shifting events have transformed disease treatment in medical history, such as the invention of surgery and the discovery of antibiotics. The next major revolution is likely to be gene therapy, which is now maturing. In 2017, the FDA approved Luxturna, the first therapy for an inherited eye disease. In the near future, more gene therapies will transition from laboratory to clinic, ushering in a new medical revolution that may fundamentally alleviate previously incurable genetic diseases.

In principle, small molecule or protein-based therapies require repeated administration (e.g., diabetic patients require repeated insulin injections), whereas

repairing a patient' s defective genes could produce sustained therapeutic effects from a single treatment, achieving control or even reversal of genetic diseases. Gene therapy has two prerequisites: (1) a vector for delivering new genes, with retroviruses and adeno-associated virus vectors showing the most clinical promise; and (2) tools for editing and repairing defective genes to mediate gene addition, deletion, correction, and other highly targeted genomic modifications, typically employing three major gene editing technologies—zinc finger nuclease (ZFN), transcription activator-like effector nuclease (TALEN), and clustered regularly interspaced short palindromic repeats (CRISPR).

Gene therapy can refer to in situ treatment of monogenic diseases carried by somatic cells, or to ex vivo modification of blood cells for reinfusion (also called cell therapy). Since it involves artificial design and modification of cells to achieve specific therapeutic functions, it falls within the scope of synthetic biology. Gene therapy holds tremendous clinical potential and represents a major achievement of synthetic biology in medicine, offering hope for many incurable diseases. Recent cases include:

In 2017, Sangamo Therapeutics conducted the world' s first in vivo gene editing treatment in humans[13]. Brian Madeux, a 44-year-old patient with Hunter syndrome, a congenital metabolic disorder, received intravenous injection of billions of copies of corrective genes and precise gene editing tools. Researchers reported good therapeutic effects with no serious side effects or safety concerns.

In February 2018, Sangamo was approved to perform in vivo gene therapy on a second patient.

Pfizer is using zinc finger transcription factors (ZFP-TFs) in clinical trials to treat amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig' s disease, and another neurodegenerative condition called frontotemporal lobar degeneration (FTLD), both caused by C9ORF72 gene mutations[14].

Bioverativ, a subsidiary of Sanofi, collects hematopoietic stem cells (HSCs) from patients and uses ZFN technology to precisely cut the erythroid enhancer that regulates the BCL11A gene, reducing BCL11A expression. The gene-edited HSCs are then reinfused into patients, where they can proliferate and differentiate into mature red blood cells, alleviating symptoms in sickle cell disease patients. The advantage of this autologous cell therapy is that it does not rely on viral vectors for gene editing, thus avoiding potential side effects from viral vectors[14].

Shire Pharmaceuticals has developed a gene therapy for Huntington' s disease, which is caused by specific mutations in the single htt gene. This therapy uses zinc finger proteins to specifically downregulate the mutant htt gene, selectively suppressing expression of mutant huntingtin protein (HTT) while maintaining normal gene copy expression, aiming to reverse Huntington' s disease symptoms and prevent disease progression. The therapy is currently in preclinical research[14].

Beyond therapeutic purposes, biohackers are also using gene therapy technologies to modify their own cells in pursuit of enhanced human capabilities. For example, former NASA scientist Josiah Zayner used CRISPR technology to introduce a Myostatin gene mutation in his own body to enhance muscle growth. Other biohackers have introduced rhodopsin genes into their retinas in attempts to enhance vision[15].

Artificial Cells for Tumor Diagnosis and Treatment

Artificially engineered immune T cells are becoming powerful cancer therapeutics. Tumor cell immunotherapy was considered the biggest biotech trend globally in 2017, and cancer immunotherapy won the 2018 Nobel Prize in Physiology or Medicine. Following the success of immune checkpoint inhibitors represented by PD-1/PD-L1, CAR-T therapy has emerged as a heavyweight weapon with potential to defeat cancer. CAR-T technology involves ex vivo engineering of T cells with artificially synthesized chimeric antigen receptors (CARs). Unlike natural physiological antigen receptors, CARs can be engineered to recognize tumor-specific proteins, glycolipids, HLA-peptide complexes, and other targets. The engineered T cells are expanded and reinfused into patients to specifically recognize, bind to, and kill cancer cells, achieving targeted therapy.

In clinical applications, CAR-T therapy currently has 307 ongoing clinical trials worldwide, with 164 from China. In July 2018, the US approved Novartis' s Kymriah (formerly CTL019), the first CAR-T product globally, for treating acute lymphoblastic leukemia, priced at $475,000. Kite Pharma' s Yescarta (KTE-C19) followed shortly after, priced at $373,000.

Chinese research institutions hold their own in intense global competition, accounting for about half of all clinical trials. Targets include not only CD19 but also newer targets such as MCU-1, EPCAM, and GPC3. The Chinese PLA General Hospital (301 Hospital) began related clinical trials in 2014 and has the most extensive development pipeline. Other institutions including West China Hospital of Sichuan University, Southwest Hospital of the Third Military Medical University, Shanghai Cancer Institute, and Renji Hospital have also achieved good therapeutic outcomes. Domestic companies in the CAR-T field include JW Therapeutics, Fosun Kite, Shanghai Kunlang, Shanghai Bion, CARsgen, Ucarer, Hengrunda, VCANBIO, Nanjing Legend, Nanjing Kedi, Beijing Malino, CBMG, and Boyalife.

CAR-T technology still requires continuous improvement in new target identification, solid tumor treatment, and universal production. In basic research, scientists are using synthetic biology to design increasingly sophisticated artificial cells. Recent advances include:

A team from Shenzhen Second People' s Hospital designed genetic circuits using logic "AND gates" and "signal connectors" for bladder cancer cell identification and therapy. Using the CRISPR-Cas9 system, they successfully constructed a genetic circuit carrying a logic "AND gate" that employs bladder cancer-specific

promoters UP and telomerase reverse transcriptase as input signals: only when both promoters are activated can downstream signals (such as luciferase) be expressed, effectively distinguishing bladder cancer cells from other cells. When the output signal is an apoptosis-related molecule, specific killing can also be achieved. This study provides a standardized synthetic biology platform for bladder cancer and other tumor detection and treatment, offering important guidance for developing new tumor biotherapeutic devices. The team also creatively proposed the "signal connector" concept, which links aptamer RNA that recognizes endogenous proteins with guide gRNA that recognizes target mRNA. This novel "signal connector" can specifically identify key protein molecules in cancer signaling pathways within cells and further use gRNA to target and inhibit translation efficiency of cancer signaling molecules. This enables quantitative detection of multi-level biological signals for diagnosis to comprehensively assess disease status of bladder cancer cells, and for therapy, uses reconstructed genetic circuits to replace damaged gene circuits in bladder cancer cells, restoring them to normal states and achieving precise diagnosis and specific, efficient tumor treatment[16].

A team from the University of California synthesized cellular sensing molecules —Notch (containing novel external sensing components and new gene activation modules) for killing tumor cells. Studies show that Notch molecules enable cells to recognize multiple molecules in vivo and activate specific gene responses. For example, cells can sense molecular damage and activate genes that stimulate repair, or sense cancer-related molecules and activate functional genes that prompt the immune system to kill tumor cells. They can also be used to sense proteins on tiny artificial scaffolds, directing cells to differentiate and fill bladders, livers, or generate replacement organs[17].

Based on significant differences in gene expression profiles between cancer and normal cells, an MIT team developed synthetic genetic circuits that automatically detect cancer signals, specifically distinguishing cancer cells from normal cells to trigger immune system attacks on cancer. After delivering the genetic circuit to target cells using viral vectors, synthetic promoters bind to specific active proteins in tumor cells to activate themselves. Only when two cancer promoters are simultaneously activated does the genetic circuit automatically turn on, after which various functional proteins (cell surface proteins, cytokines, chemokines, etc.) guide T cells to recognize and kill tumor cells[18].

Artificial Cells for Metabolic Disease Diagnosis and Treatment

Artificially designed cells can sense metabolic disease-specific or artificial signals and specifically express reporter molecules or release therapeutic drugs, enabling monitoring of physiological metabolic states and diagnosis and treatment of typical diseases.

A team from ETH Zurich used human kidney cells (HEK-293) to design artificial HEK-β cells with normal β-cell function. These cells can directly sense

blood glucose concentrations and secrete sufficient insulin to lower blood sugar when glucose exceeds a certain threshold. The team also constructed optogenetic synthetic biology circuits to effectively control blood glucose in diabetic mice. Furthermore, by integrating nanotechnology, biotechnology, and information technology, the response regulation system can be remotely activated using radiofrequency signals or smartphone applications to control insulin release. These studies provide strong support for dynamic regulation of artificial cells in human diabetes treatment[19].

A team from East China Normal University constructed a stable cell line HEKIR-Adipo containing an insulin sensor and transplanted it into various hyperinsulinemic mouse models including insulin-resistant diabetes, obesity, and diet-induced obesity using microencapsulation technology. The artificial cells could efficiently recognize and self-regulate blood insulin levels through feedback mechanisms. When blood insulin exceeded a certain threshold, the cells regulated expression of adiponectin (Fc-adiponectin) to alleviate insulin resistance symptoms and achieve therapeutic effects. The researchers also designed and synthesized customized cells with far-red light-controlled gene expression. These customized cells can be activated under far-red light illumination to express any desired reporter or therapeutic protein genes, such as green fluorescent protein or insulin. When the far-red light-controlled insulin-expressing customized cells were transplanted subcutaneously into diabetic mice, direct far-red light illumination activated the transplanted cells to express insulin and effectively lower blood glucose[20]. The team further designed and developed an intelligent integrated diabetes diagnosis and treatment system: blood glucose values read from a glucometer are wirelessly transmitted via Bluetooth to a customized smart controller and smartphone. When glucose values exceed a preset safety threshold, the smart controller can illuminate a hydrogel-LED composite containing customized cells transplanted in the mouse, activating the customized cells to produce insulin or GLP-1 to lower blood sugar and maintain stable glucose levels, ultimately achieving automatic diagnosis and precise treatment[21]. In another study, the team combined the pharmacological activity of oleanolic acid (OA) with glucagon-like peptide (shGLP-1) that improves insulin resistance, liver function, and pancreatic function. By constructing optimized genetic circuits, OA induction could precisely regulate shGLP-1 expression, simultaneously improving multiple metabolic abnormalities in a hepatogenous diabetes mouse model, including glucose tolerance, insulin resistance, hyperglycemia, lipid metabolism disorders, and liver dysfunction[22].

Human Germ Cell Design and Modification

Designing and modifying genes in human germ cells or embryos can eliminate fatal diseases before birth, offering broad future prospects. However, due to the manipulation of human embryos and the heritability of modified genes, this research carries certain safety risks and ethical-legal controversies.

In 2015, researchers from Sun Yat-sen University used CRISPR gene editing

technology to correct the gene causing β-thalassemia in human embryos. The study used discarded, non-viable embryos from volunteers[23].

In April 2016, a team from the Third Affiliated Hospital of Guangzhou Medical University successfully used CRISPR editing technology on human embryos, precisely deleting 32 bases of the CCR5 gene to confer HIV immunity to some embryos[24]. In August 2017, a collaborative team from Oregon Health & Science University and other institutions used the CRISPR gene editing system to safely correct the MYBPC3 gene mutation causing hypertrophic cardiomyopathy in human embryos[25]. This technology can also edit and repair other high-penetrance disease genes, such as those causing cystic fibrosis or breast cancer. Combining embryo gene editing technology with in vitro fertilization and preimplantation genetic diagnosis could completely prevent transmission of genetic diseases to future generations.

Advances in Enabling Technologies for Artificial Cell Design and Animal Models

Human cells, as advanced eukaryotic cells, possess special properties including diversity, multicellularity, complex regulation, cellular differentiation, and subcellular compartmentalization, offering infinite possibilities for synthetic biology. The maturation of enabling technologies such as efficient DNA delivery and targeted stable integration, gene editing, artificial chromosome design and synthesis, diverse gene regulation, and compartmentalized metabolic reactions will greatly facilitate the application of artificially designed human cells in medicine. Due to space limitations, these will not be detailed here.

Animal model research is essential for clinical studies, providing technical paradigms and theoretical foundations. Scientists have combined gene editing with somatic cell nuclear transfer technology to construct various animal models. Since the birth of Dolly, the first somatic cell cloned sheep in 1996, successful somatic cell cloning has been achieved in mice, cattle, goats, pigs, cats, rabbits, mules, horses, rats, and other mammals, though cloning dogs and monkeys has been more challenging. Chinese scientists have recently made breakthroughs in these areas. A team from the Institute of Neuroscience, Chinese Academy of Sciences, successfully bred the world' s first pair of somatic cell cloned monkeys, with the establishment of primate models being extraordinarily significant for brain disease research[26]. A team from the Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, used ear skin fibroblasts from ApoE gene-knockout dogs as nuclear transfer donors to construct the world' s first gene-knockout somatic cell cloned dog model of atherosclerosis[27]. The same team also pasted the human mutant huntingtin gene into the pig huntingtin gene, obtaining pig models that exhibit not only selective death of specific neurons in the brain but also dance-like abnormal behaviors. These pathological features and abnormal behaviors can be stably inherited by offspring, providing reliable animal models for clinical research[28].

Future Prospects for Synthetic Biological Diagnostics and Therapeutics

Artificially engineered live bacteria, cells, and viruses/phages may be the most complex "drugs" ever developed by humans, with potential to solve diseases that have long baffled the medical community. Based on recent trends, synthetic biological diagnostics and therapeutics will see more clinical trials and commercial development in the future.

While conducting large-scale clinical trials, we must not forget to refocus attention on basic research as synthetic biology is still an emerging discipline. For example, although CAR-T tumor immunotherapy is currently very popular, a shocking clinical case of relapse after CAR-T treatment was recently published in the top journal *Nature Medicine*[29]. This technology accidentally created "CAR-cancer cells" : the CAR (chimeric antigen receptor), which should have been added to T cells to help them specifically recognize CD19 and capture cancer cells, was accidentally added to the patient' s cancerous B cells, forming "CAR-cancer cells." The CAR on cancer cells binds to CD19 on the cancer cell surface, causing CAR-T cells to lose their target for recognizing cancer cells. The patient ultimately died due to massive proliferation of these "CAR-cancer cells."

Therefore, more in-depth research is needed on rational design of artificial living drugs, precise quantitative control, prediction of in vivo and in vitro functions, mechanisms from gene to phenotype, and the fate of living drugs after entering the human body. Design and modification of living drugs should fully consider host cells, expression systems, genetic circuit control, and system robustness to achieve better temporal and spatial controllability, making treatments more targeted. In the future, the depth of basic research in synthetic biology will largely determine the success of clinical applications.

As synthetic biological diagnostic and therapeutic technologies advance, policy and ethics research must also keep pace to ensure healthy and orderly development of this revolutionary technology. Compared to other treatment modalities, synthetic biology diagnostics and therapeutics involve new ethical and safety issues. In 2016, the US FDA issued a guideline[30] that clarified how live biotherapeutic products (LBPs), which can legally enter the market as foods or dietary supplements, can be used in research, and explained the manufacturing requirements researchers must meet in early clinical trials to use probiotics as drugs.

China still needs to accelerate regulatory layout in this area to ensure modern, efficient evaluation policies are established within the existing regulatory framework. This will both fully realize the potential benefits of live biotherapeutic products and understand their specific risks. Synthetic biological diagnostic and therapeutic products require more research and development and well-designed clinical trials to ensure their safety and efficacy, thereby unleashing the potential of synthetic biology in medical applications for the benefit of individual and

public health.

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Note: Figure translations are in progress. See original paper for figures.