Quantitative Biology 2021, 9(3): 267–291
https://doi.org/10.15302/J-QB-021-0253

REVIEW

Recent progress in research and application of engineered implanted cells for biomedical applications

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Received October 26, 2019; Revised January 7, 2021; Accepted January 19, 2021

***Background*: The core concept of cell engineering is the use of synthetic biological methods to engineer and design genetically modified cells with specific functions, which has revolutionized the biotechnology industry and cell therapy. Implanted cells play an important role in the cell therapy, but the currently used implanted cells are unable to fully meet the needs of researchers and clinicians. Therefore, the construction of engineered implanted cells has become a new research area, with many groups exploring the working principles of implanted cells, allowing them to better exert their repair function.**

***Results*: Based on the existing cell engineering platforms, this paper summarizes the main types of chassis cells used in implanted cell engineering, progress in the development of gene editing tools and delivery systems, as well as strategies for the construction of engineered implanted cells.**

***Conclusions*: The rational use of synthetic biology methods to program and control the function of implanted cells with high spatiotemporal accuracy provides new ideas for the development of cell therapy, and opens up new possibilities for exploring the mechanism of implanted cell action to allow them to better exert their role in promoting the progress of repair.**

Keywords: chassis cells; cell therapy; engineered implanted cells; synthetic biology

Author summary: The engineering transformation allows implanted cells to achieve optimal therapeutic effect in cell therapy. This review introduces the application of engineered implanted cells in the cell therapy, and how to equip the chassis cell with new functions to enhance or redirect their natural ability to achieve corresponding medical effects. We expect this review to draw attention to the use of synthetic biology ideas in the field of cell therapy that the rational use of synthetic biology methods provides new ideas for the development of cell therapy and new possibilities for exploring the mechanism of the implanted cell.

INTRODUCTION

Cell therapy refers to infuse cells into the body to cure or alleviate diseases. In combination with biological engineering methods and/or *in vitro* expansion, special culture and other treatments, these therapeutic cells obtained from individuals can modulate the immune system, kill pathogens and tumor cells, promote the regenerative and physical rehabilitation of tissues and organs, or other mechanisms. With the rapid development of cell biology and molecular biology, cell therapy has gradually moved from pure research towards clinical applications [1]. In recent years, cell therapy has made significant progress in many areas such as inflammation [2], tissue regeneration [3–6], anti-aging, and especially in cancer treatment [7–15]. A number of research breakthroughs have been made

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on medical problems [16,17], such as immune regulation [18–21], or the repair of degenerative organ damage [22–28], and the specific molecular mechanisms of the corresponding diseases have been further explained, which makes a significant contribution to human health and disease treatment.

Tissue engineering and regenerative medicine are important areas of cell therapy applications [3–6], and such cells are currently widely used in research on the regeneration and repair of bone, cartilage, blood vessels, skin, heart and liver tissue. Tissue engineering technology is used for the regeneration and repair of a variety of tissues and organs through its bionic and cellular functions. The three most important elements in the field of tissue repair engineering are seed cells, scaffolds and growth factors. Scaffolds and cytokines are considered an important part of the seed-cell microenvironment [29–33]. Seed cells can directly differentiate into specific cells damaged by chronic tissue injury to achieve tissue regeneration. At the same time, they can also secrete a large number of factors and extracellular vesicles to regulate the immune microenvironment [34–36].

Engineered cell therapy refers to the rapid development of clinical technology by utilizing the natural functions of cells, including migration, signal transduction, biosynthesis and secretion [37]. As our ability to build more complex cellular programs increases, assessing and improving the extent to which cell-based therapies are expected to be implemented in the body will become increasingly important considerations and opportunities for technological advancement. Engineered cells can incorporate genetic material and various genetic systems into chassis cells to endow them with specific functions, and then use the engineered cells as “living drugs” and implant them into the body to exert the desired therapeutic effect. The construction and application of engineered cells has also recently achieved success in clinical trials, including CAR-T cell-based cancer therapies [38], as well as the combined treatment with engineered hematopoietic stem cells [39] and adenosine deaminase [40] for the treatment of beta thalassemia immunodeficiency. For the transformed cells must not elicit an immune response from the body, these genetic circuits need to be tested and optimized *in vitro* in the same cell line that will be used in the body before implantation, increasing the possibility that the treatment circuit will function as designed. The clinical development of genetic and engineered cell therapies offers synthetic biologists the opportunity to create new therapies using synthetic gene circuits. These approaches are expected to make genetic and engineered cell therapies safer and more effective, and provide treatment options for diseases that are currently difficult to treat, such as hereditary diseases, certain cancers or other challenging diseases.

In this review, we systematically analyzed the challenges encountered during the application of engineered implanted cells in cell therapy and the synthetic biological strategies with which the implanted cells were modified to fill the gap. The application of biochemically treated cells to explore the role of implanted cells in cell therapy is also discussed (Fig. 1).

CHASSIS CELLS IN ENGINEERED CELL THERAPY

Chassis cells can be defined as a biology platform that embrace the incorporation of biological components with more or less specialized optimization [41]. In addition to be able to play a therapeutic role after implantation, the selected chassis cells should also have a series of desirable

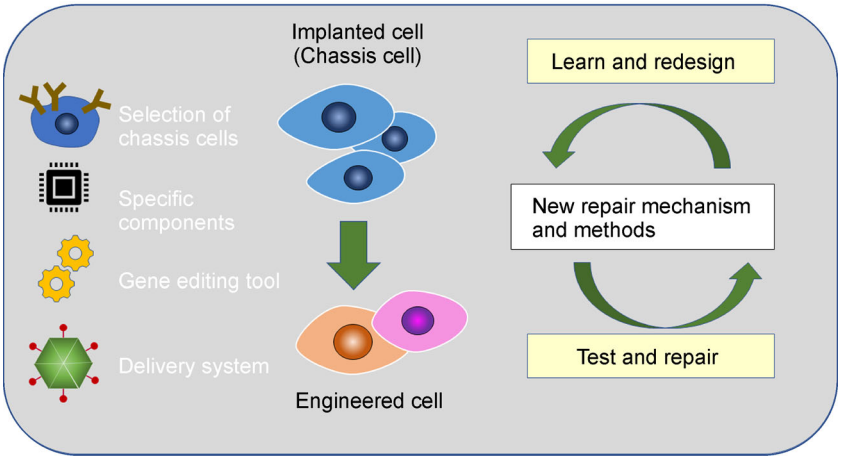

Figure 1. Engineered implanted cells for cell therapy. The strategy for the construction of engineered implanted cells revolves around the selection of chassis cells, the selection and application of gene editing tools and specific elements, as well as the selection of element delivery systems.

features, such as their amenability for synthetic biology. If biological components, DNA synthesis and assembly technology, as well as genome editing tools are used as accessory tools, then the selection and optimization of the main chassis cells will be more effective in view of the efficient synthesis of target products and the establishment of complex genetic circuits. The main idea of constructing ideal chassis cells is to optimize the necessary functions on the basis of maintaining the host cell's basic self-replication and metabolism capabilities to obtain a synthetic biology platform with good orthogonality, high robustness, and universality. Compared with microorganisms and yeasts used as chassis cells for synthetic biological transformation, mammalian cells have significantly weaker development and applications as chassis cells due to their complex genetic background and limited gene editing tools. Selecting suitable implanted cells is a key step in the translation of cell therapy research from the laboratory to clinical application. Implanted cells that can be used for cell therapy are widely available, each with their own advantages and disadvantages. According to the source of implanted cells, they can be divided into autologous, allogeneic and xenogeneic cells. At present, the use of implanted cells in cell therapy are mostly based on the extraction and further use of primary cells *in vivo* [42–48]. Therefore, in the process of constructing engineered implanted cells in the field of cell therapy, the selection of appropriate chassis cells is the basis of successful results. This section will focus on the basic requirements for the selection of chassis cells in engineered cell therapy, the types of existing engineered cells, and their use in medical research, to provide a constructive basis for the selection of suitable chassis implanted cells.

Basic requirements for chassis cell selection of engineered implanted cell

The choice of chassis cells will have a great impact on the use of engineering methods and the effect of cell therapy, thereby affecting the performance of therapy based on engineered implanted cells. In order to give full play to the role of engineered implanted cells, new requirements have been put forward for the selection of chassis cells. On the one hand, an optimal chassis cell must fulfill the basic functions of implanted cells, including the ability to proliferate and differentiate, exert therapeutic functions, and at the same time have a certain immunosuppressive ability to achieve low immunogenicity, and thereby avoid provoking a strong immune response [34–36]. On the other hand, it needs to possess the basic characteristics of chassis cells in terms of robustness and modifiability. Robustness is the basic requirement that determines that engineered cells can successfully achieve therapeutic functions *in vivo*. However, transformation with implanted genetic elements will affect the stability of implanted cells, resulting in certain heterogeneity in engineered cells after transformation. This heterogeneity can greatly affect the construction and performance of engineered implanted cells. There are many sources of this heterogeneity. First, when cells come from different donors, donor-to-donor heterogeneity may occur. Second, when the same cell product is applied to different patients, performance differences can also occur. Third, there can be differences in the performance of cell products in different body locations of a single individual (including off-target and on-target effects). Finally, the way in which each engineered cell responds to the same environmental factors will also vary [37]. Therefore, it is very important to choose robust chassis cells.

In addition, the choice of chassis cells for engineering implanted cells should also pay attention to ethical and safety issues. The application of stem cells that can be used as implanted cells may have certain ethical issues. For example, the generation and clinical application of human embryonic stem cells (hESCs) have been a unique focus of stem cell ethics [49,50]. In addition, poor differentiation and malignant transformation are the main safety issues of induced pluripotent stem cells (iPSCs) and iPSC-derived cell transplants [51,52]. Mesenchymal stem cells (MSCs) are often provided globally as universal human therapies, but may promote tumor growth and metastasis [53,54]. Although the applications of cell therapy are becoming increasingly widespread, the safety of cell therapy is still an issue that we must pay attention to, and the safety and ethics of engineered cell therapy should be considered first [55].

Immune cells in engineered cell therapy

Immune cells are responsible for the main defense functions against infectious diseases and cancer. They have the ability to migrate to the site of disease, secrete immunomodulatory molecules and lyse target cells. Most research in the field of immune cell engineering has focused on T cells because they are relatively easy to obtain, can be genetically engineered and expanded *in vitro* [56]. The most successful application of synthetic biology to cell engineering is the iterative development and upgrading of T cell immunotherapy based on chimeric antigen receptors. In 2011, Porter *et al*. directly fused the single-chain antibody V region of a specific immune antigen targeting chronic lymphocytic leukemia, with the T chain ξ chain, thereby programming the patient's own immune cells to directly target cancer cells [57]. Later, this method was also confirmed in a variety of solid tumors, and the application prospect cannot be overstated. CRISPR/Cas9 has been used to further

improve cellular behavior in the therapeutic context. For example, PD1 knockout is an important way to alleviate the PD cell response and enhance the activity of CAR-T cells in the immunosuppressive tumor microenvironment [58]. Other studies have shown that integrating CARs into the natural genomic TCR locus can prevent premature depletion of T cells, and knocking out the endogenous TCR can improve the performance of transgenic TCRs [59,60]. The development of CAR-T cells reflects the classic process of using synthetic biology to transform cells. In this case, native T cells were used as chassis cells for transformation. At present, CAR-T has developed to the fifth generation, and the first three generations have focused on co-stimulatory factors (CD28, 4/1BB, OX40) to improve the proliferation and lethality of CAR-T cells. In the fourth generation, cytokines or co-stimulatory ligands were added on the basis of the third generation to enhance the expansion capacity of CAR-T cells and prolong their residence time *in vivo*. In the past two years, the transformation and design of CAR-T solid tumor treatments has led to the fifth-generation of CAR-T cells, which are mainly manifested in dual-specific CAR, multi-target CAR, iCAR with non-activating signals, co-expression of fusion proteins to reconstruct the tumor microenvironment, CAR structure controlled by molecular switches, and general-purpose CAR. Thus, a series of optimized combinations considered in terms of safety, effectiveness and industrial production have been continuously developed [61–65]. In addition to reforming the CAR structure itself, scholars also seek other innovations, such as combining CAR-T with immune checkpoint inhibitors, using TGF-β to transform the tumor microenvironment, and using iPSCs (induced pluripotent stem cells) to induce T cells as a source of CAR-T cells (Fig. 2).

Furthermore, researchers are not limited to T cells, but also expanded the modified chassis to NK, NKT, macrophages, and DCs [66,67]. Dendritic cells and natural killer (NK) cells are two other major cell types that play a role in immune defense against cancer. NK cells equipped with CAR for targeted tumor cell lysis are replacing CAR-T cells. Allogeneic T cells usually cause graft-versus-host disease (GVHD) mediated through TCR while there is little risk for NK cell to cause that. Therefore, NK cell could be generated from donors in batches to shorten the course of treatment [68]. Due to the bottlenecks in obtaining a sufficient number of cells and genetically engineering these cells, the clinical translatability of engineered NK cells lags behind T cells. Therefore, genetic engineering of iPSCs with CAR receptors, and then differentiating them into NK cells is an important step towards generating a sufficient number of functional CAR-NK cells [69]. Li *et al*. successfully developed CAR-NK cell therapy. Optimized CAR receptors with NK-specific co-stimulatory domains have further improved the efficiency of tumor-targeted NK cells [13]. This new type of therapy is expected to become a new favorite in the immunotherapy community. DC cells are the dominant antigen presenting cells of the immune system. Accordingly, administrating DC cells loaded with tumor-associated antigens can elicit a tumor-specific immune response [70,71], Moreover, generic modification of DC cells can be used to modulate the immune response and thereby improve cancer immunotherapy [72,73].

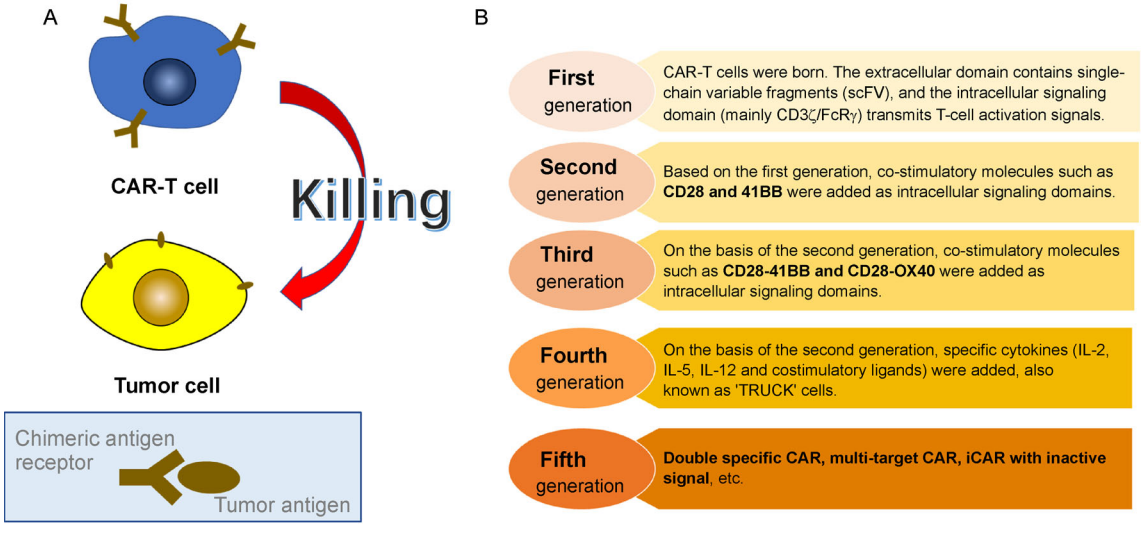

Figure 2. Engineering of CAR-T cells. (A) The principle of CAR-T cell therapy. The basic design of CAR includes a tumor-associated antigen (TAA) binding region (usually derived from the scFv antigen-binding region of a monoclonal antibody), an extracellular hinge region, a cross-membrane region and an intracellular signal region. The CAR-T target antigen is a tumor-specific antigen expressed only on the surface of tumor cells. (B) The development of the first to fifth generation of CAR-T cells.

Due to the sensitivity of macrophages to the immunosuppressive tumor microenvironment, they are generally less effective in cancer treatment. However, a new study shows that combining inhibitory receptors on macrophages with pre-loaded tumor-specific antibodies can be a very effective strategy for overcoming this problem [74]. Drug-loaded nanoparticles are another promising way to modify tumor-associated macrophages [75,76]. Beyond cancer therapy, genetically modified macrophages were construct in attempts to treat a range of other disease. For instance, Gaucher disease is an inherited disease caused by mutations in the glucocerebrosidase gene, leading to a harmful buildup of glycolipids in cell types with active glycolipid metabolism, especially in macrophages. Glucocerebrosidase expression cassettes can be introduced in human hematopoietic stem and progenitor cells (HSPCs) for specific expression in the macrophage, and glucocerebrosidase positive macrophage can then be generated from edited HSPCs [77].

Engineering these cells to perform important functions in the tumor microenvironment is an effective option for enhancing immunotherapy and may be further utilized in the future. Therefore, immune cells are currently one of the most commonly used chassis cells for cell engineering. Apart from cancer, the immune system also takes an active part in other disease conditions such as tissue repair. It's believed that immune response promotes wound healing but suppresses regenerative capacity [78]. Additionally, the scavenging capacity of immune system is prone to friendly fire the normal tissue in chronic inflammation diseases [79]. Therefore, controlling immune response to promoter regeneration has received increasing concerns, and engineered immune cells could have application for cell therapy [80].

Stem cells in engineered cell therapy

Stem cells have many useful properties, including their ability to self-renew, migrate, differentiate, and secrete a variety of therapeutic molecules, such as immunomodulatory factors. As a result, many preclinical and clinical studies have utilized stem cell-based therapies and demonstrated their great potential for treating various human diseases and conditions. At present, one of the most realistic and reliable sources of implanted cells are the stem cell including adult stem cells, embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC). Adult stem cells reside in various tissues and can give rise to numerous types of cells depending on their source. ESCs are derived from the inner cell mass of developing blastocyst embryos and almost totipotential [81]. iPSCs are ESC-like reprogramed somatic cells, and their derivation was first reported in 2006 by Takahashi and Yamanaka, who induced adult fibroblasts into pluripotent stem cells using the four factors *Oct3/4, Sox2, c-Myc,* and *Klf4* [82]. Engineered stem cells are widely used for the regeneration of various tissues, including the central nervous system, muscle, cartilage, and heart. In order to improve the efficacy of stem cell therapy, stem cells can be specifically engineered to control cell differentiation or provide molecules that can further support tissue repair. Recently, much research focused on engineering stem cells to further enhance their innate abilities and to give them new functions like the ability to deliver genes or small molecules.

Engineered implanted cells for trophic action

Although stem cells were initially expected to differentiate into functional cells to replace injured ones, in many cases there is evidence that stem cells contribute to tissue repair and regeneration mainly through paracrine mechanisms [83–85], rather than by directly differentiating and fusing with the injured tissue [86–88].

To improve the efficacy of stem cell therapy, stem cells can be engineered to enhance their capacity to specifically secrete and provide molecules that can further guide differentiation or revascularization, and thereby greatly increase the efficacy of stem cell therapy. Early in 1993, fibroblasts were used as a source of engineered cells for the secretion of NT-3, which was shown to promote neuroregeneration, differentiation and migration during brain development, reversing the main symptoms of Parkinson's disease [89]. After the paracrine effects of stem cell therapies was recognized, Kumagai and colleagues demonstrated that they can augment stem cell types with trophic activities to treat nerve damage, and they used a series of powerful genome editing technologies such as the CRISPR–Cas9 system to design MSCs that secrete neurotrophin MNTS1 using lentiviral vectors [90].

In cardiac repair, Deuse *et al*. induced mouse MSCs to secrete hepatocyte growth factor (HGF) or vascular endothelial growth factor (VEGF) via lentiviral vectors [91], and transplanting them into mice, which significantly improved myocardial function. Similarly, Guo *et al*. demonstrated that engineered MSCs using HGF can significantly enhance angiogenesis through VCAM-1 expression [92]. Liu *et al.* designed MSCs to express angiotensin, a heparin-binding protein that interacts with endothelial cells, promotes cell proliferation and induces angiogenesis [93,94].

A commonly used implanted cell design for enhancing bone regeneration focuses on genetically modified cells expressing the bone morphogenetic protein (BMP) series of genes, which induce osteogenesis with high efficiency [95,96].

Engineering stem cells to give rise to tissue cells

Nevertheless, attempts to guide stem cell differentiation were also evaluated for therapeutic applications in some diseases, such as bone tissue regeneration [97]. MSCs, ESCs, and iPSCs were demonstrated to be able to promote musculoskeletal regeneration and form tissues composed of multiple cell types. There is a broad scope of research on stem cell therapies that more accurately guide musculoskeletal regeneration. This can be done by engineering stem cells to express factors that specifically guide the differentiation of bone or cartilage lineages. For cartilage regeneration, overexpression of the *Sox* gene family, including transcription factors such as *Sox5, Sox6*, and *Sox9*, for engineering MSCs is a commonly used implanted cell design [98,99]. Wojtowicz *et al*. introduced the *Runx2* gene, which encodes a key bone-forming transcription factor, into bone marrow mesenchymal stem cells (MSCs), greatly improving the effect of seed cells in bone repair [100]. In 2014, our team transformed implanted cells with a tetracycline-regulated cassette for the expression of *Sox9*, a key cartilage transcription factor, implanted them into animals embedded in a PHBHHx scaffold, and induced the cassette by oral tetracycline to achieve controlled inhibition of cartilage degradation [101]. And Ying Ma *et al*. seeded the engineered MSC on inducer sustained-released material to timely precisely control the expression of anti-apoptotic gene *Bcl2*, as well as chondrogenic gene *Sox9*, thus increasing the cell viability and chondrogenic performance [102]. In addition, Dalabi and colleagues designed ESCs that express *Pax3* to prove the effectiveness of ESC in skeletal-muscle differentiation and eventually applied it to muscular dystrophy [103]. *Pax3* is a transcription factor whose expression leads to the activation of the myogenic regulatory factor (MRF) genes, *Myf5, Myf6, MyoD1*, and *Myog* [104]. These studies have shown that engineered implanted cells can greatly promote the musculoskeletal tissue repair engineering, greatly improving the repair effect.

Unexpected differentiation outcomes have impeded the therapeutic effect, and it is still essential to control the cell fate of these transplants. Hwang and colleagues modified NSCs to overexpress the *Olig2* gene through antiretroviral transduction to control the direction of NSC differentiation to oligodendrocytes whose widespread apoptosis is regarded as major factor of the observed malfunction [105]. Further, Hu and colleagues demonstrated that engineering NSCs to overexpress *Olig2* and bone marrow basic protein T (MBP-T) can synergistically improve their survival following transplantation and greatly improve the therapeutic effect [106]. To direct the implanted stem cells to differentiate into cardiomyocytes, Wang and colleagues designed MSCs that secrete hypoxia-inducible factor 1 + (HIF1 +) to differentiate into cardiomyocytes [107,108].

Therefore, using well-designed gene circuits to regulate cell fate may become the new gold standard for stem cell research [109], and stem cells have become one of the most important chassis cell types for transformation.

Engineered implanted cells for therapeutic agent delivery

Interesting tumor-homing characteristics make stem cells into powerful potential carriers for antitumor agents. As a carrier of gene therapy, stem cells can carry gene therapy vectors to tumors and metastatic sites, thereby increasing the local concentration at the treatment site, while reducing the required dose and subsequent side effects [110]. Stem cells can also use genetic engineering to secrete therapeutic proteins [111–114], or enzymes [115–117], and convert individually administered non-toxic drugs into cytotoxic drugs. Using these methods, engineered stem cells are able to migrate to primary tumors and metastatic sites to produce drugs or enzymes, thereby bypassing short drug half-life and repeated drug dose restrictions [118]. In addition, researchers have recently begun to design stem cells with chemotherapy-loaded nanoparticles in order to achieve greater tumor targeting and penetration using only nanoparticle-based drug delivery methods. To achieve this, a new method of loading nanoparticles on the surface of MSCs has recently been studied [119].

Duchenne muscular dystrophy (DMD) is an inherited disorder that characterized by progressive muscle weakening due to the mutation in the gene encoding dystrophin. Taking advantage of cell fusion, Goncalves *et al*. recently designed human MSCs and demonstrated that these engineered MSCs can be fused with DMD myotubes to deliver complementary gene products, thereby re-establishing the synthesis of full-length dystrophin in DMD muscle cells [120]. Furthermore, Jia and colleagues developed the first non-viral cell delivery strategy to implement optogenetics in cardiac regeneration to specifically control the excitation and contraction of cardiac tissue, which does not rely on embryogenesis [121]. This approach also has potential for cardiac tissue regeneration in future stem cell engineering approaches.

Modification of other cell types in engineered cell therapy

Apart from stem cells and immune cells, other cell sources that can be genetically modified can also serve as chassis cells. Beyond NT-3 secretion, fibroblasts can be engineered to deliver immunoregulatory IL-12 in sarcoma, which successfully suppressed tumor growth and significantly inhibited lung metastases [122]. The

treatment of some metabolic diseases has also begun to rely on engineered cells. In 2013, researchers from the Valrose Institute of Biology reprogrammed mouse endothelial cells into insulin-secreting beta cells for the first time, which may be used to treat human type-I diabetes in the future [16]. Also, Lin *et al*. found that human umbilical vein endothelial cells (HUVEC) over-expressing miR-206 enhanced the contractile function of smooth muscle cell phenotype in a co-culture system [123]. In addition, a tandem assembly and gate that simultaneously analyzes TNF and interleukin 22 (IL-22) to control the secretion of the anti-inflammatory cytokines IL-4 and IL-10 was constructed in human embryonic kidney 293 (HEK-293) cells, with great promise for the treatment of autoimmune diseases such as psoriasis [124]. Various closed-loop control systems have been developed in highly modifiable cell lines, such as HEK-293 and HeLa, for use as therapeutic biocomputers to control liver injury [125], gouty arthritis [126], hypertension [127], diabetic ketoacidosis [128], obesity [129], and Graves' disease [130]. The discussed studies have shown that not only immune and stem cells, but also differentiated cells such as vascular endothelial cells and fibroblasts can also be used as engineered chassis cells.

THE MAJOR RESEARCH AREA FOR IMPLANTED CELL ENGINEERING

At present, the mechanism underlying the therapeutic effect of stem cells remains unclear and there are various problems of application, such as limitations of gene editing tools, small capacity and toxicities of gene delivery vectors, uncontrollable cell differentiation, problems of cell residence, lack of precise spatiotemporal control and the fact that natural cells may not have the phenotype to achieve repair and treatment. By means of synthetic biology, cells can be equipped with new functions to enhance or redirect their natural ability to achieve corresponding medical effects. Moreover, the transformation of implanted cells can provide new ideas for understanding the mechanism of their action. Therefore, the transformation of implanted cells may become one of the main research directions of cell therapy in the future. We will discuss the advances in synthetic biology that improve engineered cell in this section.

Developments in gene editing tools

Gene editing tools are the basis of engineering implanted cells. Choosing appropriate gene editing tools makes cell engineering more successful. In the past few years, several programmable nuclease platforms have been developed, including zinc finger nucleases (ZFN), transcription activator-like effector nucleases (TALENs) and CRISPR/Cas9 [131,132]. Each system uses specific rules to design modular protein domains (ZFNs and TALENs), or targets the desired sequences via complementary small guide RNAs (sgRNA, used in conjunction with Cas9) to direct nuclease activity to a specific genomic location for gene knock-in or knockout. ZFNs are the most challenging to design, with the lowest success rate (about 10%), while TALENs and Cas9/sgRNA are easier to design and the success rate is relatively high (about 20%) [132]. The most widely in recent years is the CRISPR/Cas9 system, which is particularly attractive because the specificity of sgRNA allows it to target any sequence [133,134]. However, Cas9 cutting off-target DNA may lead to heterogeneity between cells and reduce the performance of cell therapy [135–138]. Therefore, the development of computational tools and strategies to minimize potential off-target effects remains an active area of research [139–142]. New approaches in this direction include the use of paired nicking enzymes [137,143,144], truncated sgRNA [145] and purified Cas9 protein [146,147]. An alternative to nuclease-mediated genome engineering is the use of recombinases to integrate large DNA cassettes into target sites [148]. Given the power and rapid development of genome editing technology, these methods may drive the prototyping, development, and implementation of engineered implanted cells (Fig. 3).

Some cell therapies involving genome editing have been approved for use in phase I clinical trials. The earliest trials used ZFNs to knock out the CCR5 co-receptor gene in T cells of HIV-positive patients [149], making the T cells resistant to the virus. TALENs have been used to enhance the efficacy of therapeutic CAR-T cells [150], and at least two trials using CRISPR-Cas9 have been approved for this purpose [151]. These examples of engineered cells provide examples of how to choose the right gene editing tools to edit implanted cells. It is also worth noting that in all cases of engineered implanted cells, whether based on *in vitro* or *in vivo* treatment, their safety and effectiveness must be demonstrated.

Development of component delivery systems

One of the keys to the construction of engineered implanted cells is to deliver the designed gene circuit to the inside of the cell, so as to achieve the effective expression of the gene circuit and achieve the function of the corresponding element system. The choice of method for introducing new genetic material into cells will affect many attributes of engineered cell therapies, including efficacy, safety, longevity and the robustness of engineered cell function. In order to achieve the desired effect of engineered implanted cells, the therapeutic gene must

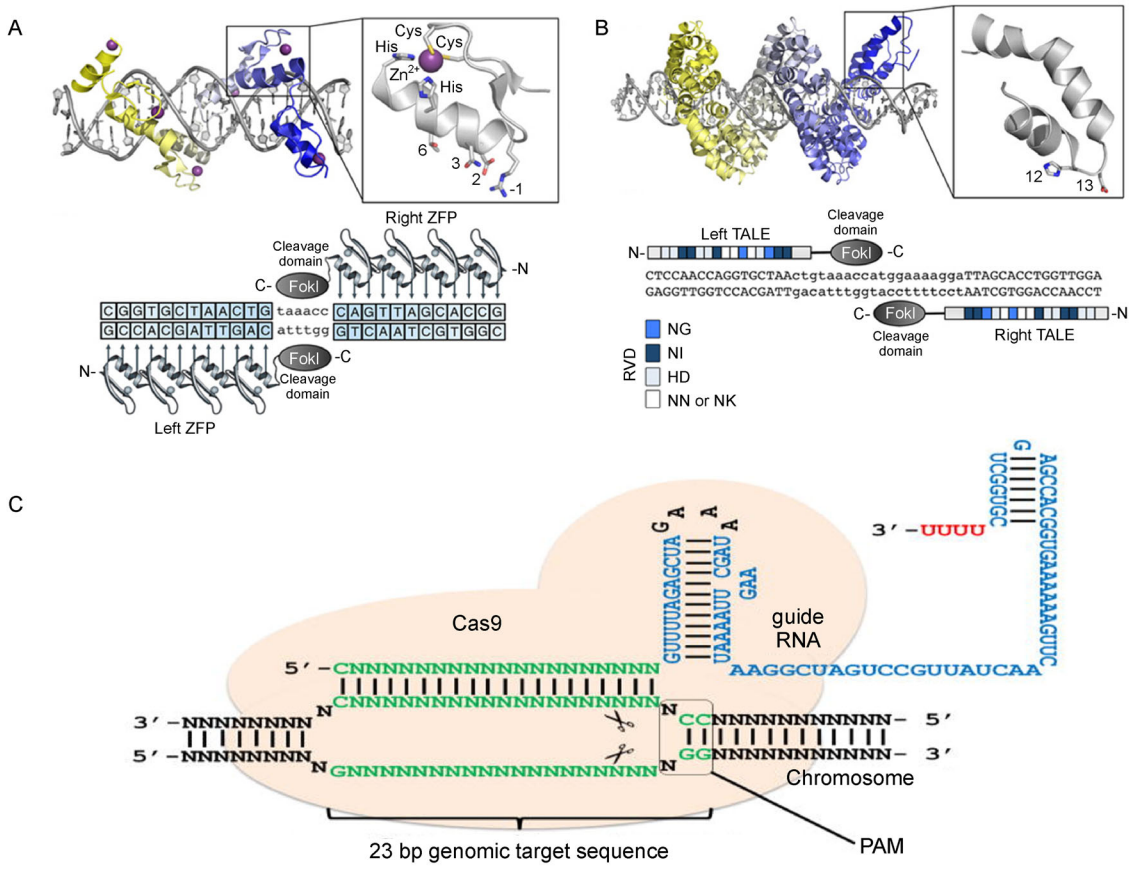

Figure 3. Gene editing tools for the construction of engineered implanted cells. (A) Designed zinc-finger protein in complex with target DNA (grey) and cartoon of a zinc-finger nuclease (ZFN) dimer bound to DNA. (B) TALE protein in complex with target DNA (grey) and cartoon of a TALE nuclease (TALEN) dimer bound to DNA. (C) RNA-guided CRISPR-Cas9 [131,132].

be carried on a safe and effective vector, which can not only specifically transfer the gene to the target cell, but also maintain its subsequent expression. Other characteristics that these vectors should possess include: 1) high transfection efficiency, 2) long-term stability without integrating into the host genome, 3) the ability to temporarily express appropriate levels of therapeutic genes, and 4) not stimulating the host's immune system or inducing malignant cell transformation [152].

Currently, the most effective and most commonly used approach for introducing gene lines into implanted cells is by means of viral vectors. The currently used viral vector delivery systems mainly include retroviruses, lentiviruses, adenoviruses, and adeno-associated viruses, each of which has distinct advantages and disadvantages, and may have different applications.

Retroviral vectors are the first type of viral vector to be developed, and they have historically been the most widely used viral vectors in clinical trials [153]. Retroviruses reverse transcribed its single-strand RNA genome to produce DNA that can be integrated into the host genome via integrases [154,155]. Though non-immunogenic, it has been found that retroviral vectors are produced at relatively low titers, require the integration of the provirus into the host chromosome for transduction, and usually only infect dividing cells. The integrated cassette of retroviral vectors can dysregulate the expression of endogenous genes through insertional mutagenesis of proto-oncogenes or tumor suppressors, leading to the malignant transformation of engineered cells [153]. Thus, the use of retroviral vectors for clinical trials has decreased in recent years [156].

Lentiviruses are currently the most promising therapeutic vectors with large-scale transgenic capabilities [157]. These vectors have been widely used in gene therapy applications, including CAR-T cell therapy, and advances in vector design and production have improved their performance and safety [158,159]. Like retroviral vectors, lentiviral vectors can be integrated into the host cell's genome. However, unlike other retroviruses, lentiviral vectors have the advantage of being able to transduce non-dividing cells. As such, these vectors are one of the most effective viral methods of gene delivery [160]. And lentiviruses are known to be less genotoxic

than other retroviral vectors [161]. But the their carcinogenic potential due to insertion mutations remains a major obstacle to the clinical application. In a clinical trial to treat β thalassemia, though no patient showed any signs of malignancy at 33 months following the implantation of the modified cells, the vector had been integrated into the high mobility AT-hook 2 (HMGA2) gene which had previously been associated with the dedifferentiation and metastasis of solid tumors in 3% of these cells [162,163]. Fortunately, the patient did not show any signs of malignancy at 33 months following the implantation of the modified cells. Finally, stem cells also exhibit a low affinity for lentiviral vectors, and thus potentially require cytokine stimulation to improve transduction efficiency [164].

Adenoviruses are non-enveloped icosahedral viruses consisting of a nucleocapsid and a double-stranded linear 36 kb DNA genome that provides ample space for large sequences to be inserted [165,166]. In addition, adenoviral vectors have high transduction efficiency in both dividing and non-dividing cells, and the vector remains episomal and therefore remain the host genome intact [156].These characteristics may be particularly useful when using stem cells as implanted cells in tissue regeneration, as transient expression of transduced genes can help prevent the overgrowth of transplanted stem cells. However, adenoviral vectors are limited by their large size and strong immunogenicity when used clinically [167]. To address the toxicity, second- and third-generation vectors with additional deletions of viral genes were developed to reduce their toxicity. But even if all viral genes are deleted and the vector is propagated using a helper-dependent packaging system [168], the vectors are not completely non-toxic, and transduction of these vectors will cause large changes in endogenous gene expression profiles [169].

Adeno-associated virus vectors are small viruses with a single-stranded DNA genome derived from the parvovirus family. A helper virus is required to propagate and package the vector [170]. It has the ability to transduce a variety of tissues and cell types, and its potency can be of great use in clinical applications [171,172]. Adeno-associated vectors are characterized by many advantages compared to adenoviral and other viral vectors, such as the ability to infect dividing and non-dividing cells. In addition, these vectors are largely episomal ($>99\%$), while $<1\%$ is predictably integrated into human chromosome 19 [173]. Finally, adeno-associated viruses are not known to cause any human disease and their immunogenicity is low. Because of these characteristics, adeno-associated virus vectors are currently the first choice for clinical viral transduction [156]. Previous studies have shown that these vectors can induce 10- to 100-fold higher levels of transgene expression *in vitro* and *in vivo* compared to other vectors. However, due to their small size (2.4–4 kb), they can only accommodate small genes, limiting their therapeutic use [174,175]. In addition, despite their low immunogenicity, a study reported that adeno-associated virus vectors were integrated near a miRNA locus known to be involved in tumorigenesis, leading to the development of hepatocellular carcinoma [176]. More importantly, a clinical trial conducted by Nathwani *et al*. showed that adenovirus-associated viral vector-mediated gene transfer in hemophilia B patients but does not cause any acute or long-term toxicity. Nevertheless, more patients and longer observation periods are needed before the effectiveness of adeno-associated virus vectors can be fully evaluated [177].

Various limitations of viral vectors, such as carcinogenicity, immunogenicity, and safety issues, have led to the development of synthetic non-viral vectors [178]. The ideal non-viral vector should be able to overcome many of the obstacles associated with systemic delivery, including: 1) targeted delivery, 2) efficient cellular uptake and endosome escape, as well as 3) biocompatible DNA release, while preventing genetic degradation. To this end, nanoparticles can provide a promising platform for delivering genes to stem cells.

Nanoparticles have many advantages over viral vectors, including: 1) lower immunogenicity, 2) the ability to deliver larger payloads, and 3) they are generally easier to prepare/synthesize [179,180]. In addition to DNA, nanoparticles can be used to deliver RNA, biomolecules (*e.g.*, peptides, proteins), small molecule drugs, and can also provide other functions (*e.g.*, heating, imaging) [181]. Due to their great potential, many nanoparticle systems have been developed to overcome the physiological obstacles faced by non-viral delivery methods. Specifically, these nanoparticles can be synthesized from a variety of materials, including lipids, polymers, metals, precious metals, semiconductors, and other inorganic materials, and can have various sizes, shapes, and characteristics [182]. However, few of these vectors have received FDA approval for clinical trials [178]. In addition, they are often hampered by lower delivery efficiency relative to viral vectors [183]. Therefore, despite the great potential of this class of delivery vehicles, there is still much room for improvement before they can be widely used in the clinic (Fig. 4).

Reprogramming of cell fate and cellular behaviors

Whether for killing cancer cell, trophic factors section or functional tissues regeneration, confining implanted cells in a particular state is requisite. Accordingly, the access to implanted cells with a specified and stable phenotype is a long-standing goal in this area, which requires precise reprogramming of human cell fate and cellular behaviors.

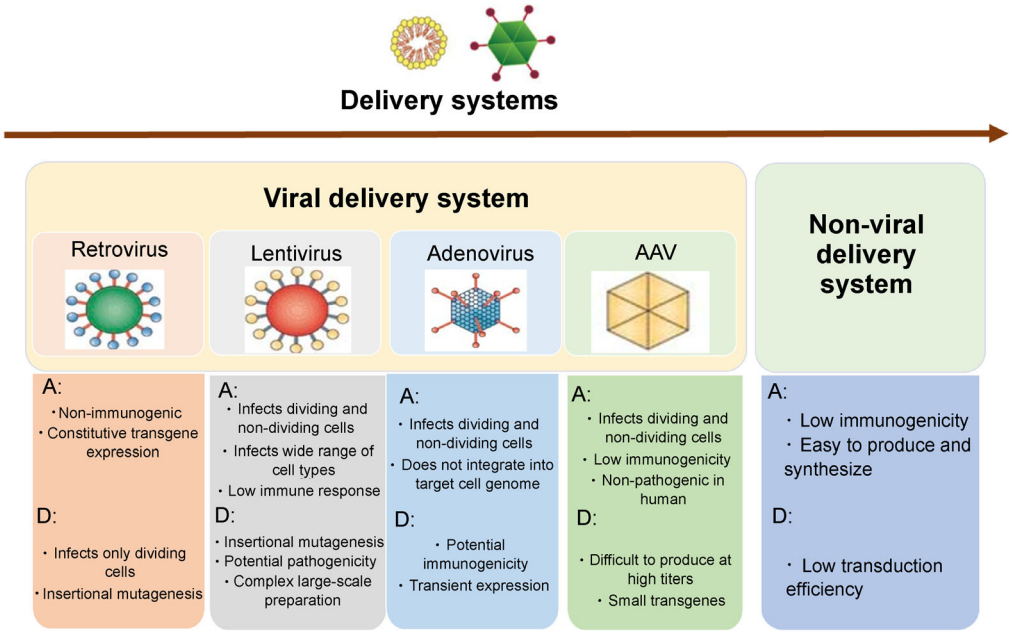

Figure 4. Various gene delivery systems with their advantages and disadvantages (A: advantages, D: disadvantages).

In the previous section it was mentioned that iPSCs can be engineered to form immune cells. The construction and induction of iPSCs is a key research area in cell reprogramming [184]. In 1998, Thomson *et al*. firstly isolated and stably culture hESC from the inner cell mass (ICM) of blastocyst-stage embryos [185], and in 2006, Takahashi and Yamanaka generated hESC-like iPSC [82]. Initially, the overexpression of the major transcription factor octamer-binding protein 4 (OCT4; also known as POU5F1), the sex-determining region Y box 2 (SOX2), Kruppel-like factor 4 (KLF4), and the MYC proto-oncogene protein can transform any cell type into a pluripotent state [186]. However, differentiation of pluripotent precursor cells into adult cell types also requires strict control. Protocols to derive disease-relevant cells from stem cell sources by providing microenvironmental components including ECM, other cells and factors have been established [187], but mimicking a cellular niche with fate-inducing activity is always challenging and it is not feasible to meticulously engineer such niches for *in vivo* cell fate conversion. The most common method to generate decision-making synthetic circuits for desired differentiation outcomes rely on the overexpression of lineage-specific transcription factors. Beyond what we have discussed in the previous section on engineering stem cells to give rise to tissue cells, the overexpression of Neurogenin-1 and Neurogenin-2 in human induced-pluripotent stem cells produced neurons with greater than 90% purity, and similar strategies were successfully applied to produce hematopoietic cells [188], cardiomyocytes [189], and other cell lines. The gene regulatory networks involved in embryogenesis or regeneration are more complicated, since regulatory factors take effect in a spatiotemporally programmed manner. Thus, more sophisticated designer gene circuits are needed. A synthetic gene circuit composed of an adjustable band-pass filter can regulate the expression of NGN3 synchronously with cell-stage specific PDX1 expression and β-cell specific MAFA transcription, which can greatly improve the efficiency of differentiation in glucose-sensitive insulin-secreting beta-like cells [190].

Furthermore, the development of a large number of sensing elements that can detect gene expression has laid the foundation to control cell fates based on intracellular transcriptional states. Implanted cells also need to sense their own intracellular changes at different times, so as to automatically coordinate cell fates and adjust cell function in response to changes in time during the process of cell therapy. An example of this approach is the protein switch engineered by Ostermeier *et al*., which only catalyzes prodrug conversion when it recognizes cancer-related hypoxia-inducible factor 1α (HIF-1α) [191]. An RNA aptamer-based sensor was also engineered to regulate gene expression or to direct protein splicing after ligand-aptamer binding to program responses to cell states, and RNA controllers have been grouped together to implement an "AND" gate, producing apoptotic proteins only when β-catenin and NFκB are detected at the same time [192].

The design and application of engineered artificial induction promoters based on intracellular transcription factors also provides new possibilities for intracellular state sensing (Fig. 5A). The typical design of artificial promoters is the combination of one or more tandem transcription factor binding sites whose activity are confined only in the cell state of interest and minimal promoter sequence [195–200]. Recently, the group of Yangming Wang from Peking University used miRNAs in the cell in combination with the CRISPRa/i system to construct a sensor element that can sense the state in the cell [194](Fig. 5B). In synthetic biology, other strategies for coupling input/output relationships of populations of cells are rapidly emerging. There is also continuous development of toggle switches [201–204], microRNA classifiers [205–207](Fig. 5C), and synthetic transcription regulators [133, 208–210] (Fig. 5D), that promote the generation of multi-input prompts for complex circuits that can be integrated to produce specified phenotype of implanted cell.

Although synthetic circuits can induce the differentiation of a specified cell lineage from pluripotent stem cells, the native gene regulatory networks guiding morphogenesis, regeneration and repair that structure mature tissue are inherently complex and incompletely understood. For example, functional regions can be found in brain organoids similar to those in fetal brains, but they are disorganized [211]. We need more quantitative information on cell differentiation and synthetic circuits that compute multiple inputs to perform complex morphogenetic behavior or direct cells to a certain state.

Engineering of cell targeting and chemotaxis

Engineering of cell targeting and chemotaxis can guide the migration of implanted cells to a specific pathological

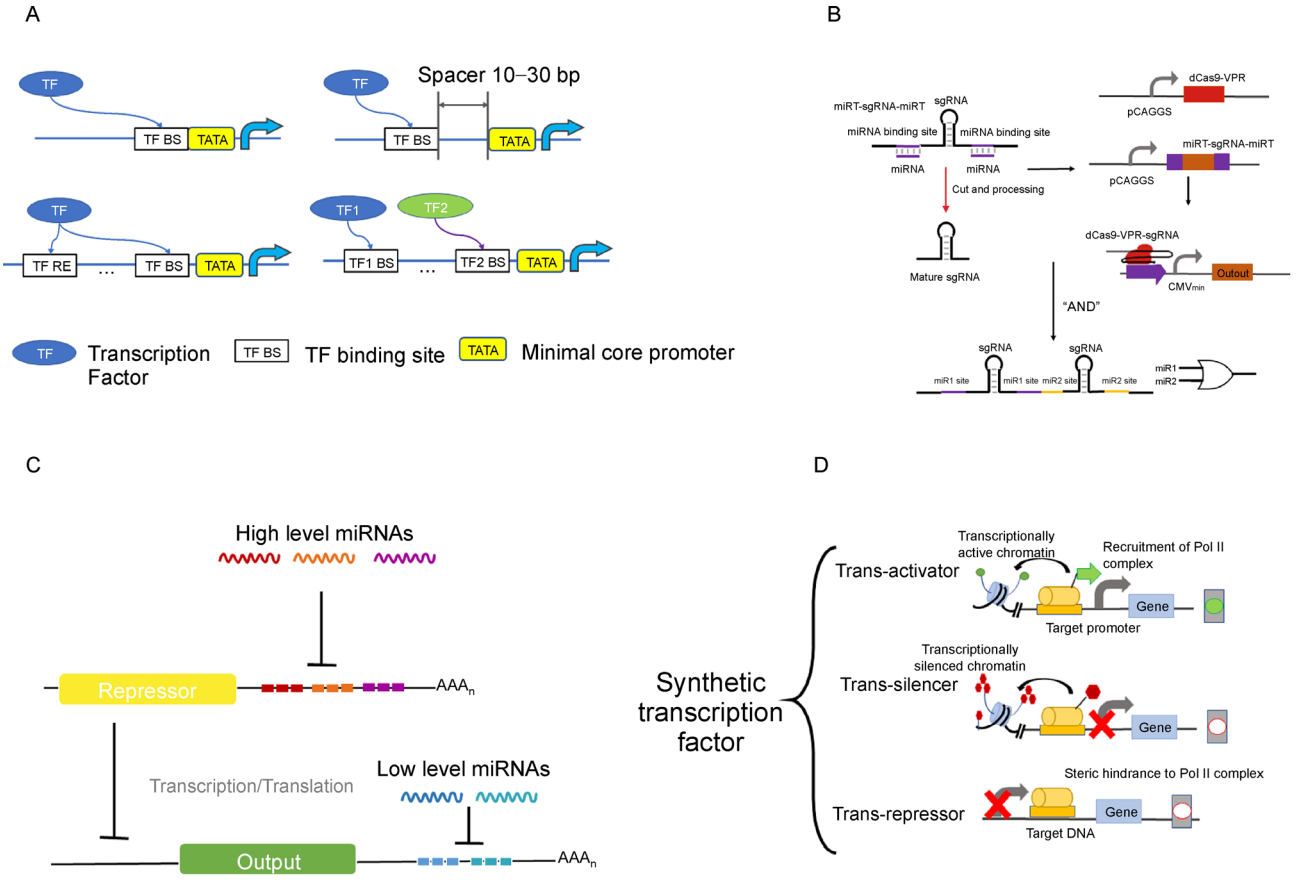

Figure 5. Intracellular state sensing for the construction of engineered implanted cells. (A) The design and working principle of synthetic transcription regulators [193]. (B) MICR, miRNA-inducible CRISPR-Cas9 platform. Schematic representation of the pre-sgRNA (miRT–sgRNA–miRT) and design of a MICR-ON system that activates gene expression upon induction by miRNAs. Expression of dCas9–VPR and pre-sgRNA is driven by a CAGGS promoter. The TRE promoter contains seven repeats of an sgRNA-binding site with GGG PAM sequences. PAM, protospacer adjacent motif. Schematic representation of pre-sgRNAs in a two-input MICR-ON-OR operator [194]. (C) miRNA classifiers ascertain cell states via the expression level of a combination of cell specific miRNAs. The miRNA can target an mRNA with complementary binding sites to facilitate its degradation. (D) Different design schemes of engineered artificial induction promoters based on transcription factors.

site, which is crucial for cell therapy. In *in situ* repair applications, accurately targeting a specific cell type or heading for the injured site are important features, and intercellular communication elements play an important role in this cellular behavior. The development of engineering tools and strategies for regulating juxtaposed cell-cell interactions is still in its infancy.

The most successful clinical use of engineered cell targeting is the development of chimeric antigen receptors (CARs) for cancer immunotherapy. Native T cell receptors (TCR) specifically bound to major histocompatibility complex (MHC) while Chimeric antigen receptors replace the variable region of TCR with a single-chain variable fragment (scFv) derived from the variable domains of antibodies. Tumor cells downregulate the expression of the MHC to avoid attack by host immune defenses. Thus, CAR-T cells are able to target originally immuno-evasive tumor cells [212–231]. However, tumor-only antigens are rare, and a single input is not sufficient to make a distinction between tumors and normal tissues.

Another recently reported strategy to engineer a completely orthogonal cell recognition system relies on the unique signaling mechanism utilized by the Notch receptor (synNotch) [232]. Each synNotch receptor contains a Notch core, flanked by modular extracellular and intracellular domains. Binding of the synNotch receptor to a ligand presented on the surface of the target cell causes the receptor to aggregate, which induces protease-mediated cleavages of the Notch intracellular domain through a natural mechanism, thereby releasing the engineered intracellular domain which could initiate downstream transcription (Fig. 6A). With this independent mechanism, multiple synNotch receptors can be connected with genetic logic gates. When integrated in CAR-T cell, synNotch could be used to switch on the expression of the CAR gene, thus constituting a sequential AND-gate to target tumor cells more precisely [234].

Apart from direct cell-cell interaction, engineered cells can be targeted to sites of injury by chemotaxis. VEGF-A, a biomarker of tumor vascularization, activate sVEGFR2 and elicits an increase of the intracellular calcium concentration. Utilizing a chimeric protein which triggers blebbing morphology and cell migration when activated by Ca^{2+}, Mills *et al.* engineered cells that migrate toward VEGF. However, not all cytokines arouse a calcium spike as does TNF-α, a pro-inflammatory factor targeted in several anti-cytokine therapies. By fusing the transmembrane and cytoplasmic domains of the VEGFR2 with the extracellular domain of TNF-α to generate transient local Ca^{2+} spikes in response to TNFα, Qudrat and colleagues constructed TNF-α seeking cells that can promote the apoptosis of TNF-α expressing cells [235].

Precisely targeting pathological sites in need of treatment reduces the side effects to a large extent and improves the efficiency significantly. The pathological sites are highly structured and patchy, which necessitates precise cell targeting or chemotaxis.

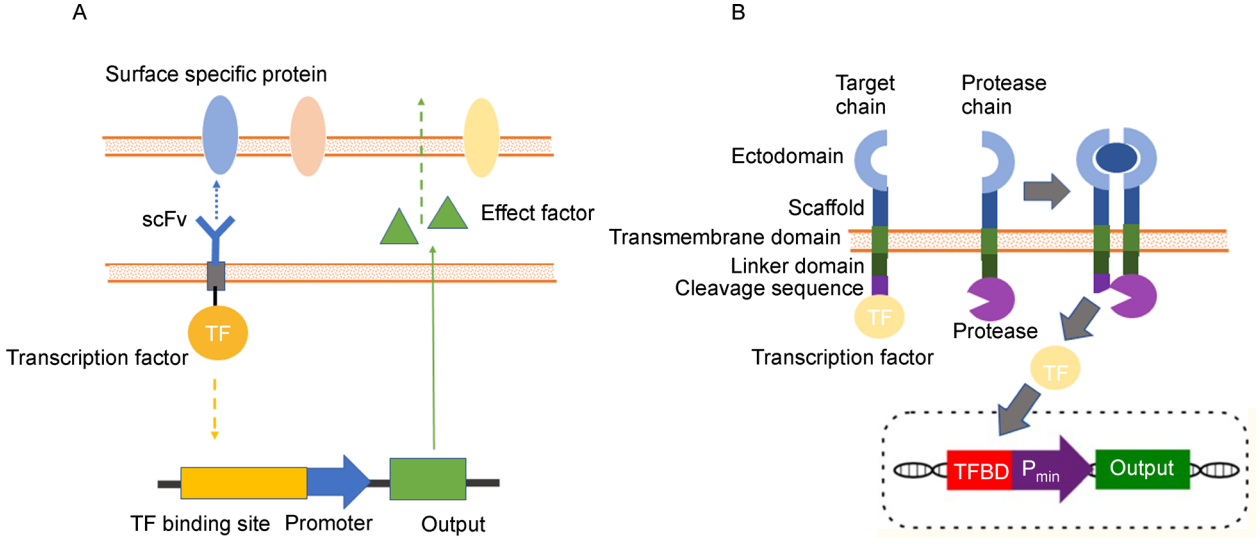

Figure 6. Synthetic receptor. (A) The intercellular recognition element can guide the migration of implanted cells to a specific pathological site, and can be used to sense elements of the microenvironment at the repair site, such as antigens, immune cells, cytokines and growth factors. (B) Modular extracellular sensor architecture (MESA) design concept: ligand binding-induced receptor dimerization causes the protease on the protease chain to cleave its cognate cleavage sequence on the target chain, which releases the transcription factor (TF) to travel to the nucleus and modulate target gene expression by binding to a TF binding domain adjacent to a minimal promoter and thereby drive the expression of the output gene [233].

Sensing and responding to the microenvironment

Implanted cells need to adjust their functions in response to the extracellular components to progress through dynamic physiological environments. Rewiring factor-sensing elements to customized outputs in cells enables the engineered cell to sense changes in the environment and respond as required.

The ability to respond to cues in the environment plays a vital role in homeostatic regulation of physiological functions. Some cells are engineered to automatically sense systemic disease markers and produce disease-specific therapeutic proteins to replace dysfunctional metabolic cell. Placement at a vascular site will allow the cell implant to continuously communicate with the host's bloodstream, enabling it to achieve automatic coordination and automatic diagnosis in the treatment and/or prevention of latent diseases, by measuring different system disease markers as trigger signal to control drug release through inducible genetic switches. For example, human cells engineered with a synthetic gene switch based on glucose-dependent calcium entry coupled with a calcium-responsive promoter that drives insulin expression have been used to autonomously restore the glucose and insulin homeostasis in diabetic mice through closed-loop control of glucose sensitivity and insulin secretion. Similarly, closed-loop insulin detection and controlled adiponectin secretion effectively targets potential and asymptomatic stages of insulin resistance and reduces the development of obesity-induced diabetes [236].

Another important environmental cue in pathological tissues is the cytokine milieu. By redirecting natural receptors to novel downstream signaling transduction pathways, engineered cell can identify and distinguish environmental factors, and exert customized functions. The natural extracellular surface receptors can be engineered to link to new intracellular events while maintaining responsiveness to natural ligands [237]. In this approach, the intracellular portion of the cell surface receptor of interest can be genetically fused to a transcription factor via a cleavable linker sequence. Homologous proteases bind to connexins genetically, and are recruited to receptors by regulating the natural mechanism by which these proteins conditionally interact with other proteins. Thus, the association of these proteins induced by ligand binding causes the proteases to cleave their target sequences, releasing transcription factors to drive the expression of transgenes such as fluorescent protein reporter genes. Baeumler *et al*. further integrated a transduction module based on split-dCas9 with a synthetic receptor to simplify the programming of output functions [238].

An alternative method is to couple sensing of new ligands to intracellular signal induction through orthogonal signaling mechanisms, such as the modular extracellular sensor architecture (MESA), which is a self-contained receptor-based signal-transduction system [239]. Each MESA receptor contains two engineered transmembrane proteins, one containing an intracellular protease domain and the other containing an intracellular transcription factor that was originally sequestered to the plasma membrane. The extracellular domain confers recognition ability for the target ligand, so that the heterodimerization of the receptor induced by ligand binding causes a trans-split response, thereby releasing the transcription factor to reach the nucleus (Fig. 6B). Importantly, this modular mechanism can be easily adapted to confer recognition of new inputs (via alternative ligand recognition domains) and regulation of new outputs (via alternative transcription factor domains). By leveraging the MESA receptor, Schwarz and co-works enabled T-cells to sense vascular endothelial growth factor (VEGF), which is richly expressed in tumors, and secrete interleukin 2 (IL-2) to locally enhance anti-tumor immunity [240].

In addition, several closed-loop control systems have been reported. They are based on intracellular ligands related to external physiological states, which determine whether engineered transcriptional regulators are recruited to target DNA sequences encoding genes that regulate host-cell physiology [241–243]. This mechanism enables the real-time monitoring and modulation of the host-cell's physiological state. For example, a synthetic device based on the bacterial transcriptional repressor HucR was used to sense and reduce the accumulation of uric acid in the blood. Engineered cells implanted in uric acid oxidase-deficient mice reduced the uric acid levels by about 2.5 times, which was comparable to the efficacy of standard drug treatments [241,244].

With the continuous development of extracellular sensors and relevant gene circuits, our ability to engineer cells to identify and distinguish environmental factors is also increasing, providing the possibility for implanted cells to sense and tailored respond to the microenvironment at pathological sites.

Precise spatiotemporal control of gene expression

Generally, artificial synthetic circuits are put under the control of chemical inducers, like tetracycline, but chemical compounds cannot achieve precise spatiotemporal and side-effect-free control due to limitations with respect to bioavailability, pharmacokinetics, and human compatibility. A new trend is to incorporate physical stimuli including radio waves, magnetic fields, light, and

electric pulses into mammalian cell manipulation. Non-invasive physical triggers have shown therapeutic potential in clinical application such as maintaining glucose homeostasis in diabetes.

Francis Crick proposed a long time ago that light might be used to precisely target one type of cells in neural systems, but it took decades for scientist to generate light-control neuron cell lines [245]. The output of photo-activatable proteins was redirected to a new signal cascade in synthetic light-responsive cells. Using the optogenetics toolkit, light-sensitive gene circuits can be constructed in engineered cells to enable guided disease modification. For example, light-gated cation channels can mediate Calcium influx when activated, resulting in calcium-dependent dephosphorylation of the nuclear factor of activated T cells (NFAT). This way, the expression of glucagon-like peptide 1, which stimulates the secretion of insulin, can be linked to illumination through NFAT driven promoters [246]. In another study, erectile optogenetic stimulator (EROS) was created in mammalian cells using a guanylate cyclase that generates cGMP upon exposure to blue light. The synthesis of cGMP shut down calcium channels and led to penile erection by relaxing smooth muscles of the corpus cavernosum. When the corpus cavernosum of male rats was transfected with an EROS-encoding expression vector, penile reflexes could be observed within a minute upon exposure to blue light [247].

Bioelectricity can affect the proliferation, differentiation, migration and apoptosis of a number of cells beyond excitable nerves and muscle [248]. While an electro-genetic system had not been reported until recently, Krawczyk and colleagues described a synthetic human β cell placed into a bioelectronic implant that can rapidly secret insulin from vesicular stores given electrical stimulation and restore normoglycemia in type 1 diabetic mice. The pancreatic β cell line was transfected with vector encoding an L-type voltage-gated channel CaV1.2 and the inwardly rectifying potassium channel Kir2.1. The co-expression of Cav1.2 and kir2.1 rewired electric stimulation to calcium influx and concurrent release of intracellular vesicles containing insulin [249].

Moreover, insulin synthesis could be induced by radio waves, magnetic fields [250] and far-red light [251] via the heating of metal nanoparticles. These physically controllable engineered cells can be directly interfaced with non-genetically encoded elements, providing a brand-new toolkit for signal transduction in biosystems.

SUMMARY AND OUTLOOK

Engineered cell-based therapy has broad development prospects in the medical field. The use of engineered immune cells to combat certain types of cancer has already achieved spectacular clinical successes. However, cancer immunotherapy represents only one application of engineered cells. The generation of customized and programmable cell functions enables safer, more effective and functionally complex treatment strategies. The latest breakthroughs in therapies that use synthetic genetic circuits have shown that engineered cell therapy has definite development potential. The application of synthetic biology to cell therapies can successfully treat some diseases that have been difficult to cure so far, including cancer and diabetes. Moreover, the literature already provides favorable evidence for the application of engineered implanted cells in cell therapy.

However, the current research on engineered cells is still focused on the transformation of immune cells and limited types of stem cells, while research on the transformation of other cell types is relatively limited. The development and utilization of new chassis cells for synthetic biology is an important research direction for seed cell engineering. Although the combination of immune cells and stem cells can provide some ideas about implanted cell transformation in cell therapy, implanted could work in conjunction with scaffold materials, which is an important difference to the direct injection of cells, and may bring some difficulties to the transformation process. Synthetic biology is expected to improve genetic and engineered cell-based therapies for a variety of diseases by precisely controlling the intensity, timing, and background of therapeutic interventions. The engineering of implanted cells has injected new vitality into the development of cell therapy. At present, some synthetic biology modules, such as safety switches, have already been introduced into clinical trials, and more complex genetic circuits have also been introduced. Synthetic biology is gradually being applied to new therapies, which provides strong evidence for the engineering of implanted cells. However, the current research on the engineering of implanted cells is still very limited. How to use the existing synthetic biology modules and develop new components or systems for the transformation of implanted cells in cell therapy remains a challenge [252]. This may also be another research focus in the development of cell therapy in the future, and it is worthy of our further exploration.

Although this article summarizes the application of some basic strategies for the engineering of implanted cells, there are still many problems with the transformation of implanted cells in cell therapy. For example, since implanted cells are mostly derived from autologous or allogeneic cells, their sources are limited. To improve efficiency, the improvement of transformation efficiency

becomes crucial. In addition, the growth state and survival of implanted cells after transformation are also problems that need to be addressed. This series of questions shows that the construction and application of engineered implanted cells will be the subject of important future research. How to improve the transformation efficiency of implanted cells, as well as improve the growth state and survival of the engineered implanted cells are important research topics for future studies on engineered cell therapy.

Although the transformation of engineered implanted cells is still in its infancy, the development prospects of cell therapy cannot be ignored. On the one hand, the engineering of implanted cells can aid the exploration of the basic biology of implanted cells. The improvement and reduction of defects, as well as the tracing of possible functions and signal pathways that implanted cells utilize will provide valuable information. With the help of synthetic biology, interfering and optimizing the signal pathways and genetic systems in implanted cells, as well as validating their function and related signal pathways by means of cell biology, can help understand the mechanisms of implanted cell action. Furthermore, engineering can solve the current problems of implanted cells and improve their efficacy in tissue repair and the treatment of cancer. Through the transformation of implanted cells, a new iterative upgrade can be added using different material scaffolds [253] and different cytokine systems. According to the therapeutical effect, new modifications are continuously being added to implanted cells, and engineered implanted cells are being continuously upgraded. Through a combination of all these strategies, the best repair effect can finally be achieved. Our own group is also devoted to research in this area. We used single-cell sequencing technology to explore cartilage tissue repair, and then used synthetic biology to transform implanted cells based on single-cell data to further explore the repair mechanism. Finally, the optimized engineered implanted cells are used for cartilage tissue repair engineering to achieve a better repair effect (Fig. 7).

Overall, the application of synthetic biology methods holds new potential for cell therapy, and it is an attractive direction for its future development. The transformation of implanted cells as chassis cells can expand the application of synthetic biology in mammalian cells, and at the same time provide new research targets and directions for the development of tissue engineering. Finally, these approaches will require the integration of multiple disciplines such as synthetic biology, cell biology, and medical science, injecting new vitality for the further development of multiple fields.

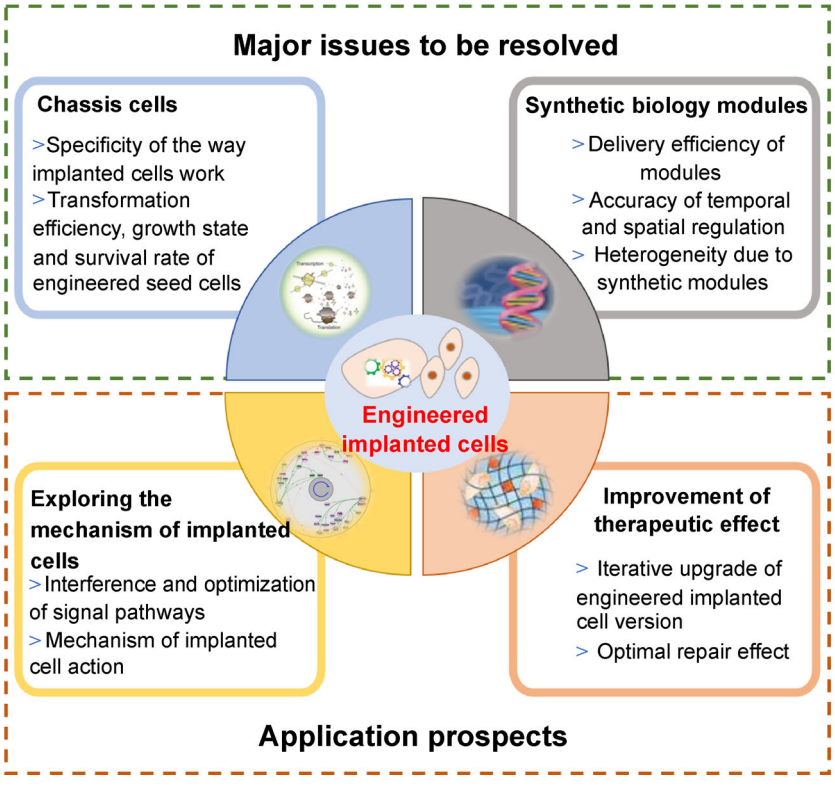

Figure 7. Major issues to be resolved and application prospects of engineered implanted cells in tissue repair.

ABBREVIATIONS

CAR-T	chimeric antigen receptor T-cell
DC	dendric cell
DMD	duchenne muscular dystrophy
ESC	embryonic stem cell
Hesc	human embryonic stem cell
HSPC	human hematopoietic stem and progenitor cell
iPSC	induced pluripotent stem cell
MHC	major histocompatibility complex
MSC	mesenchymal stromal cell
NK cell	natural killer cell
NSC	neural stem cell
TALE	transcription activator like effector
TALEN	transcription activator like effector nuclease
TCR	T-cell receptor
ZFN	zinc finger protein

ACKNOWLEDGEMENTS

This work was financially supported by National Key Research and Development Project of China (No. 2018YFA0900100), the National Natural Science Foundation of China (No. 31961133019), and National Natural Science Foundation of China (No. 31670091). The National Natural Science Foundation of China (No. 31961133019) is part of MIX-UP, a joint NSFC and EU H2020 collaboration. In Europe, MIX-UP has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 870294.

COMPLIANCE WITH ETHICS GUIDELINES

The authors Tianying Chen, Xue Zhang, and Qiong Wu declare that they have no conflict of interests.

All procedures performed in studies were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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