Engineering better stem cell therapies for treating heart diseases

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Abstract: For decades, stem cells and their byproducts have shown efficacy in repairing tissues and organs in numerous pre-clinical studies and some clinical trials, providing hope for possible cures for many important diseases. However, the translation of stem cell therapy for heart diseases from bench to bed is still hampered by several limitations. The therapeutic benefits of stem cells are mediated by a combo of mechanisms. In this review, we will provide a brief summary of stem cell therapies for ischemic heart disease. Basically, we will talk about these barriers for the clinical application of stem cell-based therapies, the investigation of mechanisms behind stem-cell based cardiac regeneration and also, what bioengineers can do and have been doing on the translational stage of stem cell therapies for heart repair.

Keywords: Stem cell therapy; ischemic heart diseases; bioengineering strategies; clinical translation

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Introduction

Stem cells are a group of primitive cells with the potential of self-replication and multi-directional differentiation (1). Under certain conditions, they can differentiate into multiple adult cells in the body (1). Stem cell therapy, also known as stem cell transplantation, is the delivery of stem cells to a specific part of the body by systemic or local injection to repair diseased or damaged tissue (2). Many human diseases are caused by abnormal lesions or tissue death within certain organs. By transplanting stem cells into these damaged regions, healthy cells can be regenerated, improving organ function and reversing diseased states (3).

For decades, stem cells and their byproducts have shown efficacy in repairing tissues and organs in numerous pre-clinical studies and clinical trials, providing hope for alternate therapies and possible cures for important diseases such as metabolic diseases (4), nervous system diseases (5), blood system diseases (6), autoimmune diseases (7), and cardiovascular diseases (8), including heart disease (9).

Stem cell therapy in heart repair

According to the recent report of American Heart Association, cardiovascular disease is still the number one cause of death worldwide (10). Coronary heart disease, dilated cardiomyopathy, and severe valvular disease can lead to heart failure (HF) due to ischemic necrosis of cardiomyocytes at the end of the disease period (11). At the same time, heart transplantation has problems such as the lack of donors, the need for long-term use of anti-rejection drugs, and high medical expenses.

Since existing treatments still have limited ability to reverse the HF process after myocardial infarction, regeneration of cardiomyocytes has become the direction of many scientists' research. An increasing number of stem cell types have been demonstrated to be visible in cardiac repair, including skeletal muscle progenitor cells, bone marrow stem cells [mesenchymal stem cells (MSCs), hematopoietic stem cell (HSCs), monocytes, etc.], adipose-derived stem cells, bone marrow and blood-derived endothelial

progenitor cells, cardiac stromal cells (CSCs), etc. (12).

In August 2016, the biotech company CardioCell announced effective results in the application of stem cells for the treatment of chronic HF indications at the European Society of Cardiology Congress. This was the world's first phase 2a clinical trial to study the effects of intravenous ischemic tolerance to mesenchymal stem cells (itMSCs) in the treatment of chronic HF (13). The result of this trial turned out to be safe and well-tolerated, but only with marginal efficacy. During the same year, at the annual meeting of the Society for Cardiovascular Angiography and Interventions (SCAI), a number of professors and experts jointly announced promising results for the RENEW (Efficacy and Safety of Targeted Intramyocardial Delivery of Auto CD34+ Stem Cells for Improving Exercise Capacity in Subjects With Refractory Angina) trial (14) and the ATHENA (Autologous adipose-derived regenerative cells for refractory chronic myocardial ischemia with left ventricular dysfunction) trial (15). Although the results of these trials did not sufficiently show significant efficiency due to the early termination (14) and limited sample size (15), they could still be promising development demonstrating the potential for viable stem cell-based heart therapies.

Current limitations and challenges from bench to bedside

Stem cell transplantation has great potential. In theory, stem cells can be differentiated into almost all types of human cells. However, according to the International Society for Stem Cell Research, stem cell transplantation is currently recognized as safe and effective only in the treatment of hematopoietic systems (16). Other widely used stem cell therapies are applied to the skin (in the case of burns) (17), bone (18) and corneal diseases (19), and bone-marrow transplantations (16).

For decades, stem cells have been widely studied in preclinical animal models and clinical trials. However, few of the trials have been approved by the FDA and successfully reached the market. For heart disease like cardiovascular ischemia, stem cell therapies are making headway in clinical trials but have not yet reached the clinic (*Table 1*). Other cell-based therapies for HF or cardiomyopathy include DREAM-HF (phase 3 trial of mesenchymal precursor cells in chronic HF) (20), CONCERT-HF (combination of mesenchymal and c-kit+ cardiac stem cells as regenerative therapy for heart failure) (21), ELPIS (allogeneic human MSC injection in patients with hypoplastic left heart syndrome) (22), and POSEIDON-DCM (comparison of allogeneic *vs.* autologous MSCs for non-ischemic dilated cardiomyopathy) (23). These ongoing trials are expected to have available results in 2020.

Increasing research on stem cell therapies for acute myocardial infarction (AMI) has put in doubt the traditional notion that the heart cannot be repaired. By extension, the enthusiasm for stem cell therapies that target cardiovascular disease continues to rise. But unfortunately, either the pre-clinical research of stem cell-based cardiac regeneration or clinical trials of stem cell therapies still have a number of limitations (24). Why does this advanced therapeutic option encounter barriers before being able to benefit the public, and what are the challenges that the scientific community must overcome before implementation is possible?

Long-term efficacy

Despite evidence of short-term improvements in heart performance, it is not clear whether heart stem cell therapies have long-term benefits. In April 2009, Meyer *et al.* published a long-term (5-year) follow-up of a clinical trial involving bone marrow cell transplantation to promote ST-segment elevation myocardial infarction regeneration (BOOST) (25). The results showed that left ventricular function, measured by left ventricular ejection fraction (LVEF), was significantly improved compared with the control group after 6 months. However, there was no significant difference in improvement in left ventricular cardiac function or major adverse cardiovascular events (MACEs) between the two groups long-term follow-up at 5 years after the treatment was applied. The investigators believed that despite the faster recovery of LVEF in the treatment group, the lack of long-term improvement of left ventricular systolic function in AMI patients who received stem cell transplantation needs to be addressed (25).

Uncontrollable biodistribution

The poor engraftment of stem cells at the site of injury or disease is considered to be a primary explanation for the low efficacy of some stem cell trials (26,27). The traditional systemic delivery of stem cells, accomplished through intravenous injection, while facile, is not particularly good at getting cells where they need to be. What's more, a larger portion of the injected cells accumulate in other organs, such as the lungs (28). One alternative method is to directly inject cells or byproducts into the injury tissue. This has been a popular research strategy for heart repair. We and many others usually administer therapeutic stem cells into

Table 1 Twenty NIH-funded recruiting and completed clinical trials in stem cell-based treatment for heart diseases (until November 2019). Source: https://clinicaltrials.gov/

NCT number	Title	Status	Study results	Conditions	Sponsor/collaborators	Phases	Start date
NCT00243776	Molecular and Cellular Characterization of Cardiac Tissue in Postnatal Development	Recruiting	No results available	Congenital heart disease/ tetralogy of Fallot	Emory University/National Heart, Lung, and Blood Institute (NHLBI)		April 2005
NCT00824005	Effectiveness of Stem Cell Treatment for Adults With Ischemic Cardiomyopathy (The FOCUS Study)	Completed	Has results	Chronic ischemic heart disease/left ventricular dysfunction/angina/ ischemic cardiomyopathy	The University of Texas Health Science Center, Houston/ National Heart, Lung, and Blood Institute (NHLBI)/Cardiovascular Cell Therapy Research Network (CCTRN)	Phase 2	March 2009
NCT02408432	Bone Marrow Derived Mesenchymal Stem Cells in Improving Heart Function in Patients With Heart Failure Caused by Anthracyclines	Recruiting	No results available	Cardiomyopathy/heart failure	M.D. Anderson Cancer Center/ National Cancer Institute (NCI)	Phase 1	January 11, 2016
NCT00352209	Cardiomyopathy Following Stem Cell Transplantation	Completed	No results available	Cardiomyopathy	National Institutes of Health Clinical Center (CC)		May 2, 2006
NCT00043628	Stem Cell Mobilization to Treat Chest Pain and Shortness of Breath in Patients With Coronary Artery Disease	Completed	No results available	Coronary artery disease	National Heart, Lung, and Blood Institute (NHLBI)/National Institutes of Health Clinical Center (CC)	Phase 2	August 2002
NCT01392625	Percutaneous Stem Cell Injection Delivery Effects On Neomyogenesis in Dilated Cardiomyopathy (The POSEIDON-DCM Study)	Completed	Has results	Non-ischemic dilated cardiomyopathy	Joshua M Hare/National Heart, Lung, and Blood Institute (NHLBI)/ University of Miami	Phase 1/ phase 2	May 19, 2011
NCT00013975	Endothelial Progenitor Cells and Risk Factors for Coronary Artery Disease	Completed	No results available	Coronary arteriosclerosis	National Heart, Lung, and Blood Institute (NHLBI)/National Institutes of Health Clinical Center (CC)		March 2001
NCT00684060	Use of Adult Autologous Stem Cells in Treating People 2 to 3 weeks After Having a Heart Attack (The Late TIME Study)	Completed	Has results	Left ventricular dysfunction	The University of Texas Health Science Center, Houston/National Heart, Lung, and Blood Institute (NHLBI)	Phase 2	July 2008
NCT00308633	Endothelial Progenitor Cells and Nitric Oxide in Cardiac Rehabilitation Program Participants	Completed	No results available	Coronary artery disease (CAD)	National Heart, Lung, and Blood Institute (NHLBI)/National Institutes of Health Clinical Center (CC)		March 23, 2006

Table 1 (*continued*)

Table 1 (*continued*)

NCT number	Title	Status	Study results	Conditions	Sponsor/collaborators	Phases	Start date
NCT00053456	The Effect of Exercise on Stem Cell Mobilization and Heart Function in Patients Undergoing Cardiac Rehabilitation	Completed	No results available	Coronary arteriosclerosis	National Heart, Lung, and Blood Institute (NHLBI)/National Institutes of Health Clinical Center (CC)		January 2003
NCT00090558	Effect of Nitric Oxide Donor on Endothelial Progenitor Cells in Patients With Coronary Artery Disease	Completed	No results available	Coronary artery disease	National Heart, Lung, and Blood Institute (NHLBI)/National Institutes of Health Clinical Center (CC)	Phase 2	August 2004
NCT02077218	Computed Tomography and Biomarker Analysis in Diagnosing Coronary Artery Disease in Asymptomatic Patients Who Have Undergone Stem Cell Transplant	Completed	No results available	Cancer survivor/diabetes mellitus/hypertension	City of Hope Medical Center/ National Cancer Institute (NCI)	Not applicable	February 2014
NCT00684021	Use of Adult Autologous Stem Cells in Treating People Who Have Had a Heart Attack (The TIME Study)	Completed	Has results	Left ventricular dysfunction	The University of Texas Health Science Center, Houston/National Heart, Lung, and Blood Institute (NHLBI)	Phase 2	July 2008
NCT02348515	Cardiovascular Disease Protection Tissue	Completed	No results available	Myocardial ischemia	University of Florida/National Institutes of Health (NIH)/National Heart, Lung, and Blood Institute (NHLBI)		June 2013
NCT00893360	Cardiosphere-Derived autologous Stem Cells to Reverse ventricular dysfunction	Completed	No results available	Recent myocardial infarction/ventricular dysfunction	Cedars-Sinai Medical Center/ National Institutes of Health (NIH)/ National Heart, Lung, and Blood Institute (NHLBI)/Johns Hopkins University/The Emmes Company, LLC	Phase 1	May 2009
NCT00417417	Rilonacept to Improve Artery Function in Patients With Atherosclerosis	Completed	Has results	Coronary artery disease/ atherosclerosis/ inflammation/endothelial dysfunction	National Heart, Lung, and Blood Institute (NHLBI)/National Institutes of Health Clinical Center (CC)	Phase 2	December 2006
NCT01557088	Lp-PLA2 and Coronary Atherosclerosis in Humans	Completed	No results available	Coronary atherosclerosis/ endothelial dysfunction	Mayo Clinic/National Institutes of Health (NIH)/National Institute on Aging (NIA)	Not applicable	February 2009

Table 1 (*continued*)

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NCT number	Title	Status	Study results	Conditions	Sponsor/collaborators	Phases	Start date
NCT02706639	Williams Syndrome (WS) and Supravalvular Aortic Stenosis (SVAS) DNA and Tissue Bank	Recruiting	No results available	Williams syndrome/ supravalvular aortic stenosis/cardiovascular disease	National Heart, Lung, and Blood Institute (NHLBI)/National Institutes of Health Clinical Center (CC)		January 1, 2016
NCT02277613	A Phase 2 Trial of AMI MultiStem® Therapy in Subjects With Non-ST Elevation Acute Myocardial Infarction	Recruiting	No results available	Heart attack/NSTEMI	Athersys, Inc/National Heart, Lung, and Blood Institute (NHLBI)	Phase 2	June 2015
NCT00378352	REVEAL: Reduction of Infarct Expansion and Ventricular Remodeling With Erythropoietin After Large Myocardial Infarction	Completed	Has results	Acute ST elevation myocardial infarction	National Institute on Aging (NIA)/ National Institutes of Health Clinical Center (CC)	Phase 2	September 2005

the infarct border zone of the heart via intramyocardial injections (29,30). An obvious shortcoming of this method is that it generally requires an open-chest surgery, leading to increased post-operative pain and general risk to the patient.

Another clinical obstacle that must be addressed is the low survival rate of stem cells *in vivo* (26). In many of the clinical trials of stem cell-based heart repair, autologous cells are intravenously or intracoronarily injected into the patient (31). Somehow, after 24 to 48 hours of transplantation, usually only a small fraction of cells (about 5%) remain in the transplanting site. Four to six weeks after transplantation, 99% of the retained cells do not survive (31). One of the reasons believed to cause the diminished viability of the cells is the harsh environment in the heart or other organs, which threatens their proliferation, accelerating apoptosis and migration to other issues (26).

Risk of tumorigenicity and immunogenicity

In May 2001, an Israeli nine-year old boy was diagnosed with ataxia-telangiectasia, a rare neurological disease that unfortunately has no treatment. He received embryonic stem cell injection in his brain in Moscow with the last remaining hope of improving his condition. Various regions of his brain were injected with the embryonic cells. Four years later, tumors were found in his brain. And two embryonic stem cells were detected among the tumor cells (32). This story, which is the first-reported case of stem cell therapy causing a brain tumor, engendered a rejection to stem cell treatment by the local people. Fortunately, the tumor was diagnosed to be benign and safely removed.

The risk of tumorigenicity, remaining a terrifying concern for the public, limits their acceptance to stem cell-based therapy. The concern is not unwarranted either. Stem cells are biologically similar to tumor cells in many respects (33). They exhibit sustained proliferation, insensitivity to apoptosis, and similar growth regulation mechanisms as tumor cells. It has been found from animal models that human embryonic stem cells or induced pluripotent stem cells can cause both benign teratomas and malignant teratomas (33). Their pluripotency is considered to be the biological basis of tumor formation. Understanding this biological basis better and more fully is key to preventing future cases of tumor formation, as illustrated by the young patient's case above.

Host immunity is a serious challenge to consider when injecting non-autologous cells or agents into patients. On

the other hand, autologous products do not risk immune rejection, but must be collected from the patient and expanded/manipulated before infusing back into the patient. The collection of cells, which usually requires a biopsy, from already diseased patients presents an added health risk.

Mechanisms of stem cell-based therapy for heart repair

In order to address the safety and efficacy issues faced by cell-based cardiac therapies, we need to firstly elucidate the therapeutic mechanisms involved. According to FDA regulations, every drug comes into the market after its therapeutic mechanisms and safety profiles have been widely accepted (34). However, with stem cells, the therapeutic mechanisms are illusive; even more so when it comes to their application for the treatment of heart diseases. In other words, transplanted cells may have unpredictable and uncontrollable behaviors in tissues/organs, such as heart, as a result of their developmental pluripotency. Popular and promising as they are, stem cell therapies still lack elucidation. Among the many questions left to answer are: how do they move *in vivo*? Where do they go? Why do they behave the way they do?

Initially, the stem cell therapy field had two schools of thought when it comes to the treatment of heart disease. One is the "replacement" theory. In this scenario, transplanted stem cells differentiate into cardiomyocytes, replacing the cells that were lost due to myocardial infarction (35). Patients can lose up to half billion cardiomyocytes in a major heart attack event. Studies on the differentiation from stem cells to cardiomyocytes have been accumulating since the theory was first established (36). However, the low survival rates and engraftment efficiency recorded in many studies have put into question the importance of this mechanism. Meanwhile, a number of studies have shown that adult stem cells can not differentiate into cardiomyocytes, but this seems not affect their ability of repairing damaged myocardium, and improving myocardial function (2). An ever-improving array of detection techniques continue increase the odds that the mechanisms behind their behavior will be understood.

The other point of view is the "awake" theory, in which stem cells secrete cytokine nutrients, promote endogenous cell proliferation, and thereby reduce the number of cells that are dying due to myocardial infarction (2). Paracrine activity is an important process for cells to communicate with other nearby cells. It appears to be especially valuable for active intercellular process in the body such as stem cell-based regeneration (37). Indeed, there is now a large body of evidence supporting the hypothesis that paracrine mechanisms are crucial for tissue regeneration (37-39). In recent years, more evidence suggests that transplanted stem cells exert their therapeutic effects by secreting biologically active proteins, or paracrine factors, to resident cells (39). In the heart, there are also various types of paracrine factors playing key roles in cardiac repair, including growth factors and chemokines, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (FGF), hepatocyte growth factor (HGF), insulin-like growth factor-1 (IGF-1), and secreted frizzled related protein 2 (Sfrp2) (37,39).

The heightened interest in paracrine signals has spurred the increased focus on extracellular vesicles (EVs) transporting those molecules and a move away from the use of cells themselves. The most popular of these vesicles is the exosome, which is also the means by which most of the molecules are transmitted (40). Exosomes, as the functional paracrine units of therapeutic cells, can partially reproduce the reparative properties of their parental cells (41,42). Exosomes have become a popular research focus for us and many others in the past five years because of their primary role in cell-cell communication, including stem cell-derived exosome's therapeutic role in heart repair (41). The cargo carried by these exosomes, as well as the membrane proteins that characterize them vary by cell type and cellular microenvironment (40). Our lab has isolated exosomes from explant-derived CSCs sourced from patients with HF (FEXO) or from normal (non-diseased) donor hearts (NEXO) and compared their regenerative activities *in vitro* and *in vivo* (43). The results suggest that the HF altered the miRNA cargos of cardiac-derived exosomes and impaired their regenerative activities. We demonstrated that miR-21-5p contributes to exosome-mediated heart repair by enhancing angiogenesis and cardiomyocyte survival through the phosphatase and tensin homolog/Akt pathway. Many of these experiments were conducted using conditioned media, which is the media that nourishes the cell *in-vitro*, absorbing the proteomic and exosomal output that the cells release after a number of days. When conditioned media was injected into infarcted cardiac tissue, reparative and regenerative effects comparable to direct cell transplantation where observed.

Surprisingly, there is another important intracellular communication method that is likely to be ignored by many studies in the past few years. Direct cell-cell interaction has been reported to be crucial to the functional regeneration

of stem cell therapies (44-46). As early as 2003, Fukuhara *et al.* has co-cultured bone marrow stromal cells (BMCs) with cardiomyocytes and found that direct cell-cell contact with cardiomyocytes was important for BMCs to trigger some potential environmental factors of differentiation *in vitro* (44). Later on, in 2017, when some researchers co-transplanted MSCs and HSCs to MI mice heart, their results demonstrated that mechanism of HSCs promoting cardiac regeneration lay in their angiogenesis ability. Meanwhile, transplanted MSCs showed the capability for intercellular communication with surrounding cardiomyocytes by gap junctional signaling (45). However, in-depth *in vivo* studies are still needed to furtherly confirm the importance of direct cell-cell crosstalk in stem cell-based cardiac remodeling. Specifically, *in vivo* gap junction blocking approach, combined with *in vitro* cell co-culture may give us a better understanding of the interaction process between transplanted stem cells and neighboring cardiac cells.

New era: bioengineering strategies

Bioengineers have been able to utilize the limited mechanistic information available to develop advanced therapeutic strategies using stem cells. Engineering methods that aim to realize the multifunctionalization of stem cell therapies have been thriving in the past five years (47). With the collaboration of physicians, chemists, and biologists, bioengineers are able to develop stem cell-based therapies that combine stem cells, or their byproducts, with biomaterials in order to enhance therapeutic efficiency.

Improving targeting ability

The first challenge stem cell therapy faces is the effective delivery. To be specific, similar to regular drug-based treatment, it is important to send cells to the injury site in a targeted manner. Achieving this is one of the goals of bioengineering. Previously, our lab has used FDA-approved ferumoxytol nanoparticles to attempt magnetic targeting in the body (48,49). As a technique continuously improved in this field, an externally introduced magnetic field was set up near the injury spot in the heart. During the injection of iron-labelled (ferumoxytol) stem cells, the magnetic field attracting them directly to the injured cardiac tissue. However, the use of a strong magnetic field during an operative procedure may have unexpected consequences on the equipment as well as the patient. The development of a more biosafe targeting strategy was needed.

Thus, we began to focus on the platelet, a unique component in blood which can also accumulate and bind directly to injured endothelial cells on blood vessels. Previously, our group has developed an innovative method to decorate the surface membrane of CSCs with platelet nanovesicles (PNVs) (50). Our engineered PNV-fused CSCs were demonstrated to express platelet surface markers that are associated with platelet adhesion to injury sites, enhancing the targeted vascular delivery of CSCs to the site of myocardial infarction.

In addition, platelet membranes indicate an alternative solution to adhere injected stem cells to the injured endothelium (51). Recently, our group successfully synthesized a platelet-inspired nanocell (PINC) that incorporates both prostaglandin E2 (PGE2)-modified platelet membrane and cardiac stromal cell-secreted factors (43). The natural infarct-homing ability of platelet membranes and the overexpression of PGE2 receptors in the injury microenvironment of heart after myocardial ischemia/reperfusion, gave us the inspiration to design this unique combo. Our PINCs have been demonstrated to achieve the targeted delivery of therapeutic payloads to the injured cardiac tissue.

Moreover, platelets can be functionalized on their membrane which generates another promising solution for targeted delivery. Those more specific units are antibodies. Studies from our lab and others have also demonstrated that antibodies against biomarkers that specifically express under heart ischemic diseases, such as CD34, can serve as a targeted mediator, not only navigating transplanted stem cells to the injured heart, but recruiting circulating endogenous stem cells to the ischemic site (52,53). Specifically, taking advantage of the natural infarct-homing ability of platelets and their ability to bind to circulating CD34+ progenitors in patients and improve prognosis, we engineered CD34 antibody-linked platelets (P-CD34) to capture circulating CD34-positive endogenous stem cells and direct them to the site of myocardial infarction (53). Similarly, CD41 antibodies, binding to platelets, can also be used to target the MI area. Taking advantage of pre-targeting and bioorthogonal chemistry (PTBC), we engineered a PTBC system using bioorthogonal click reaction to link these two antibodies (CD34 and CD41) *in vivo*, engaging endogenous stem cells with circulating platelets (54). As a result, the platelets redirected the stem cells to the injured cardiac tissue and enhanced repairing efficiency.

Bispecific antibodies (BsAbs), promising therapeutic agents used in cancer immunotherapy, can also be utilized to treat cardiovascular diseases. In the most recent study, our lab designed BsAbs by the chemical cycloaddition of F(ab')2 fragments from monoclonal anti-CD34 and anti-cardiac myosin heavy chain (CMHC) antibodies, which specifically target circulating CD34-positive cells and injured cardiomyocytes simultaneously (55). However, the major disadvantage of antibody-based targeting is that some particular biomarkers are only expressed during AMI.

Overcoming low cell retention

After achieving more efficient targeted delivery of the cellular therapy, the next obstacle to overcome is improving cell engraftment in the injury site. To improve the low retention and survival rates of transplanted stem cells, many innovative biomaterials have been developed in the past decade that encapsulate them and protect them once injected. Injectable hydrogels have been designed with different types of materials and combined with particular stem cells inside. Previously, our lab demonstrated the safety and efficacy of encapsulating CSCs in thermosensitive poly(N-isopropylacrylamide-co-acrylic acid) or P(NIPAM-AA) nanogels in mouse and pig models of myocardial infarction (MI) (56). In a recent study, we created a hydrophilic and negatively charged microenvironment using poly(N-isopropylacrylamide-co-itaconic acid), which is favorable for maintaining high viability of CSCs (57). The results revealed the treatment promoted MI heart repair through angiogenesis and inhibition of apoptosis with an improved cell retention rate. What is more, the other advantage of many hydrogels is that they do not elicit systemic inflammation or local T cell infiltrations in immunocompetent mouse models.

In addition to the idea of hydrogels, another revolutionary biomaterial used in the area of cardiac regeneration is the cardiac patch. The delivery of therapeutic cells in a cardiac patch increases cell retention and represents other functionalization aims. We have reported a novel strategy for creating a vascularized cardiac patch featuring biomimetic microvessels (BMVs) in a fibrin gel and spiked with human CSCs (58). Our results showed that the endothelialized BMVs could mimic the natural architecture and function of capillaries and that the vascularized cardiac patches (BMV-CSC patch) have great regenerative potential. Meanwhile, a shortcoming of the epicardial patches remains their slow integration with host myocardium. To address this issue, our group engineered a microneedle patch integrated with cardiac stromal cells (MN-CSCs), utilizing polymeric MNs to create communication channels between host myocardium and therapeutic CSCs (59).

The cardiac patch strategies normally require open-chest surgery, which is risky for the patient and requires a long recovery. In comparison, hydrogels can be injected directly to the site of injury using a minimally-invasive operation. Ventrix, a subsidiary of the University of California, San Diego, completed the first successful, minimally invasive human trial using a heart-repairing hydrogel approved by the FDA (60). The trial was the first to test a hydrogel used to repair heart tissue and also the first to test a hydrogel made from the natural scaffold of myocardial tissue, also known as extracellular matrix (ECM). Results have shown that this hydrogel, called VentriGel, can be safely injected through a catheter into patients who have had a heart attack in the past 2 to 36 months (60). Once injected into the damaged myocardium, VentriGel forms a scaffold that creates a healing environment for healthy cell migration, promoting new cardiac tissue formation. Ventrix is currently preparing for phase II clinical trials (60).

Cell-free therapy

An unavoidable risk with stem cell transplantations is that of tumorigenicity or immunogenicity, as we discussed above. Thus, many in the field have gravitated toward the study of bioactive agents released from stem cells, which have had comparable therapeutic effects, suggesting the possibility of a promising alternative to stem cell therapies. The most important bioactive agents currently being studied are EVs, including microvesicles and exosomes, which contain the biologically active components [mRNA, miRNAs, proteins (growth factors)] found in stem cells. These have been shown to have salutary effects (similar to cell therapies) on myocardial repair after injury. Compared to other EVs, exosomes have been more widely regarded as candidates for cell-free therapy and have been tested by our lab and others in the treatment of pulmonary fibrosis, cancer therapy, myocardial infarction, etc. For example, our lab has found that exosomes obtained from atorvastatin-pretreated MSCs have significantly enhanced therapeutic efficacy in treating MI (9). Nevertheless, many preclinical or clinical protocols for the application of exosomes have not been standardized, including their extraction and purification. Thus, much work is left to be done before exosomes are successful in clinical trials.

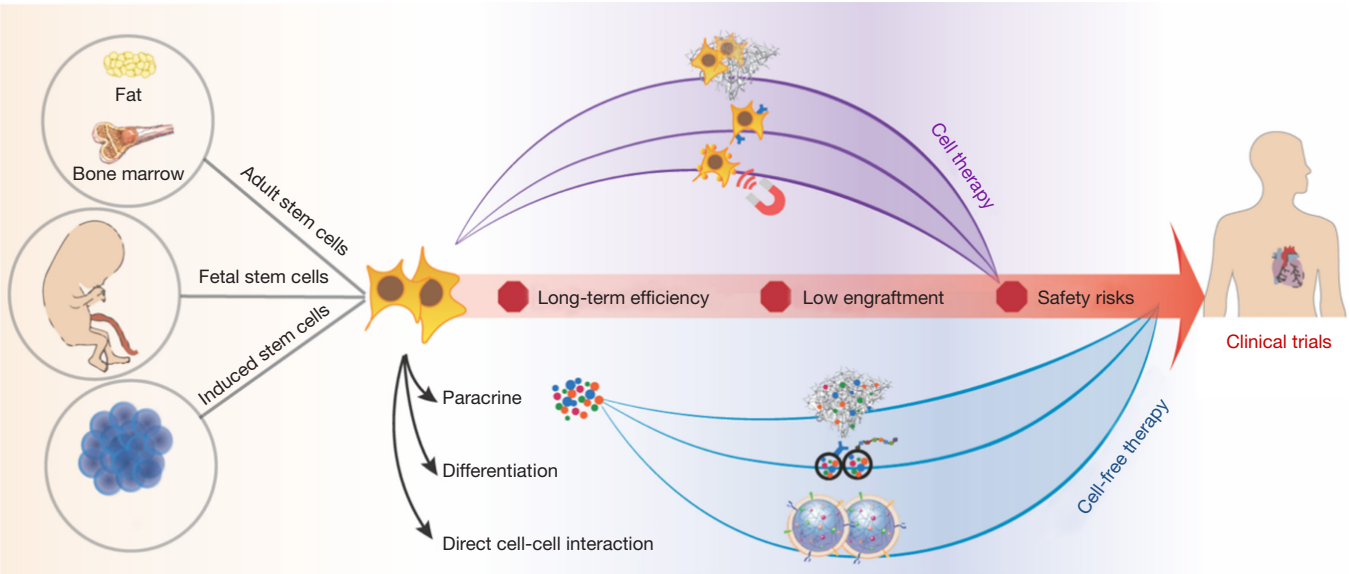

Figure 1 Bioengineering strategies to accelerate clinical translation of stem cell therapies.

Biomimetic strategies in stem cell-based therapy

While the therapeutic strategies discussed above have commonly focused on the functionalization of stem cells, there is another groundbreaking innovation based on the creation of 'super stem cells', by which we mean the construction of synthetic stem cells or cell-mimicking composites (61). This idea has recently been attempted twice by our group, for the treatment of heart diseases. For the first time, we reported a 'core-shell' design for a therapeutic microparticle (MP) which mimicked stem cell biointerfacing during regeneration (62). Named cell-mimicking MP (CMMP), this artificial stem cell contained control-released stem cell factors in its polymeric core and was cloaked with stem cell membrane fragments on the surface. In our mouse model of myocardial infarction, injection of CMMPs resulted in a similar augmentation of cardiac functions in comparison to direct CSC therapy. What is more important, CMMPs did not stimulate T cell infiltration in immuno-competent mice, suggesting their great potential for clinical trials. Subsequently, we sought to create a more complex stem cell-mimicking composite. We successfully packaged secreted factors from human bone marrow-derived MSC into Poly(lactic-co-glycolic acid) PLGA microparticles and then coated them with MSC membranes (63). These therapeutic particles, "synthetic MSC" (or synMSC), demonstrated their regenerative potential in mice with AMI.

Conclusions

Throughout decades of stem cell pre-clinical studies and clinical trials, challenges and risks exist, also, prospects and innovations exist. How to overcome the most conspicuous shortcomings with more innovative strategies is a question for every bioengineer, which has been trying to do and needs to be done in the future (*Figure 1*). In any case, an indispensable premise is a more comprehensive interpretation and understanding of the mechanism under which stem cells benefit cardiac regeneration. As we mentioned before, in addition to the paracrine effect, we still believe that direct cell-cell contact plays a vital role in this process. Therefore, the combination of the paracrine effect and the potential activation of the intrinsic program of cardiac cells, which is triggered by cell-cell crosstalk, followed by further observation of cell fate, cell niche, and cell *in situ* migration, are our top priorities for the next decade of advancing stem cell therapy for heart repair.

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