

# Stem Cell Angiogenesis in Myocardial Infarction Treatment: Efficacy and Future Directions

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## Abstract:

Myocardial infarction (MI) stands as a grave consequence of coronary heart disease, stemming from an imbalance between oxygen demand and supply. This review elucidates the clinical manifestations, complications, and treatment strategies associated with MI, emphasizing the critical role of angiogenesis in myocardial repair post-infarction. Factors influencing angiogenesis following MI are outlined alongside therapeutic applications aimed at cardiac regeneration. Stem-cell therapy emerges as a promising intervention, leveraging various cell types to bolster vascular support and myocardial regeneration. The intricate processes of wound healing and angiogenesis post-MI are examined, shedding light on their pivotal roles in tissue repair. Understanding these mechanisms holds promise for advancing therapeutic approaches in clinical practice, offering hope for improved outcomes in patients with MI.

## INTRODUCTION

Myocardial infarction is particularly the utmost severe manifestation of coronary heart disease and it is a final outcome of either acute or chronic myocardial ischemia that results due to a mismatch in oxygen demand and oxygen supply. Myocardial infarction leads to myocardial injury or necrosis which is characterized by a rise in cardiac biomarkers, along with supporting clinical evidence conforming electrocardiogram changes, or imaging validation of new injury of viable myocardium or acute abnormality in regional wall motion. The clinical manifestations of myocardial infarction include severe and sustained chest pain, often together with breathlessness, nausea, and sweating. Myocardial infarction causes ischemic attacks, angina, severe to severe life-threatening arrhythmias and congestive heart failure as complications.

Acute myocardial infarction (AMI) remains the main cause of mortality worldwide. Despite surgery and medical treatment, the non-regeneration of dead cardiomyocytes and the limited contractile ability of scar tissue can lead to heart failure. Therefore, restoring blood flow in the infarcted area is important for the repair of myocardial injury. The objective of the present review was to summarize the factors influencing angiogenesis after AMI, and to describe the application of angiogenesis for cardiac repair. Collectively, this review may be helpful for relevant studies and to provide insight into future therapeutic applications in clinical practice.

Thus, clinical doubt of myocardial infarction should lead to aggressive treatment depending on the severity and type of infarction as well as supportive care and employing secondary prevention strategies. Prognosis mainly depends on the severity of clinical signs observed, the presence of concurrent disease if any, and

response to initial therapy by the patient.

Stem-cell therapy has been focused on interventions in acute myocardial infarction (AMI), postinfarct cardiac support including ischemic cardiomyopathy, and refractory angina. Such therapy appears to protect the heart against ischemic injury and decrease the development of cardiac fibrosis.

Although the term stem cell has been utilized for this area of intervention, such cells include not only bone marrow stem cells, but more mature endothelial progenitor cells (EPCs), resident cardiac stem cells (CSCs), mesenchymal stem cells (MSCs), skeletal myoblasts, and embryonic stem cells. Stem-cell therapy may lead to reversal of pathophysiologic changes in coronary heart disease (CHD) and heart failure (HF). It is utilized as an adjunct to coronary interventions to bolster vascular support of the myocardium and for regeneration of myocardial cells. Such vascular regeneration can provide relief from symptoms refractory to maximal antianginal agents. Additionally, gene therapy is being utilized to support generation and proliferation of stem cells.

Tissue damage suffered during acute MI elicits an inflammatory reaction that leads to the necrotic area being replaced with granulation tissue and eventually a collagen-rich scar. It has long been realized that the wound healing response after MI varies interindividually, with some patients experiencing early, deleterious changes in LV geometry and function before scar formation has stabilized the infarct region. Infarct healing may therefore present a window of therapeutic opportunity. Wound healing after MI involves a robust angiogenic response that commences in the border zone and extends into the necrotic infarct core. A properly coordinated angiogenic response is associated with favourable outcomes in animal models of acute MI as evidenced by smaller infarct scars, less remodelling, and better-preserved heart function. How, exactly, angiogenesis improves tissue repair is incompletely understood and difficult to dissect experimentally. Swift formation of a dense capillary network provides entry routes for inflammatory cells; it facilitates gas exchange, nutrient diffusion, and waste removal to match the high metabolic demand at the site of inflammation and may limit ongoing cardiomyocyte dysfunction and death in the infarct border zone.

During embryonic development, vessels form de novo by assembly of mesoderm-derived endothelial precursor cells that differentiate into a primitive vascular plexus; subsequent EC sprouting creates a microvascular network that remodels to form the entire vascular tree. After MI in mice, new capillary structures arise from pre-existing ECs in the infarct region, with minimal, if any, contribution from non-EC sources. Here, we review current knowledge of the cellular and molecular processes driving this angiogenic response. We focus on mechanistic studies, using loss-of-function, gain-of-function, or lineage tracing approaches. Due to space limitations, we do not discuss studies on intracellular signalling pathways, including non-coding RNAs, which may transmit or modulate EC responses to angiogenic stimuli.

After MI in mice, new microvessels form in the infarct border zone, the subendocardial space, and the epicardium. Between 2 and 4 days after coronary artery ligation, the capillary network in the border zone starts expanding, with extensive branching and vessel sprouting into the infarct core. After 7 days, most capillary ECs in the border zone cease to proliferate and some newly formed capillaries enlarge and acquire smooth muscle cell support. In a classic autopsy series in patients from the pre-reperfusion era, newly formed capillaries penetrated the infarct core from the periphery starting on Day 4; ingrowth of blood vessels was most prominent during the second week after MI, indicating that the process takes longer in patients than in the much smaller murine heart.

Angiogenesis is essential for correct healing post-infarction. The blood supply of the cells in the infarcted

area gradually decreases, which restricts oxygen transfer, nutrient absorption and removal of metabolic waste, and the cardiomyocytes gradually become necrotic; therefore, restoring the blood supply to the infarcted area is a favorable repair method. The present review outlines the progress of current research on angiogenesis in myocardial infarction repair, including the main factors affecting angiogenesis and the therapeutic methods.

### **MECHANISM OF STEM CELL ANGIOGENESIS IN MI.**

Angiogenesis is the formation of new blood vessels based on previous vasculature. The formation of blood vessels starts with the sprouting of endothelial cells (ECs), which adhere to each other and are connected to the extracellular matrix (ECM), followed by hydrolytic remodeling of ECM in the presence of various enzymes. Hydrolytic remodeling of ECM refers to the continuous process of decomposition and synthesis of the ECM under the action of various enzymes. There are three main types of ECs, namely tip, stalk and phalanx cells. Tip and stalk cells are located at the sprouting tip of blood vessels and can secrete a variety of pro-angiogenic factors, such as vascular endothelial growth factor (VEGF), fibroblast growth factor and platelet-derived growth factor (PDGF). There are numerous factors that affect the formation of blood vessels.

First step in angiogenesis in the adult vessel is dissociation of periendothelial cells (pericytes) tightly adhering to ECs. The balance between adhesion and dissociation of ECs and pericytes depends on angiopoietins, the ligands for TIE2.

Angiopoietin-1 (Ang1) deficient embryos show severe vascular abnormalities, as observed in TIE2 deficient embryos. These abnormalities include insufficient branching of the cardinal vein or capillaries in the pericardium, lack of remodeling of vessels in the yolk sac, and insufficient heart development. Moreover, ultrastructural analysis reveals that close adhesion between ECs and pericytes is inhibited in the absence of TIE2 or Ang1. A naturally occurring antagonist of Ang1, designated Ang2, blocks the ability of Ang1 to activate TIE2. Transgenic mice expressing Ang2 exhibit phenotypes similar to those observed in TIE2 or Ang1 deficient embryos. Thus, while Ang1 is expressed in mesenchyme surrounding large vessels in the embryo and is widely expressed in the adult, Ang2 is expressed only at sites of vascular remodeling. This observation suggests that Ang2 blocks an otherwise constitutive stabilizing signal by Ang1 and promotes localized vessel destabilization. Destabilization of vessels by Ang2 may contribute to either vessel regression by blocking factors required for survival of ECs produced by pericytes or new vessel sprouting. Prolonged overexpression of Ang2 without VEGF promotes vessel regression, although vessel sprouting occurs when Ang2 and VEGF are coexpressed.

The use of stem cell-based therapy is becoming increasingly recognized as having the potential to salvage damaged myocardium and to promote endogenous repair of cardiac tissue.

Review of the literature indicates, regardless of whether 'stem' or 'progenitor' cells consist of a mixture of several cell populations or selected subpopulations, that these cells have the capacity to mediate neovascularization. Kamihata et al. have shown that bone marrow mononuclear cells (BM-MNCs), which consist of numerous different types of stem cells, transplanted into ischemic myocardium mediate angiogenesis via increased expression of angiogenic ligands and cytokines such as basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), angiopoietin-1 (Ang-1), interleukin-1 beta (IL-1 $\beta$ ), and tumor necrosis factor-alpha (TNF- $\alpha$ ). Bone marrow derived stem cells (BMSCs) expressing c-kit and Sca-1 subjected to preconditioning (anoxic conditions) expressed increased amounts of activated Akt and activated eNOS, and secreted higher levels of VEGF, bFGF, insulin growth factor (IGF), and stromal

cell derived factor-1 (SDF-1) compared to cells cultured under normal culture conditions, and the myocardial transplantation of these preconditioned cells led to increased blood vessel density. Using MSCs, Kinnaird et al. demonstrated the release of several angiogenic factors, such as VEGF, bFGF, placental growth factor (PIGF), and monocyte chemoattractant protein-1 (MCP-1), into the culture media, and the injection of these cells led to an increase in vessel number without MSC incorporation in mature vessels. Using gene expression profiling, additional studies from this laboratory demonstrate that MSCs express bFGF, FGF-7, IL-1, IL-6, PIGF, transforming growth factor-beta (TGF- $\beta$ ), TNF- $\alpha$ , and VEGF, which was augmented in response to hypoxia. This increased gene expression paralleled increased secreted protein levels of VEGF, bFGF, IL-6, PIGF, MCP-1, platelet-derived growth factor (PDGF), Ang-1, plasminogen activator (PA), and metalloproteinase-9 (MMP-9). In the heart, the intramyocardial injection of MSCs led to the in vivo upregulation of bFGF, VEGF, and SDF-1 $\alpha$ , and led to increased vessel density after myocardial infarction (MI). Overexpression of VEGF in MSCs also led to increased capillary density following MI, suggesting that stem cells may be modulated to overexpress a variety of key factors that may further enhance their capacity to promote neovascularization in the heart.

Progenitor cells for endothelial cells have been identified both in peripheral blood and in bone marrow. Peripheral blood endothelial progenitor cells (EPCs) can be isolated by magnetic bead selection on the basis of the CD34 antigen, and they were found to be positive for CD34, CD133, and vascular endothelial growth factor (VEGF) receptor 2, sometimes also referred to as KDR or FLK1. EPCs originate in the bone marrow and can be mobilized either endogenously by tissue ischemia or exogenously by cytokine stimulation or HMG-CoA reductase inhibitors. CD133-selected cells from peripheral blood were also shown to have the capacity to differentiate into endothelial cells under defined conditions.

Multipotent adult progenitor cells (MAPCs) were isolated from bone marrow by depleting hematopoietic cells from the bone marrow cell fraction and plating the resulting cells. MAPCs are positive for the VEGF receptors KDR and FLT1 and dimly positive for CD44 and CD133. Besides their ability to differentiate in numerous mesenchymal tissues, they were also shown to differentiate in endothelial and neuronal cells in vitro and in vivo, indicating a greater developmental potential of MAPCs compared with MSCs.

Stem cells have two features: the ability to differentiate along different lineages and the ability of self-renewal. Two major types of stem cells have been described, namely, embryonic stem cells and adult stem cells. Embryonic stem cells (ESC) are obtained from the inner cell mass of the blastocyst and are associated with tumorigenesis, and the use of human ESCs involves ethical and legal considerations. The use of adult mesenchymal stem cells is less problematic with regard to these issues. Mesenchymal stem cells (MSCs) are stromal cells that have the ability to self-renew and also exhibit multilineage differentiation. MSCs can be isolated from a variety of tissues, such as umbilical cord, endometrial polyps, menstrual blood, bone marrow, adipose tissue, etc. This is because the ease of harvest and quantity obtained make these sources most practical for experimental and possible clinical applications. Recently, MSCs have been found in new sources, such as menstrual blood and endometrium.

Pluripotent stem cells, including both embryonic stem cells (ESCs) and induced pluripotent stem (iPS) cells, hold immense promise for cardiac regeneration because they possess unparalleled differentiation ability.

Stem and progenitor cells derived from bone marrow or from pluripotent stem cells have shown therapeutic benefit in boosting angiogenesis as well as restoring tissue function. Notably, adult stem and progenitor cells including mononuclear cells, endothelial progenitor cells, and mesenchymal stem cells have progressed into clinical trials and have shown positive benefits. In this review, we overview the major

classes of stem and progenitor cells, including pluripotent stem cells, and summarize the state of the art in applying these cell types for treating myocardial infarction and peripheral arterial disease.

### **Stem cell therapy for clinical treatment of MI**

The treatment of myocardial injury has been increasingly strategized using stem cell approaches. Over 100 clinical trials involving stem cell therapy for the treatment of MI have been reported. Depending on the stage of injury, stem cell therapies have sought to prevent the progression of acute myocardial ischemic cardiomyopathy and congestive heart failure, or restore function in failing or chronically ischemic hearts. Since stem cell clinical trials in MI patients have been described elsewhere, below we will briefly describe several selected clinical trials.

The results of recent United States clinical trials performed by the Cardiovascular Cell Therapy Research Network indicate the lack of therapeutic efficacy of BM-MNCs for treatment of acute MI. Briefly, 120 patients were randomly assigned to either BM-MNC therapy or placebo at different times of introduction (i.e., 3 or 7 days after percutaneous coronary intervention). After 6 mo, MRI-based evaluation of LVEF revealed that those treated with BM-MNCs did not show significant improvement compared with the placebo group. Thus, because of the lack of promise of BM-MNCs from several clinical trials, a shift in focus has been made toward other stem cell sources in the past several years.

### **3. Types of Stem Cells Used in MI Treatment**

Various types of stem cells have been investigated for their efficacy in this regard, including embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and mesenchymal stem cells (MSCs).

#### **Embryonic Stem Cells (ESCs)**

ESCs are pluripotent stem cells derived from the inner cell mass of blastocysts. They have the ability to differentiate into any cell type in the body. In preclinical studies, ESCs have shown remarkable potential for cardiac regeneration. They can differentiate into cardiomyocytes, endothelial cells, and smooth muscle cells, contributing to the formation of new blood vessels and myocardial tissue. However, their clinical translation has been hindered by ethical concerns related to their derivation from human embryos and the risk of teratoma formation.

In terms of promoting angiogenesis, ESCs have demonstrated the ability to release angiogenic factors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). These factors stimulate the proliferation and migration of endothelial cells, leading to the formation of new blood vessels. Additionally, ESC-derived endothelial cells can directly integrate into existing blood vessels, enhancing their functionality.

#### **Induced Pluripotent Stem Cells (iPSCs)**

iPSCs are reprogrammed somatic cells that have been induced to revert to a pluripotent state similar to ESCs. They offer the advantage of patient-specific therapy without the ethical concerns associated with ESCs. iPSCs have been investigated for their potential in cardiac regeneration, including MI treatment. In preclinical studies, iPSC-derived cardiomyocytes and endothelial cells have shown promising results in improving cardiac function and promoting angiogenesis.

Similar to ESCs, iPSCs have been shown to secrete pro-angiogenic factors such as VEGF and bFGF. These factors play crucial roles in stimulating angiogenesis and enhancing blood flow to the ischemic myocardium. Additionally, iPSC-derived endothelial cells have been shown to form functional blood vessels when transplanted into infarcted hearts, further supporting their role in promoting angiogenesis.



### Mesenchymal Stem Cells (MSCs)

MSCs are multipotent stem cells found in various tissues, including bone marrow, adipose tissue, and umbilical cord blood. They have the capacity to differentiate into multiple cell types, including cardiomyocytes and endothelial cells. MSCs have been extensively studied for their therapeutic potential in MI treatment, both in preclinical and clinical settings.

In preclinical studies, MSCs have been shown to promote angiogenesis through paracrine mechanisms. They secrete a variety of angiogenic factors, including VEGF, bFGF, and hepatocyte growth factor (HGF), which stimulate the proliferation and migration of endothelial cells. Additionally, MSCs have been shown to directly incorporate into newly formed blood vessels, enhancing their stability and functionality.

### Comparison of Efficacy in Promoting Angiogenesis

When comparing the efficacy of ESCs, iPSCs, and MSCs in promoting angiogenesis, several factors should be considered:

1. **Differentiation Potential:** ESCs and iPSCs have the advantage of being able to differentiate into endothelial cells directly, which may enhance their ability to promote angiogenesis compared to MSCs, which typically exert their effects through paracrine signaling.
2. **Immunogenicity:** MSCs have been shown to have immunomodulatory properties, allowing them to evade immune rejection and potentially exert longer-lasting effects compared to ESCs and iPSCs, which may trigger immune responses.
3. **Safety Concerns:** ESCs and iPSCs carry the risk of teratoma formation, which has raised safety concerns in clinical applications. MSCs, on the other hand, have a favorable safety profile and have been widely used in clinical trials for various indications, including MI treatment.
4. **Patient-specific Therapy:** iPSCs offer the advantage of patient-specific therapy, allowing for personalized treatment approaches tailored to individual patient needs.

### Preclinical studies

Preclinical studies on stem cell angiogenesis typically involve investigating the potential of stem cells to stimulate the growth of new blood vessels (angiogenesis) in laboratory settings or animal models. These studies are crucial for understanding the mechanisms involved and assessing the safety and efficacy of stem cell-based therapies for various medical conditions, including cardiovascular diseases, wound healing, and ischemic conditions.

Acute myocardial infarction (MI), despite significant progress in its treatment, remains a leading cause of chronic heart failure and cardiovascular events such as cardiac arrest. Promoting angiogenesis in the myocardial tissue after MI to restore blood flow in the ischemic and hypoxic tissue is considered an effective treatment strategy. The repair of the myocardial tissue post-MI involves a robust angiogenic response, with mechanisms involved including endothelial cell proliferation and migration, capillary growth, changes in the extracellular matrix, and stabilization of pericytes for neovascularization. In this review, we provide a detailed overview of six key pathways in angiogenesis post-MI: the PI3K/Akt/mTOR signaling pathway, the Notch signaling pathway, the Wnt/ $\beta$ -catenin signaling pathway, the Hippo signaling pathway, the Sonic Hedgehog signaling pathway, and the JAK/STAT signaling pathway. We also discuss novel therapeutic approaches targeting these pathways, including drug therapy, gene therapy, protein therapy, cell therapy, and extracellular vesicle therapy. A comprehensive understanding of these key pathways and their targeted therapies will aid in our understanding of the

pathological and physiological mechanisms of angiogenesis after MI and the development and application of new treatment strategies.

In early murine studies mobilization of myeloid clonogenic cells from spleen and bone marrow (BM) was observed during wound healing<sup>8</sup>. Later discoveries noted the effects of neovasculogenesis after endothelial progenitor cells (EPCs) mobilized secondary to hind limb ischemia. Rabbits mobilize EPCs specifically from the BM after hind limb ischemia; which was enhanced following GM-CSF administration<sup>9</sup>. These findings paved the way for use of progenitor cell to treat disease. During these early studies, there was no notion of intrinsic self-renewing cardiac cells. In 2003 this paradigm changed; cardiac stem cells that are self-renewing, clonogenic, and multipotent were observed in adult rat hearts<sup>10</sup>. Thus, began the concept that, with some help, the heart could heal itself. The controversy concerned the nature of that help. For many, the answer was which type of stem cell should be used to treat heart disease. The safety, efficacy and fate of each cell line needed further research in animal models to determine not only which model was best to simulate human cardiac response but which of these various cell types should be studied further.

### Small animal studies

For preclinical development, an appropriate animal model that accurately reflects human pathological conditions is essential. Cell and molecular studies provide important mechanistic data and toxicity studies evaluate candidate drugs, but a working heart is needed to evaluate and optimize treatments.

### Large animal studies

Cardiac repair studies show larger effects in rodents, increased left ventricular ejection fraction (LVEF) up to 20% and normalization of LV function, in contrast to large animal studies (mean LVEF improvement ~5–7%). This moderate benefit corresponds better to the results of clinical trials, giving realistic insight into the expected benefit of human cell-based cardiac therapies. The presence or absence of collateral coronary circulation is an important factor for choosing an adequate animal model for a particular study. Large animals such as pigs, dogs, or sheep satisfy many of these criteria. Dogs have an extensive collateral coronary circulation, while pigs and sheep have no functionally relevant vascular adaptation system, similar to humans. Thus, a dog model is suitable for studying vascular adaptation to myocardial ischemia, while pigs and sheep are generally regarded as appropriate to assess the direct myocardial effects of hypoxic injuries.

Here's a general outline of what such preclinical studies might involve:

**Cell Culture Studies:** Researchers often start by culturing stem cells in vitro (in a lab dish). They might use various types of stem cells, such as embryonic stem cells, induced pluripotent stem cells (iPSCs), or adult stem cells like mesenchymal stem cells (MSCs). These studies aim to understand how stem cells behave and interact with other cells, including endothelial cells that form blood vessels.

**Angiogenic Assays:** Researchers use various assays to assess the angiogenic potential of stem cells. These assays measure parameters like endothelial cell proliferation, migration, and tube formation, which are essential processes in blood vessel formation.

**Animal Models:** Once promising results are obtained in vitro, researchers move to in vivo studies using animal models. Rodents such as mice and rats are commonly used for these studies due to their small size, short reproductive cycles, and genetic manipulability. Researchers can induce conditions like hind limb

ischemia or myocardial infarction in these animals and then transplant stem cells to observe their effects on angiogenesis and tissue regeneration.

**Imaging Techniques:** Advanced imaging techniques like MRI, CT scans, or fluorescence microscopy are often employed to track the fate of transplanted stem cells in living organisms and visualize the formation of new blood vessels over time.

**Functional Assessments:** Researchers evaluate the functional outcomes of stem cell therapy, such as improved blood flow, tissue perfusion, and recovery of organ function. This may involve physiological measurements, such as blood pressure, cardiac function tests, or tissue oxygenation levels.

**Safety and Long-Term Effects:** Researchers also assess the safety profile of stem cell therapies, including the potential for tumor formation (teratoma formation with pluripotent stem cells) or immune rejection. Long-term studies are essential to understand the persistence of therapeutic effects and any adverse events associated with stem cell transplantation.

Overall, preclinical studies play a vital role in providing the necessary evidence to support the translation of stem cell angiogenesis therapies from the laboratory to clinical trials in humans. These studies help establish the feasibility, safety, and efficacy of such treatments, ultimately contributing to the development of novel regenerative medicine approaches.

Effects of basal molecules involved in angiogenesis after MI on angiogenesis, vascular homeostasis, and vascular permeability.

Basal mediators	Angiogenesis	Vascular homeostasis	Vascular permeability	Other
<b>VEGF and VEGFR</b>				
<b>VEGF-A</b>	Induced tip cells and promotes filopodia formation ; Enriched expression in endothelial tip cells and promoted tip cell invasiveness ; Controlled cytoskeleton rearrangement, adhesion, and EC polarization to open lumen in new blood vessels	Protected neovascular EC by inducing the expression of anti-apoptotic proteins and the production of NO	Increased permeability via adherens junctions, particularly VE-cadherin	Regulated immune cells
<b>VEGF-B</b>	Displaced VEGF-A from VEGFR-1, resulting in enhanced angiogenesis	Stimulated mitochondrial biogenesis and controlled EC fatty acid uptake	ND	Potent antioxidant
<b>VEGF-C and VEGF-D</b>	ND	ND	ND	Involved in the generation and



				remodeling of lymphatic vessels
<b>PIGF</b>	Promoted angiogenesis	ND	ND	ND
<b>VEGFR-1</b>	Negative regulator of angiogenesis ; Decoy receptor for VEGF-A	Promoted pericyte coverage	ND	ND
<b>VEGFR-2</b>	Primary receptor for VEGF-A	ND	Increased permeability via adherens junctions, particularly VE-cadherin	ND
<b>VEGFR-3</b>	Negative regulator of angiogenesis	ND	ND	Mediated lymphangiogenesis

### Clinical Studies

Clinical studies on stem cell angiogenesis in myocardial infarction (MI) aim to evaluate the safety and efficacy of stem cell therapies for promoting angiogenesis and tissue repair in patients who have suffered from a heart attack. These studies typically involve transplanting stem cells into the damaged heart tissue to stimulate the growth of new blood vessels, improve blood flow, and enhance cardiac function. Here's an overview of what such clinical studies might entail:

**Patient Selection:** Participants in clinical trials are usually individuals who have recently experienced a myocardial infarction or have chronic ischemic heart disease with impaired cardiac function. They undergo thorough screening to ensure they meet specific eligibility criteria, such as age, overall health status, and severity of cardiac impairment.

**Stem Cell Type:** Various types of stem cells have been investigated in clinical trials for MI, including bone marrow-derived mesenchymal stem cells (MSCs), cardiac progenitor cells, and induced pluripotent stem cell-derived cardiomyocytes. The choice of stem cell type depends on factors such as availability, safety profile, and potential for cardiac regeneration.

**Delivery Methods:** Stem cells can be delivered to the heart tissue via different routes, including intracoronary infusion, transendocardial injection, or intramyocardial injection during open-heart surgery. Each delivery method has its advantages and challenges, and clinical studies may compare different approaches to determine the most effective and safest delivery strategy.

**Outcome Measures:** Clinical studies typically evaluate various endpoints to assess the efficacy of stem cell therapy. These endpoints may include improvements in cardiac function parameters such as left ventricular ejection fraction (LVEF), myocardial perfusion, exercise capacity, and quality of life. Imaging techniques such as echocardiography, cardiac MRI, and nuclear imaging are commonly used to measure these endpoints.

**Safety Monitoring:** Safety assessments are crucial in clinical trials to monitor for any adverse events associated with stem cell transplantation, such as arrhythmias, immune reactions, or tumor formation.

Participants are closely monitored throughout the trial period, and long-term follow-up is often conducted to assess the persistence of therapeutic effects and any delayed adverse events.

**Randomized Controlled Trials (RCTs):** Many clinical studies are designed as randomized controlled trials, where participants are randomly assigned to receive either stem cell therapy or a placebo/control treatment. This design helps minimize bias and provides robust evidence on the safety and efficacy of stem cell angiogenesis therapies for MI.

**Regulatory Approval:** Clinical trials on stem cell therapy for myocardial infarction must adhere to strict regulatory guidelines set by health authorities such as the FDA (in the United States) or the EMA (in Europe). Researchers must obtain approval from these regulatory bodies before initiating clinical trials, and they are required to adhere to Good Clinical Practice (GCP) guidelines to ensure the ethical conduct of research and patient safety.

## Application of angiogenesis for cardiac repair

### Mesenchymal stem cells (MSCs)

MSCs are stromal cells that have self-renewal ability and show multilineage differentiation. MSCs can be isolated from a variety of tissues, such as umbilical cord, endometrial polyps, menses blood, stem cells, bone marrow and adipose tissue. Due to their powerful function and easy access, MSCs have been widely used by researchers in recent years, especially in the study of ischemic heart disease. For example, placenta-derived MSCs can be used to promote therapeutic angiogenesis. Such MSCs can differentiate into vascular-like cells and secrete some provascular factors to promote angiogenesis. These cytokines include VEGF, basic fibroblast growth factor, IL-6, IL-8, HGF, insulin-like growth factor (IGF) binding protein (IGFBP)2, IGFBP3 and IGFBP6. These factors generate blood vessels by activating key provascular-related signaling pathways

### Exosomes

Exosomes are small extracellular vesicles that are only 50-150 nm in diameter, surrounded by a lipid bilayer membrane and contain components derived from their original cells. Exosomes have a relatively rich source, existing in various tissues and cells throughout the body, such as embryos, adipose tissue and bone marrow

The extraction methods for exosomes include ultracentrifugation, immunoprecipitation, size-based isolation techniques and commercial rapid extraction reagents. The most widely used methods are ultracentrifugation and rapid extraction reagents. These methods have both advantages and disadvantages, and thus the appropriate method to extract exosomes should be selected according to the research needs. Another important point is the identification of exosomes, and the quality of exosomes plays a crucial role in research.

As early as 2014, the International Association of Extracellular Vesicles proposed that the identification of exosomes can be divided into three levels: Transmission electron microscopy (TEM), nanosight particle size and protein markers. In general identification, there must be  $\geq 3$  vesicle-positive protein makers, including  $\geq 1$  transmembrane or lipid-binding protein and one cytoplasmic protein,  $\geq 1$  vesicle-negative protein maker. The identification of a single vesicle requires two different but complementary methods, such as TEM or atomic force microscopy plus nanoparticle tracking analysis

## Biomaterials

In recent years, additional research has focused on repair by using biomaterials. Compared with traditional cell therapy, biomaterials have more advantages, such as being degradable, easy to obtain and free to regulate the repair process. Biomaterials are mainly divided into natural and artificial synthetic materials. Natural materials mainly refer to the various components of mammalian ECM, such as collagen, fibrinogen, Matrigel and gelatin. Natural materials also include some ingredients extracted from plants or animals, such as chitosan and cellulose. Artificial synthetic materials are easier to obtain and are more plastic.

Biomaterials can be combined with cells or factors to promote wound healing. Biomaterials with loaded stem cells and immunomodulating and tissue-regenerating factors can be used to ameliorate inflammation, improve angiogenesis, reduce fibrosis and generate functional cardiac tissue. For example, the encapsulation of VEGF in polylactic acid glycolic acid NPs improves the therapeutic efficacy of VEGF and promotes angiogenesis. Liu et al reported that co-transplantation of chitosan thermosensitive hydrogel and bone-marrow-derived MSCs can ameliorate the inflammatory response and promote cardiac functional recovery. Citrate hydrogels can be injected as an angiogenic biomaterial to improve cardiac function after AMI and increase the number of blood vessels in the infarcted area. In addition to the injectable biomaterials, some researchers have developed thermal plastic poly (glycolic acid) surgical sutures and mussel-inspired conductive particle adhesion into highly elastic, conductive spring-like coils. The polypyrrole-coated biospring acts as an electrode and is assembled into a solid-state supercapacitor. After being injected through a syringe needle (0.33-mm inner diameter), the tangled coils form an elastically conductive three-dimensional (3D) network to modulate angiogenesis. Additionally, a cardiopatch has been created with adipose-tissue-derived progenitor cells seeded into an engineered bioimplant consisting of 3D bioabsorbable polycaprolactone scaffolds filled with a peptide hydrogel. This treatment decreases fibrosis, limits infarct scar expansion and reduces postischemic ventricular deformity. Similarly, the use of chitosan/calcium silicate heart patches showed increased angiogenesis in rats after myocardial infarction. The alginate scaffold modified with RGDfK (Arg-Gly-Asp-D-Phe-Lys) peptide can be used for cell transplantation and cardiac neovascularization. It is also interesting to note that by changing the stiffness of hydrogel, VEGF secretion of MSCs can be improved, thus further promoting vascular formation.

Overall, clinical studies on stem cell angiogenesis in myocardial infarction represent a promising approach for improving outcomes in patients with heart disease. While some trials have shown encouraging results, ongoing research is needed to optimize treatment protocols, identify the most effective stem cell types and delivery methods, and address remaining safety concerns.

## 6. Challenges and Limitations

### Challenges in Stem Cell Survival:

The survival of transplanted stem cells within the ischemic myocardium poses a significant challenge in angiogenesis therapy for MI. Upon transplantation, stem cells encounter a hostile microenvironment characterized by limited oxygen and nutrient availability, inflammation, and oxidative stress. These adverse conditions contribute to poor cell viability and functionality, undermining the therapeutic efficacy of the treatment. Strategies to improve stem cell survival include preconditioning techniques, such as hypoxic preconditioning and genetic modifications, aimed at enhancing cell resistance to ischemic injury.

Additionally, the development of biomaterial-based scaffolds provides a supportive microenvironment for transplanted cells, promoting their survival and integration into the ischemic tissue.

### **Immune Rejection:**

Immune rejection represents another formidable challenge in stem cell angiogenesis therapy, particularly in allogeneic transplantation settings. Transplanted stem cells are recognized as foreign entities by the host immune system, eliciting immune-mediated responses that can lead to graft rejection and treatment failure. While immunosuppressive regimens are commonly employed to mitigate immune rejection, long-term immunosuppression poses risks of infection, organ toxicity, and systemic side effects. Alternative strategies, such as the use of autologous or immune-evasive stem cells, offer potential solutions to overcome immune-related challenges in MI therapy. Furthermore, the induction of immune tolerance through regulatory T cells or tolerogenic dendritic cells presents an intriguing avenue for promoting immune acceptance of transplanted cells.

### **Optimization of Delivery Methods:**

The effective delivery of stem cells to the ischemic myocardium is critical for the success of angiogenesis therapy in MI treatment. Conventional delivery methods, including intramyocardial injection and intracoronary infusion, face limitations in achieving targeted and uniform cell distribution within the infarcted tissue. Moreover, challenges such as cell entrapment, embolization, and limited retention further complicate optimal delivery. Innovative approaches, such as tissue engineering strategies and advanced imaging-guided techniques, are being explored to enhance the precision, efficacy, and safety of stem cell delivery. Biomimetic scaffolds offer a promising platform for controlled cell release and improved engraftment, while imaging modalities enable real-time monitoring of cell distribution and retention, facilitating optimized delivery in MI therapy.

## **7. Future Directions**

### **Emerging research explores various avenues to optimize this therapy:**

1. **Biomaterials:** Integrating stem cells with biomaterials to enhance their retention, survival, and paracrine signaling within the ischemic tissue, thereby maximizing angiogenic potential.
2. **Gene Editing:** Utilizing gene-editing techniques like CRISPR-Cas9 to modify stem cells for enhanced angiogenic properties, such as overexpressing pro-angiogenic factors or improving resistance to hostile microenvironments.
3. **Exosome Therapy:** Investigating the therapeutic potential of stem cell-derived exosomes, which contain bioactive molecules capable of inducing angiogenesis and tissue repair, as a cell-free alternative to traditional stem cell transplantation.
4. **Combination Therapies:** Exploring synergistic effects of combining stem cell therapy with other modalities, such as growth factors, cytokines, or small molecules, to amplify angiogenesis and improve clinical outcomes.

### **Potential Strategies to Enhance Stem Cell Angiogenesis Therapy for MI:**

1. **Preconditioning:** Pre-treating stem cells with growth factors, hypoxia, or pharmacological agents to prime them for survival, proliferation, and angiogenic activity upon transplantation.
2. **Optimized Delivery:** Developing novel delivery methods, including injectable hydrogels, nanoparticles, or tissue-engineered scaffolds, to improve stem cell retention, engraftment, and spatial distribution within the infarcted myocardium.

3. Remote Conditioning: Inducing remote ischemic conditioning in patients prior to stem cell transplantation to create a pro-angiogenic microenvironment in the heart, thereby enhancing the therapeutic efficacy of transplanted cells.
4. Immunomodulation: Modulating the immune response to mitigate inflammation and immune rejection, while promoting tissue healing and angiogenesis, through strategies such as immunosuppressive drugs or regulatory T cell therapy.
5. Patient-Specific Approaches: Implementing personalized medicine approaches, including genetic profiling and imaging modalities, to tailor stem cell therapy to individual patient characteristics and optimize treatment outcomes.

#### **Novel Technologies and Approaches:**

1. Microfluidic Platforms: Utilizing microfluidic devices to culture and manipulate stem cells in a controlled microenvironment, enabling high-throughput screening of angiogenic factors and optimization of cell-based therapies.
2. 3D Bioprinting: Employing 3D bioprinting techniques to fabricate complex tissue constructs composed of stem cells, biomaterials, and vasculature, mimicking the native cardiac microenvironment for enhanced angiogenesis and tissue regeneration.
3. Nanotechnology: Harnessing nanoscale materials and drug delivery systems to target stem cells specifically to the ischemic myocardium, while minimizing off-target effects and improving therapeutic efficacy.
4. Machine Learning: Applying machine learning algorithms to analyze large-scale omics data and identify predictive biomarkers of stem cell responsiveness and patient outcomes, facilitating personalized treatment strategies.
5. Bioinformatics: Leveraging bioinformatics tools to integrate multi-omics data, including genomics, transcriptomics, and proteomics, to gain insights into the molecular mechanisms underlying stem cell angiogenesis and identify novel therapeutic targets.

#### **Conclusion**

Neovascularization within the infarcted tissue is an integral critical component of the cardiac remodeling process. The formation of the new dense capillary network would favor gas exchange, nutrient diffusion, and waste removal, which would attenuate cardiac myocyte dysfunction in the peri-infarcted zone. Understanding the complex mechanisms behind angiogenesis after MI may create multiple therapeutic opportunities. As summarized in this review, we briefly Several miRNAs might be attractive candidates for modulating certain therapeutic targets in MI, although more work is still required to explore new targets and to develop efficient delivery vehicles into the infarcted heart.

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