Cardiac Protective Engineering

Shu Q. Liu
Biomedical Engineering Department,
Northwestern University,
2145 Sheridan Road,
Evanston, IL 60208-3107
e-mail: sliu@northwestern.edu

Cardioprotective engineering is an emerging bioengineering discipline aiming to develop engineering strategies to optimize cardioprotective actions against cardiac injuries and disorders. Although there exist innate cardioprotective mechanisms capable of supporting cardiomyocyte survival in response to an insult, not all these mechanisms are optimized in promptness and effectiveness, suggesting the necessity of cardioprotective engineering. Various cardioprotective strategies have been developed and used in experimental and clinical investigations; however, few of these strategies have exerted a significant clinical impact. There are two major challenges in cardioprotective engineering—understanding the innate cardioprotective mechanisms and developing engineering strategies for precise control of the types, levels, timing, and coordination of cardioprotective actions to facilitate recovery from injuries and disorders. Understanding the innate mechanisms is the foundation for developing cardioprotective engineering strategies. Here, ischemic myocardial injury is used as an example to demonstrate the concept of cardioprotective engineering. [DOI: 10.1115/1.4043434]

Introduction

Protective engineering is an emerging bioengineering discipline aiming to understand the innate mechanisms of protection against injuries and disorders and develop engineering strategies to optimize protective processes. Nature has developed various protective mechanisms in response to genetic and environmental insults during evolution at all levels of structure—molecules, cells, organs, and systems [1]. Examples of protective mechanisms include nonhomologous deoxyribonucleic acid (DNA) end joining and homologous recombination for repairing double-stranded DNA breaks induced by irradiation and chemicals [2,3]; cell and organ regeneration in response to the loss of cell and extracellular matrix structures [1,4–6]; and systems inflammatory responses to micro-organism infections and severe organ damages [7,8]. However, not all naturally occurring protective mechanisms are optimized in promptness and effectiveness—lethal gene mutations occur in spite of the presence of gene repair mechanisms; the expression of protective genes often lags behind injuries, missing the early period for optimal protection [1]; vital cells such as the neurons and cardiomyocytes possess a limited capacity of protection; and inflammatory responses are commonly over-activated to cause excessive matrix production and fibrosis, imposing adverse impacts on cell function and regeneration [1,7,8]. Protective engineering is developed to correct natural deficiencies by optimizing protective actions.

Protective engineering is related to another bioengineering discipline—regenerative engineering, addressing the development of engineering strategies to control cell regenerative processes for restoring the structure and function of disordered organs [5,9,10]. Protective engineering aims to support cell survival prior to cell death, whereas regenerative engineering addresses cell proliferation and differentiation after cell death in injuries and disorders [1,5]. In nature, cell survival, proliferation, and differentiation involve similar regulatory processes [11,12], all supporting the continuation of the life of organisms. In a broader sense, regenerative engineering is protective—protecting an organ or the entire organism from death by regenerating functional cell and matrix structures. Here, ischemic myocardial injury is used to demonstrate the concept of cardioprotective engineering. In particular, two fundamental topics are to be covered: Naturally occurring cardioprotective mechanisms and potential engineering strategies for optimizing cardioprotective actions in ischemic myocardial injury. Understanding the cardioprotective mechanisms is the foundation for developing cardioprotective engineering strategies.

Manuscript received February 6, 2019; final manuscript received April 2, 2019; published online August 2, 2019. Assoc. Editor: X. Edward Guo.

Foundations of Cardioprotective Engineering

The Concept of Systems Cardioprotective Mechanisms. Myocardial ischemia, although causing myocardial infarction, can activate two types of innate mechanisms that protect the cardiomyocytes from injury and death—regional and distant cardioprotective mechanisms [1]. Both mechanisms are collectively defined as *systems cardioprotective mechanisms* (Fig. 1). The regional mechanisms include upregulation and release of paracrine protective factors, leukocyte activation and infiltration, resident stem cell activation, and fibroblast proliferation and extracellular matrix production [1]. The distant mechanisms include upregulation and release of endocrine protective factors and mobilization of protective cells from the bone marrow, spleen, liver, and potentially other organs [1]. Both regional and distant cardioprotective mechanisms act in coordination and synergy to protect cardiomyocytes from injury and facilitate the restoration of myocardial structure and function. Systems protective mechanisms are the foundation of cardioprotective engineering.

Regional Cardioprotective Mechanisms

Paracrine Cardioprotective Actions. Within the ischemic myocardium, various cardioprotective mechanisms can be activated to protect the cardiomyocytes from injury and death. These mechanisms have been recognized from experimental models of ischemic preconditioning, a procedure inducing nonlethal ischemic episodes in the heart or a remote organ, resulting in alleviation of subsequent lethal myocardial infarction [13–15]. Ischemic preconditioning is the most effective and reproducible approach to date for myocardial protection in experimental and clinical investigations [16–20], providing convincing evidence for the presence of innate cardioprotective mechanisms. It has now been understood that the cardioprotective effect of ischemic preconditioning is mediated by paracrine protective factors released from injured cardiac cells. A number of such factors have been identified, including adenosine, opioids, bradykinin, vascular endothelial growth factor (VEGF), insulin-like growth factor (ILGF), hepatocyte growth factor (HGF), interleukin 6 (IL6), and stromal cell-derived factor 1 (SDF-1) [21–31]. Based on the time of action following ischemic myocardial injury, these factors are classified into two categories: early- and late-phase cardioprotective factors. Adenosine, opioids, and bradykinin are defined as early phase factors, and the others as late-phase factors [24–31]. The early phase factors are released from injured cardiac cells during the first several hours after ischemic myocardial injury without the involvement of de novo gene expression, whereas the late-phase factors

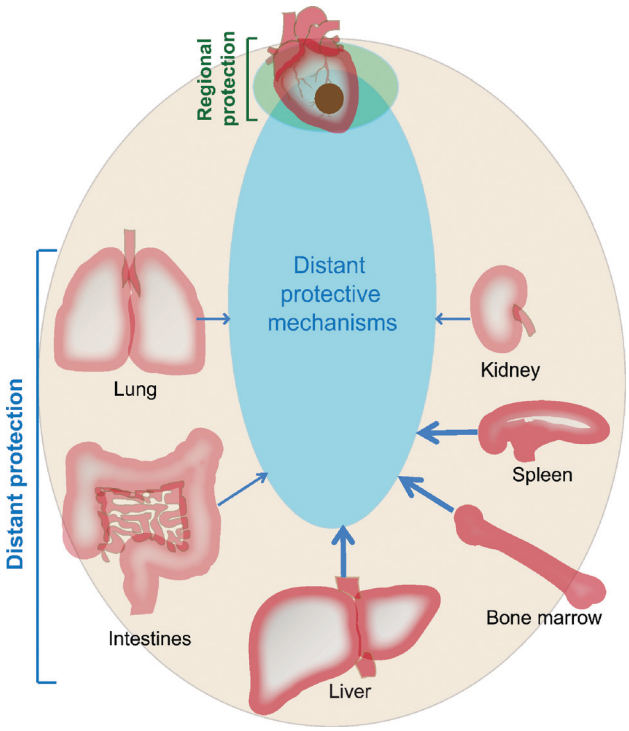

Fig. 1 Naturally occurring systems protective mechanisms activated in response to ischemic myocardial injury. The largest oval indicates the coverage of cytokines and other endocrine factors released from ischemic cardiac cells; the second largest oval indicates the coverage of distant cardioprotective mechanisms involving endocrine protective factors and cells mobilized from distant organs; and the smallest oval indicates the coverage of regional cardioprotective mechanisms. The thick arrows represent distant cardioprotective mechanisms from organs experimentally confirmed, and the thin arrows indicate potential distant cardioprotective mechanisms from organs that have not been confirmed.

are produced and released from 12 h to several days, requiring de novo gene expression [1,21]. The time-dependent activation and release of multiple factors provide a wide time window of protection. However, there are two potential problems: the ischemic myocardium may not be well protected during the early period prior to the release of the protective factors, when cardiomyocyte injury and death occur, and not all protective factors can reach optimal levels for effective protection, as suggested by the observations that administration of exogenous protective factors alleviate ischemic myocardial injury [1,20,32]. Correction of these deficiencies is the goal of cardioprotective engineering.

Signaling Mechanisms of Paracrine Cardioprotective Actions. A fundamental question is how the paracrine cardioprotective factors act. The early phase factors (adenosine, opioids, and bradykinin) exert a cardioprotective effect by activating the G protein-coupled receptor signaling networks, whereas the late-phase growth factors (VEGF, ILGF, and HGF) and cytokines (IL6 and SDF-1) act through the protein tyrosine kinase receptor and cytokine receptor signaling networks, respectively [1,33]. Among the early phase cardioprotective factors, adenosine is a purine nucleoside, can bind to the adenosine A1 receptor [29], causing recruitment of guanosine diphosphate (GDP)-bound trimeric G protein to the intracellular domain of the receptor. The G protein is modified to substitute guanosine triphosphate (GTP) for GDP and separate into a monomeric α unit and a dimeric $\beta\gamma$ unit. The $\beta\gamma$ unit can activate the phospholipid kinase phosphoinositide 3-kinase (PI3K), an enzyme capable of phosphorylating phosphoinositides (PIs), generating phosphatidylinositol (3,4)-bisphosphate (PIP2) and phosphatidylinositol (3,4,5)-trisphosphate (PIP3) [34]. PIP2 and PIP3 mediate the interaction of phosphoinositide-dependent protein kinase-1 with Akt, resulting in Akt phosphorylation and activation [35]. Activated Akt in turn phosphorylates the cell death-inducing protein Bcl-XL/Bcl-2-associated death promoter (BAD), suppressing the activity of BAD and thereby reducing cardiomyocyte death [1,33]. The other early cardioprotective factors opioids and bradykinin are short peptides capable of binding to their cognate G protein-coupled receptors, exerting cardioprotective actions following similar signaling mechanisms [33].

The late-phase cardioprotective growth factors (VEGF, ILGF, and HGF) act by interacting with protein tyrosine kinase receptors [1,36]. Each growth factor can bind to specific receptor(s), causing receptor dimerization and tyrosine autophosphorylation on the receptor kinase domains. Phosphotyrosine residues serve as docking sites for the recruitment of the SH2 domain-containing protein complex growth factor receptor bound protein 2 (Grb2)/son of sevenless (SOS, a guanine nucleotide exchange factor). Grb2/SOS can activate Ras small GTPases, which in turn activate a cascade of kinases, resulting in the phosphorylation of extracellular signal-regulated kinases 1/2 (ERK1/2) and c-Jun N-terminal kinases (JNK). These kinases can phosphorylate *c*-Fos and *c*-Jun, respectively, transcription factors capable of activating cell mitogenic and survival genes and protecting cardiomyocytes from injury and death [5].

The cytokines IL6 and SDF-1 act through different signaling networks. IL6 is able to bind to IL6 receptor α (IL6Rα), a step causing receptor conformational changes and recruitment of two gp130 coreceptors. The IL6 receptor/coreceptor complex can recruit a Janus kinase (JAK) to each gp130 coreceptor, causing gp130 phosphorylation on tyrosine residues that recruit the transcription factors signal transducer and activator of transcriptions (STATs). JAKs can phosphorylate STATs, resulting in STAT dimerization. STAT complexes serve as transcription factors, inducing the expression of genes encoding cell mitogenic and survival genes and protecting cardiomyocytes from injury and death [1,5]. SDF-1 is a chemokine (C-X-C motif chemokine 12) capable of activating cardioprotective mechanisms via the G protein-coupled receptor signaling networks described above [1].

Inflammation. Another regional cardioprotective mechanism is inflammation in the ischemic myocardium. Inflammation involves several processes activated in response to ischemic injury—elevation of endothelial permeability, edema in the interstitial space, upregulation and release of cytokines and growth factors from injured cells, extravasation of leukocytes, endothelial cell proliferation and angiogenesis, fibroblast proliferation, and over-production of extracellular matrix, ultimately leading to fibrosis [7,8]. These are protective mechanisms to deploy cardioprotective and angiogenic factors to the injury sites, clean dead cell debris, prevent microorganism infections, provide mechanical supports to the injury-weakened myocardium, enhance angiogenesis, and promote recovery from injury. Elevation of endothelial permeability is induced in response to histamine released from injured endothelial cells, mast cells, and basophils. Histamine can cause opening of the endothelial cell tight junctions, thereby increasing endothelial cell permeability, causing fluid leakage, and facilitating leukocyte extravasation. Injured cardiac cells and leukocytes can express and release cytokines, which can attract leukocytes to the injury sites to clean dead cell debris, release additional cytokines to boost inflammation, and prevent microorganism infection. Upregulated growth factors, especially VEGF, can cause endothelial cell proliferation, inducing angiogenesis. Growth factors can also stimulate fibroblast proliferation and over-production of collagen matrix, resulting in fibrosis, a critical process preventing myocardial rupture in ischemic injury. Thus, inflammation is a class of protective mechanisms in injury. However, inflammation is overactivated in most injury cases, exerting adverse impacts on myocardial function—edema retards myocardial contractile

activities and fibrotic tissue hinders resident stem cell migration and cardiomyocyte regeneration.

Distant Cardioprotective Mechanisms

Endocrine Cardioprotective Actions. In addition to the regional cardioprotective mechanisms described above, ischemic myocardial injury can induce expression and release of endocrine proteins from the liver, including α1-acid glycoprotein type 2 (AGP2), bone morphogenetic protein-binding endothelial regulator (BMPER), fibroblast growth factor 21 (FGF21), neuregulin 4 (NRG4), and trefoil factor 3 (TFF3) [32,37]. These proteins have been identified by systematic differential gene expression profiling and protein analyses in experimental ischemic myocardial injury. These proteins are concurrently elevated in the circulatory system and are able to enter the ischemic myocardium in the presence of increased endothelial permeability, supporting cardiomyocyte survival [1,32]. Administration of the recombinant form of each liver-derived protein alleviates significantly myocardial infarction, confirming the cardioprotective role of these proteins. A combination of all five liver-derived proteins exerts a higher level of cardioprotective action [32].

The five liver-derived endocrine proteins have been identified previously for regulating various functions. AGP2 is a glycosylated acute-phase serum protein that is upregulated in response to inflammatory cytokines such as IL6 and tumor necrosis factors [38,39]. The primary function of AGP2 is to regulate inflammatory responses by suppressing T cell and neutrophil activities and promote fibroblast proliferation for wound healing [38,40]. BMPER has been known as a secreted protein that interacts with bone morphogenetic protein (BMP) 4, a transforming growth factor (TGF)β family member responsible for controlling embryonic morphogenesis of the skeletal and cardiovascular systems [41,42]. BMPER binding to BMP4 enhances the activity of BMP4 at a relatively low level by promoting BMP4 interactions with its receptors, but may inhibit BMP4 at a high level by masking the binding sites of BMP4 [43]. FGF21, one of the 22 FGF family proteins [44,45], is expressed primarily in the liver and, to a lesser extent, the adipose tissue and pancreas [46,47]. Whereas the majority of the FGF family proteins regulate cell proliferation and differentiation in embryogenic and pathogenic processes, FGF21 has been reported to stimulate glucose intake in a variety of cell types [48,49], induce insulin expression in pancreatic β cells [50], and regulate lipid metabolism [51–53]. NRG4, a member of the neuregulin family, has been found in the skeletal muscle and pancreas [54,55] and causes cell proliferation, neuronal morphogenesis, and pancreatic islet cell formation [55,56]. TFF3 is a protein characterized by the presence of a trefoil motif and was originally discovered in the intestinal epithelial cells [57,58]. This protein has been known to protect intestinal cells from injury and promote wound healing [58,59].

The expression and release of AGP2, BMPER, FGF21, NRG4, and TFF3 are possibly regulated by cytokines upregulated in ischemic myocardial injury. Cytokines can activate a number of transcription factors, including activator protein 1 (AP-1) and the STAT family of proteins through cognate signaling pathways [1]. The genes encoding three of the five liver-derived cardioprotective proteins, AGP2, FGF21, and TFF3, contain consensus binding site(s) in the enhancer regions for cytokine-activated transcription factors [1]. The AGP2 gene contains binding sites for the transcription factors STAT1, STAT2, STAT3, STAT4, STAT5, and STAT6 [1]; the FGF21 gene contains binding sites for AP-1, STAT3, and STAT5 [1]; and the TFF3 gene contains binding sites for AP-1, STAT1, and STAT3 [1]. These analyses suggest that cytokines activating the transcription factors listed above may cause upregulation of these liver-derived proteins in ischemic myocardial injury. For the other two liver-derived cardioprotective proteins, BMPER and NRG4, few binding sites for transcription factors activated by common cytokines have been found in their genes. It remains unclear how the expression of these two proteins is regulated.

Mobilization of Cardioprotective Cells. Ischemic myocardial injury can cause cell mobilization from various organs, including the bone marrow [60–62], spleen [63], and liver [1,64]. The mobilized cells can enter the ischemic myocardium, exerting cardioprotective actions. The bone marrow has been found to discharge hematopoietic stem cells and endothelial progenitors into the circulatory system in response to ischemic myocardial injury [60–62]. These cells, once entering the ischemic myocardium, can release cytokines and growth factors, supporting cardiomyocyte survival. Endothelial progenitor cells can transform into endothelial cells in the ischemic myocardium, initiating and promoting angiogenesis [65]. Bone marrow cell mobilization is regulated by secreted proteins, including granulocyte colony stimulating factor [66,67], stromal cell-derived factor 1 [68,69], and vascular endothelial growth factor [70]. These factors are possibly upregulated in response to ischemic myocardial injury and act on cognate signaling pathways in bone marrow cells, causing cell mobilization. Ischemic myocardial injury can also cause mobilization of splenic monocytes to the circulatory system and ischemic myocardium to regulate inflammatory responses and wound healing [63]. Cytokines are potential factors mediating monocyte mobilization from the spleen.

The liver is another organ that can mobilize cells to the circulatory system in response to ischemic myocardial injury [1,64]. Major cell types mobilized include hepatocytes and biliary epithelial cells [1,64]. Hepatic cell mobilization is primarily found from 3 to 10 days after ischemic myocardial injury with a peak population of circulatory hepatic cells about 2% of leukocytes at 5 days [64]. Circulatory hepatic cells can be identified by using a genetic marker such as the yellow fluorescent protein gene driven by the albumin gene promoter, which induces gene expression primarily in hepatocytes and biliary epithelial cells [64]. The mobilized hepatic cells may enter the ischemic myocardium, potentially contributing to myocardial protection and repair by expressing and releasing cardioprotective proteins as discussed above. Hepatic cell mobilization is regulated by a series of cytokine-related activities [64]. Ischemic myocardial injury causes upregulation and release of IL6, a cytokine that activates circulatory leukocytes, inducing leukocyte retention in the liver parenchyma. The liver-retained leukocytes can upregulate and release matrix metalloproteinase 2 (MMP2), an enzyme that degrades collagen matrix, resulting in liberation and mobilization of hepatocytes and biliary epithelial cells in the vicinity of leukocytes. Other cell types, including Kupffer cells and Ito cells may also be mobilized. However, the cardioprotective role of these cell types has not been investigated largely because of the lack of identification markers.

Strategies of Cardioprotective Engineering

Cardioprotective engineering strategies can be designed and implemented to boost cardioprotective actions at three levels with currently available technologies—molecular, cellular, and tissue levels. Molecular cardioprotective engineering is to initiate and promote cardioprotective gene expression, suppress adverse gene expression, and control signaling processes to facilitate recovery from ischemic myocardial injury. Cell-based cardioprotective engineering is to provide needed cell types for delivery of cardioprotective factors and regeneration of functional cardiomyocytes and other cardiac cell types. Tissue-level cardioprotective engineering is to provide functional supports to the myocardium to boost contractile activities and prevent myocardial rupture in ischemic injury.

Molecular Cardioprotective Engineering

Promoting Cardioprotective Gene Expression by Gene Transfer. Gene transfer is an approach for boosting the expression of genes encoding cardioprotective and regenerative proteins, thereby reducing cardiomyocyte death, enhancing cardiomyocyte regeneration, and facilitating recovery from ischemic myocardial

injury [5,71]. Candidate cardioprotective genes include the growth factor genes, such as the VEGF, HGF, epidermal growth factor (EGF), and FGF genes [72–75], and selected cytokine genes, such as the SDF-1 and cardiotrophin 1 (a member of the IL6 cytokine family) genes [76–78]. The biological foundation of gene transfer is that mammalian cells are capable of endocytosing genes and the endocytosed genes can be expressed once reaching the nucleus [5,71]. However, the rate of naturally occurring gene endocytosis is low. Thus, plain gene transfer is not effective to produce sufficient proteins for cardioprotection.

Several methods have been developed and used to facilitate gene transfer into cardiac cells, including virus-, liposome-, and receptor-mediated gene transfers [5,71]. Viruses are the most effective mediators for gene transfer. Protective genes can be integrated into the viral genome by recombinant biotechnology and introduced to target cells via virus infection. Liposomes are phospholipid vesicles that can bind DNA fragments and fuse into target cells to facilitate gene transfer. Selected protein ligands, such as transferrins, can form complexes with DNA fragments and bind to cognate receptors to cause internalization of the gene-transferrin-receptor complexes, thereby facilitating gene transfer. All these methods are applicable to cardiac gene delivery. The cardioprotective gene-containing carriers can be delivered into the ischemic and intact myocardium by direct injection during heart surgery, such as coronary artery reconstruction. Alternatively, gene carriers can be delivered into the myocardium by direct myocardial or intrapericardial injection during percutaneous coronary interventions such as angioplasty and stent placement [79]. The delivered genes, once reaching the nucleus, can be expressed from 1 day to several weeks following gene transfer.

It is important to note that a time window of 12 h to 1 day is required for gene expression following gene transfer. The ischemic myocardium is not sufficiently covered with cardioprotective proteins from the transferred gene(s) during this window. A supplementary strategy is to deliver concurrently cardioprotective proteins encoded by the genes transferred. The proteins can start cardioprotective actions immediately following delivery and remain effective for about 12–24 h from a single dose. With protein and gene delivery together, the ischemic myocardium can be covered immediately following the ischemic insult up to several weeks. This is the most critical period for myocardial protection and regeneration because cell death, inflammatory responses, stem cell activation, fibroblast proliferation, and fibrosis all occur during this period. After this period, the ischemic myocardium becomes stabilized, the penumbrous cardiomyocytes are recovered from injury, and the infarcted areas, if any, are replaced by fibrotic tissue.

The effectiveness of the molecular protective strategies depends on the types of gene selected for transfer and the timing, levels, and coordination of gene expression. In principle, genes that are activated in response to ischemic myocardial injury and directly involved in innate cardioprotective processes are the candidates of choice. These genes can be identified by a systematic differential gene expression profiling approach such as RNA sequencing (RNA-seq). A challenge for this approach, however, is the analysis of a large amount of information with a large number of ischemia-altered genes from the profiling tests. It is often difficult to identify the most effective genes that can be used for cardioprotective therapies. One practical approach is to classify the upregulated or downregulated genes into categories such as secreted protein genes (for instance, growth factor and cytokine genes), receptor genes, protein kinase genes, transcription factor genes, and others. The next step is to screen the genes of a selected category by using functional assays in vitro or in vivo. For in vitro assays, each selected gene can be introduced to cultured animal neonatal cardiomyocytes subjected to a hypoxic insult, the level of the encoded protein can be measured at selected time points, and the cardioprotective impact can be evaluated based on the rate of cardiomyocyte survival or death under a given hypoxic condition. The most effective cardioprotective genes can be selected by comparison analyses between different genes. For in vivo assays, ischemic myocardial injury can be induced in an animal model, and a similar protocol can be used to identify the most effective genes based on various measures such as the fraction of myocardial infarcts, the rate of cardiac cell death, and the relative activities of caspases 3, 8, and 9. Proteins encoded by the selected genes can also be used for these tests with or without concurrent gene transfer.

Suppressing Adverse Gene Expression by siRNAs. Ischemic myocardial injury is associated with expression of genes imposing adverse impacts on cardioprotection, such as the tumor necrosis factor superfamily genes encoding cell death-inducing proteins and the transforming growth factor β family genes responsible for extracellular matrix over-production. An important task of cardioprotective engineering is to control the expression of these genes to desired levels. The RNA interference method by using small interfering RNAs (siRNAs) can be used to suppress or silence adverse genes [80,81]. A siRNA is a double-stranded RNA sequence about 21–22 base pairs in length, originally discovered in the plant petunias [82,83] and the nematode worm *C. elegans* [84]. It is capable of recognizing and interacting with a specific target mRNA, cleaving the target mRNA, and thereby knocking down the synthesis of the corresponding protein [85]. A siRNA can also physically block mRNA transcription [85]. These features have made RNA interference a useful approach for post-transcriptional silencing of selected target genes. A siRNA can be constructed based on the sequence of a selected gene and delivered to target cells to knockdown gene expression. Once inside the cell, double-stranded siRNAs can form complexes with a protein structure known as RNA-induced silencing complex. This complex can separate the two siRNA strands, reject the sense strand, and keep the antisense strand. The antisense strand can bind to a target mRNA based on a complementary match, and the RNA-induced silencing complex can cleave the target mRNA into smaller fragments, thereby preventing protein translation.

Gene Editing-Mediated Control of Gene Expression. Gene editing is another potential approach that can be used to enhance or suppress the expression of selected genes. A representative gene editing method is clustered regularly interspaced short palindromic repeat (CRISPR)/cas-based gene editing. CRISPRs are DNA sequences originally found in bacteria and archaea for acquired defensive immunity against viral and plasmid invasion [86]. These DNA sequences are about 23–47 base pairs each and are alternated with DNA sequences about 21–72 base pairs in length known as spacers [86]. The spacers are derived from the genome of viruses and plasmids that invade the host microorganisms and integrated into the host CRISPR repeats. The function of the spacers, together with CRISPR repeats, is to retain memory of the viruses or plasmids from previous infections and guide host nucleases to the genome of the same types of virus or plasmid for genomic destruction in new invasions [86,87]. CRISPR repeats are associated with cas genes that encode various types of enzyme, including nucleases, polymerases, and helicases [86]. A nuclease is an enzyme that cleaves nucleic acids at phosphodiester bonds, a polymerase catalyzes synthesis of nucleic acids, whereas a helicase is capable of modifying and unpackaging nucleic acids. CRISPRs and Cas enzymes together form CRISPR/Cas systems.

The action of the CRISPR/Cas system involves several steps [86]. An established CRISPR/spacer gene is first transcribed to a linear RNA, known as pre-CRISPR RNA (pre-crRNA). The pre-crRNA sequence is cleaved by Cas enzymes into short fragments known as CRISPR RNAs (crRNAs), each containing a spacer at the center and two short partial CRISPR fragments on the sides [86]. Cas9 is a representative enzyme capable of processing a pre-crRNA to crRNAs ([88,89]). A crRNA, working together with a Cas enzyme, can recognize a target viral or plasmid genome, which is subsequently cleaved by the associated Cas enzyme, a

potent mechanism for destroying and eliminating viruses and plasmids [86].

The mechanisms described above, although occur in bacteria and archaea, can be applied to mammalian cells to modify and edit target genes responsible for pathogenic events. An example is the application of the CRISPR/Cas9 system to mammalian cells [88,89]. This system consists of three basic elements—crRNA, trans-activating crRNA (trRNA), and Cas9 [89]. Among these elements, crRNA is able to recognize a target gene to be edited; trRNA is a sequence partially complementary to the crRNA required for Cas9 action [89] and is also involved in the formation of crRNAs from a pre-crRNA [88]; and Cas9 is an enzyme capable of causing DNA double-strand breaks, a process initiating DNA repair or replacement by homologous recombination [89]. Gene editing can be initiated by simply introducing crRNA, trRNA, and Cas9 into target cells to induce double-stranded DNA breaks on a target gene at sites recognized by the gene-specific crRNA [89]. In the presence of an exogenous gene, double-stranded DNA breaks activate homologous recombination mechanisms, resulting in replacement of the damaged target gene with the introduced exogenous gene [90,91]. In the absence of an exogenous gene, double-stranded DNA breaks cause nonhomologous DNA end joining to repair the damage. By using these mechanisms, a cardioprotective gene with enhanced expression capacity (induced by using a stronger gene promoter) can be introduced to a specified target locus on the genome to boost cardioprotective gene expression. A gene imposing an adverse impact on cardioprotection can be replaced by a modified gene with reduced expression capacity. Thus, CRISPR/Cas editing is a potentially effective method for cardioprotective engineering.

Cell-Based Cardioprotective Engineering. Cell-based cardioprotective engineering strategies can be designed and used to induce and promote regeneration of cardiomyocytes and other needed cell types such as endothelial and smooth muscle cells, and can also be used to deliver cardioprotective factors to the ischemic myocardium. A fundamental method of cell-based cardioprotective engineering is cell transplantation into the ischemic myocardium. Several cell types can be used for cardiac cell protection and regeneration—embryonic stem cells, induced pluripotent stem cells, cardiac resident stem cells, and somatic cells. For myocardial regeneration, embryonic, induced pluripotent, and resident stem cells are the candidates of choice. Somatic cardiomyocytes are not usually used because these cells are not able to survive the transplantation procedures and host environment. For myocardial protection, stem and somatic cells, such as bone marrow progenitor cells, skeletal muscle progenitor cells, and hepatic cells, can be engineered to overexpress selected cardioprotective gene(s) and used for cell transplantation. A fundamental method of cell transplantation is direct cell injection into the ischemic myocardium or the circulatory system, from which the injected cells can home to the ischemic myocardium. The effectiveness of cell-based engineering therapies has been demonstrated in previous investigations [92–94]. However, there are problems for cell-based protective and regenerative engineering. The transplanted stem and somatic cells often die at a considerably high rate in the heart within several days. A small fraction of the transplanted stem cells may differentiate into cardiomyocytes; however, the newly generated cardiomyocytes may not able to integrate into the native myocardium to exert synchronized contractile actions. These problems may be resolved when the mechanisms of stem and somatic cell survival and communication are fully understood.

Tissue-Level Cardioprotective Engineering. Tissue-level cardioprotective engineering strategies can be designed and used to assist in myocardial performance, protect the weakened ischemic myocardial wall from rupture, and deliver cardioprotective factors to the ischemic myocardium. To assist in myocardial performance, it is necessary to establish a myocardium-mimicking construct integrated with functional cardiomyocytes possessing synchronized contractile activities. The construct can be implanted onto the exterior surface of the ischemic myocardium to enhance the pumping function of the ischemic ventricle [95–98]. To protect the weakened ischemic myocardium from rupture, an extracellular matrix or synthetic polymer construct can be established and implanted onto the exterior surface of the ischemic myocardium to provide a mechanical support [95–98]. To deliver cardioprotective factors, an extracellular matrix or synthetic polymer construct can be integrated with cells engineered to express selected cardioprotective genes and implanted onto the exterior surface of the ischemic myocardium. This approach provides controlled and sustained release of cardioprotective proteins from the cells of the implant. However, problems discussed for cell-based protective engineering also exist for tissue-level protective engineering.

Concluding Remarks

Nature has developed various mechanisms for myocardial protection and regeneration in ischemic myocardial injury; however, not all mechanisms are optimized in promptness and effectiveness. Cardioprotective engineering is developed to correct natural deficiencies and optimize cardioprotective effectiveness by controlling cardioprotective actions. Various cardioprotective engineering strategies have been established at the molecular, cellular, and tissue levels and used in experimental and clinical investigations for protection against ischemic myocardial injury, but few of these strategies have exerted a significant clinical impact. There are two major challenges in cardioprotective engineering—understanding the innate cardioprotective mechanisms and developing protective engineering strategies for precise control of the types, levels, timing, and coordination of cardioprotective actions in ischemic myocardial injury. Understanding the innate cardioprotective mechanisms is the foundation for developing such strategies.

Funding Data

- National Science Foundation (Funder ID: 10.13039/501100008982).

References

[1] Liu, S. Q., Ma, X. L., Qin, G. J., Liu, Q. P., Li, Y. C., and Wu, Y. H., 2015, "Trans-System Mechanisms Against Ischemic Myocardial Injury," Comp. Physiol., **5**(1), pp. 167–192.

[2] Cannan, W. J., and Pederson, D. S., 2016, "Mechanisms and Consequences of Double-Strand DNA Break Formation in Chromatin," J. Cell Physiol., **231**(1), pp. 3–14.

[3] Jasin, M., and Rothstein, R., 2013, "Repair of Strand Breaks by Homologous Recombination," Cold Spring Harb. Perspect. Biol., **5**(11), p. a012740.

[4] Alvarado, A. S., and Yamanaka, S., 2014, "Rethinking Differentiation: Stem Cells, Regeneration, and Plasticity," Cell, **157**(1), pp. 110–119.

[5] Liu, S. Q., 2007, *Bioregenerative Engineering: Principles and Applications*, Wiley-Interscience, Hoboken, NJ, pp. 420–455.

[6] Tanaka, E., and Reddien, P. W., 2011, "The Cellular Basis for Animal Regeneration," Dev. Cell, **21**(1), pp. 172–185.

[7] Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., Li, Y., Wang, X., and Zhao, L., 2018, "Inflammatory Responses and Inflammation-Associated Diseases in Organs," Oncotarget, **9**(6), pp. 7204–7218.

[8] Rock, K. L., and Kono, H., 2008, "The Inflammatory Response to Cell Death," Annu. Rev. Pathol., **3**, pp. 99–126.

[9] Gardiner, D. M., 2018, *Regenerative Engineering and Developmental Biology: Principles and Applications* (CRC Press Series in Regenerative Engineering), 1st ed., CRC Press, Boca Raton, FL.

[10] Laurencin, C. T., and Khan, Y., 2018, *Regenerative Engineering*, 1st ed., CRC Press, Boca Raton, FL.

[11] Basson, M. A., 2012, "Signaling in Cell Differentiation and Morphogenesis," Cold Spring Harb. Perspect. Biol., **4**(6), p. a008151.

[12] Duronio, R. J., and Xiong, Y., 2013, "Signaling Pathways That Control Cell Proliferation," Cold Spring Harb Perspect. Biol., **5**(3), p. a008904.

[13] Birnbaum, Y., Hale, S. L., and Kloner, R. A., 1997, "Ischemic Preconditioning at a Distance: Reduction of Myocardial Infarct Size by Partial Reduction of

Blood Supply Combined With Rapid Stimulation of the Gastrocnemius Muscle in the Rabbit," Circulation, **96**(5), pp. 1641–1646.
[14] Kloner, R. A., and Rezkalla, S. H., 2006, "Preconditioning, Postconditioning and Their Application to Clinical Cardiology," Cardiovasc. Res., **70**(2), pp. 297–307.
[15] Murry, C. E., Jennings, R. B., and Reimer, K. A., 1986, "Preconditioning With Ischemia: A Delay of Lethal Cell Injury in Ischemic Myocardium," Circulation, **74**(5), pp. 1124–1136.
[16] Granfeldt, A., Lefer, D. J., and Vinten-Johansen, J., 2009, "Protective Ischaemia in Patients: Preconditioning and Postconditioning," Cardiovasc. Res., **83**(2), pp. 234–246.
[17] Hausenloy, D. J., and Yellon, D. M., 2011, "The Therapeutic Potential of Ischemic Conditioning: An Update," Nat. Rev. Cardiol., **8**(11), pp. 619–629.
[18] Schwarz, E. R., Whyte, W. S., and Kloner, R. A., 1997, "Ischemic Preconditioning," Cur. Opin. Cardiol., **12**(5), pp. 475–481.
[19] Vinten-Johansen, J., Zhao, Z., Jiang, R., Zatta, A. J., and Dobson, G. P., 2007, "Preconditioning and Postconditioning: Innate Cardioprotection From Ischemia Reperfusion Injury," J. Appl. Physiol., **103**(4), pp. 1441–1448.
[20] Yellon, D. M., and Downey, J. M., 2003, "Preconditioning the Myocardium: From Cellular Physiology to Clinical Cardiology," Physiol. Rev., **83**(4), pp. 1113–1151.
[21] Banai, S., Shweiki, D., Pinson, A., Chandra, M., Lazarovici, G., and Keshet, E., 1994, "Upregulation of Vascular Endothelial Growth Factor Expression Induced by Myocardial Ischaemia: Implications for Coronary Angiogenesis," Cardiovasc. Res., **28**(8), pp. 1176–1179.
[22] Hashimoto, E., Ogita, T., Nakaoka, T., Matsuoka, R., Takao, A., and Kira, Y., 1994, "Rapid Induction of Vascular Endothelial Growth Factor Expression by Transient Ischemia in Rat Heart," Am. J. Physiol., **267**(5 Pt. 2), pp. H1948–H1954.
[23] Kawata, H., Yoshida, K.-I., Kawamoto, A., Kurioka, H., Takase, E., Sasaki, Y., Hatanaka, K., Kobayashi, M., Ueyama, T., Hashimoto, T., and Dohi, K., 2001, "Ischemic Preconditioning Upregulates Vascular Endothelial Growth Factor mRNA Expression and Neovascularization Via Nuclear Translocation of Protein Kinase C in the Rat Ischemic Myocardium," Circ. Res., **88**(7), pp. 696–704.
[24] Bell, S. P., Sack, M. N., Patel, A., Opie, L. H., and Yellon, D. M., 2000, "Opioid Receptor Stimulation Mimics Ischemic Preconditioning in Human Heart Muscle," J. Am. Coll. Cardiol., **36**(7), pp. 2296–2302.
[25] Bell, R. M., and Yellon, D. M., 2003, "Bradykinin Limits Infarction When Administered as an Adjunct to Reperfusion in Mouse Heart: The Role of P18K, Akt and eNOS," J. Mol. Cell Cardiol., **35**(2), pp. 185–193.
[26] Fryer, R. M., Hsu, A. K., and Gross, G. J., 2001, "ERK and p38 MAP Kinase Activation Are Components of Opioid-Induced Delayed Cardioprotection," Basic Res. Cardiol., **96**(2), pp. 136–142.
[27] Goto, M., Liu, Y., Yang, X. M., Ardell, J. L., Cohen, M. V., and Downey, J. M., 1995, "Role of Bradykinin in Protection of Ischemic Preconditioning in Rabbit Hearts," Circ. Res., **77**(3), pp. 611–621.
[28] Liu, G. S., Thornton, J., Van Winkle, D. M., Stanley, A. W., Olsson, R. A., and Downey, J. M., 1991, "Protection Against Infarction Afforded by Preconditioning Is Mediated by A1 Adenosine Receptors in Rabbit Heart," Circulation, **84**(1), pp. 350–356.
[29] Thornton, J. D., Liu, G. S., Olsson, R. A., and Downey, J. M., 1992, "Intravenous Pretreatment With A1-Selective Adenosine Analogues Protects the Heart Against Infarction," Circulation, **85**(2), pp. 659–665.
[30] Vinten-Johansen, J., and Shi, W., 2011, "Preconditioning and Postconditioning: Current Knowledge, Knowledge Gaps, Barriers to Adoption, and Future Directions," J. Cardiovasc. Pharmacol. Ther., **16**(3–4), pp. 260–266.
[31] Ytrehus, K., Liu, Y., and Downey, J. M., 1994, "Preconditioning Protects Ischemic Rabbit Heart by Protein Kinase C Activation," Am. J. Physiol., **266**(3 Pt. 2), pp. H1145–H1152.
[32] Liu, S. Q., Tefft, B. J., Roberts, D. T., Zhang, L.-Q., Ren, Y., Li, Y. C., Huang, Y., Zhang, D., Phillips, H. R., and Wu, Y. H., 2012, "Cardioprotective Proteins Upregulated in the Liver in Response to Experimental Myocardial Ischemia," Am. J. Physiol. Heart Circ. Physiol., **303**(12), pp. H1446–1458.
[33] Liu, S. Q., Tefft, B. J., Zhang, D., Roberts, D., Schuster, D. J., and Wu, A., 2011, "Cardioprotective Mechanisms Activated in Response to Myocardial Ischemia," MCB, **8**(4), pp. 319–338.
[34] Leevers, S. J., Vanhaesebroeck, B., and Waterfield, M. D., 1999, "Signalling Through Phosphoinositide 3-Kinases: The Lipids Take Centre Stage," Cur. Opin. Cell Biol., **11**(2), pp. 219–225.
[35] Datta, S. P., Brunet, A., and Greenberg, M. E., 1999, "Cellular Survival: A Play in Three Akts," Genes Dev., **13**(22), pp. 2905–2927.
[36] Torella, D., Ellison, G. M., Karakikes, I., and Nadal-Ginard, B., 2007, "Growth Factor Mediated Cardiac Stem Cell Activation in Myocardial Regeneration," Nat. Clin. Pract. Cardiovasc. Med., **4**(S1), pp. S46–S51.
[37] Liu, S. Q., Roberts, D., Kharitonenkov, A., Li, Y. C., Zhang, L.-Q., and Wu, Y. H., 2013, "Cardioprotective Action of Fibroblast Growth Factor 21 Upregulated and Released From the Liver and Adipose Tissue in Experimental Myocardial Ischemia," Sci. Rep., **3**, p. 2767.
[38] Fournier, T., Medjoubi-N, N., and Porquet, D., 2000, "Alpha-1-Acid Glycoprotein," Biochim. Biophys. Acta, **1482**(1–2), pp. 157–171.
[39] Muchitsch, E. M., Teschner, W., Linnau, Y., and Pichler, L., 1996, "In Vivo Effect of α1-Acid Glycoprotein on Experimentally Enhanced Capillary Permeability in Guinea-Pigskin," Arch. Int. Pharmacodyn. Ther., **331**(3), pp. 313–321.
[40] Liu, H. M., Takagaki, K., and Schmid, K., 1988, "In Vitro Nerve-Growth-Promoting Activity of Human Plasma α1-Acid Glycoprotein," J. Neurosci. Res., **20**(1), pp. 64–72.
[41] Heinke, J., Wehofsits, L., Zhou, Q., Zoeller, C., Baar, K.-M., Helbing, T., Laib, A., Augustin, H., Bode, C., Patterson, C., and Moser, M., 2008, "BMPER Is an Endothelial Cell Regulator and Controls Bone Morphogenetic Protein-4–Dependent Angiogenesis," Circ. Res., **103**(8), pp. 804–812.
[42] Ikeya, M., Kawada, M., Kiyonari, H., Sasai, N., Nakao, K., Furuta, Y., and Sasai, Y., 2006, "Essential Pro-Bmp Roles of Crossveinless 2 in Mouse Organogenesis," Development, **133**(22), pp. 4463–4473.
[43] Kelley, R., Ren, R., Pi, X., Wu, Y., Moreno, I., Willis, M., Moser, M., Ross, M., Podkowa, M., Attisano, L., and Patterson, C., 2009, "A Concentration-Dependent Endocytic Trap and Sink Mechanism Converts Bmper From an Activator to an Inhibitor of Bmp Signaling," J. Cell Biol., **184**(4), pp. 597–560.
[44] Kharitonenkov, A., Shiyanova, T. L., Koester, A., Ford, A. M., Micanovic, R., Galbreath, E. J., Sandusky, G. E., Hammond, L. J., Moyers, J. S., Owens, R. A., Gromada, J., Brozinick, J. T., Hawkins, E. D., Wroblewski, V. J., Li, D. S., Mehrbod, F., Jaskunas, S. R., and Shanafelt, A. B., 2005, "FGF-21 as a Novel Metabolic Regulator," J. Clin. Invest., **115**(6), pp. 1627–1635.
[45] Nishimura, T., Nakatake, Y., Konishi, M., and Itoh, N., 2000, "Identification of a Novel FGF, FGF-21, Preferentially Expressed in the Liver," Biochim. Biophys. Acta., **1492**(1), pp. 203–206.
[46] Ryde'n, M., 2009, "Fibroblast Growth Factor 21: An Overview From a Clinical Perspective," Cell Mol. Life Sci., **66**(13), pp. 2067–2073.
[47] Wente, W., Efanov, A. M., Brenner, M., Kharitonenkov, A., Köster, A., Sandusky, G. E., Sewing, S., Treinies, I., Zitzer, H., and Gromada, J., 2006, "Fibroblast Growth Factor-21 Improves Pancreatic β-Cell Function and Survival by Activation of Extracellular Signal-Regulated Kinase 1/2 and Akt Signaling Pathways," Diabetes, **55**(9), pp. 2470–2478.
[48] Dostalova, I., Haluzikova, D., and Haluzik, M., 2009, "Fibroblast Growth Factor 21: A Novel Metabolic Regulator With Potential Therapeutic Properties in Obesity/Type 2 Diabetes Mellitus," Physiol. Res., **58**(1), pp. 1–7.
[49] Kharitonenkov, A., 2009, "FGFs and Metabolism," Curr. Opin. Pharmacol., **9**(6), pp. 805–810.
[50] Kharitonenkov, A., and Larsen, P., 2011, "FGF21 Reloaded. Challenges of a Rapidly Growing Field," Trends Enocrinol. Metab., **22**(3), pp. 81–86.
[51] Badman, M. K., Pissios, P., Kennedy, A. R., Koukos, G., Flier, J. S., and Maratos-Flier, E., 2007, "Hepatic Fibroblast Growth Factor 21 is Regulated by PPARa and is a Key Mediator of Hepatic Lipid Metabolism in Ketotic States," Cell Metab., **5**(6), pp. 426–437.
[52] Hotta, Y., Nakamura, H., Konishi, M., Murata, Y., Takagi, H., Matsumura, S., Inoue, K., Fushiki, T., and Itoh, N., 2009, "Fibroblast Growth Factor 21 Regulates Lipolysis in White Adipose Tissue But is Not Required for Ketogenesis and Triglyceride Clearance in Liver," Endocrinology, **150**(10), pp. 4625–4633.
[53] Inagaki, T., Dutchak, P., Zhao, G., Ding, X., Gautron, L., Parameswara, V., Li, Y., Goetz, R., Mohammadi, M., Esser, V., Elmquist, J. K., Gerard, R. D., Burgess, S. C., Hammer, R. E., Mangelsdorf, D. J., and Kliewer, S. A., 2007, "Endocrine Regulation of the Fasting Response by PPARa-Mediated Induction of Fibroblast Growth Factor 21," Cell Metab., **5**(6), pp. 415–425.
[54] Falls, D. L., 2003, "Neuregulins: Functions, Forms, and Signaling Strategies," Exp. Cell Res., **284**(1), pp. 14–30.
[55] Hayes, N. V. L., Newsam, R. J., Baines, A. J., and Gullick, W. J., 2008, "Characterization of the Cell Membrane-Associated Products of the Neuregulin4 Gene," Oncogene, **27**(5), pp. 715–720.
[56] Harari, D., Tzahar, E., Romano, J., Shelly, M., Pierce, J. H., Andrews, G. C., and Yarden, Y., 1999, "Neuregulin-4: A Novel Growth Factor That Acts Through the ErbB-4 Receptor Tyrosine Kinase," Oncogene, **18**(17), pp. 2681–2689.
[57] Chinery, R., Poulsom, R., and Cox, H. M., 1996, "The Gene Encoding Mouse Intestinal Trefoil Factor: Structural Organization, Partial Sequence Analysis and Mapping to Murine Chromosome17q," Gene, **171**(2), pp. 249–253.
[58] Sands, B. E., and Podolsky, D. K., 1996, "The Trefoil Peptide Family," Annu. Rev. Physiol., **58**, pp. 253–273.
[59] Babyatsky, M. W., de Beaumont, M., Thim, L., and Podolsky, D. K., 1996, "Oral Trefoil Peptides Protect Against Ethanol-and Indomethacin-Induced Gastric Injury in Rats," Gastroenterology, **110**(2), pp. 489–497.
[60] Fazel, S. S., Chen, L., Angoulvant, D., Li, S. H., Weisel, R. D., Keating, A., and Li, R. K., 2008, "Activation of c-Kit is Necessary for Mobilization of Reparative Bone Marrow Progenitor Cells in Response to Cardiac Injury," FASEB J., **22**(3), pp. 930–940.
[61] Kucia, M., Dawn, B., Hunt, G., Guo, Y., Wysoczynski, M., Majka, M., Ratajczak, J., Rezzoug, F., Ildstad, S. T., Bolli, R., and Ratajczak, M. Z., 2004, "Cells Expressing Early Cardiac Markers Reside in the Bone Marrow and Are Mobilized Into the Peripheral Blood After Myocardial Infarction," Circ. Res., **95**(12), pp. 1191–1199.
[62] Ripa, R. S., Jørgensen, E., Wang, Y., Thune, J. J., Nilsson, J. C., Søndergaard, L., Johnsen, H. E., Køber, L., Grande, P., and Kastrup, J., 2006, "Stem Cell Mobilization Induced by Subcutaneous Granulocyte-Colony Stimulating Factor to Improve Cardiac Regeneration After Acute ST-Elevation Myocardial Infarction: Result of the Double-Blind, Randomized, Placebo-Controlled in Myocardial Infarction (STEMMI) Trial," Circulation, **113**(16), pp. 1983–1992.
[63] Swirski, F. K., Nahrendorf, M., Etzrodt, M., Wildgruber, M., Cortez-Retamozo, V., Panizzi, P., Figueiredo, J. L., Kohler, R. H., Chudnovskiy, A., Waterman, P., Aikawa, E., Mempel, T. R., Libby, P., Weissleder, R., and Pittet, M. J., 2009, "Identification of Splenic Reservoir Monocytes and Their Deployment to Inflammatory Sites," Science, **325**(5940), pp. 612–616.
[64] Liu, S. Q., Tefft, B. J., Liu, C., Zhang, B., and Wu, Y. H., 2011, "Regulation of Hepatic Cell Mobilization in Experimental Myocardial Ischemia," Cell Mol. Bioeng., **4**(4), pp. 693–707.

[65] Shintani, S., Murohara, T., Ikeda, H., Ueno, T., Honma, T., Katoh, A., Sasaki, K., Shimada, T., Oike, Y., and Imaizumi, T., 2001, "Mobilization of Endothelial Progenitor Cells in Patients With Acute Myocardial Infarction," Circulation, **103**(23), pp. 2776–2779.
[66] Engelmann, M. G., Theiss, H. D., Hennig-Theiss, C., Huber, A., Wintersperger, B. J., Werle-Ruedinger, A. E., Schoenberg, S. O., Steinbeck, G., and Franz, W. M., 2006, "Autologous Bone Marrow Stem Cell Mobilization Induced by Granulocyte Colony-Stimulating Factor After Subacute ST-Segment Elevation Myocardial Infarction Undergoing Late Revascularization: Final Results From the G-CSF-STEMI (Granulocyte Colony-Stimulating Factor ST-Elevation Myocardial Infarction) Trial," J. Am. Coll. Cardiol., **48**(8), pp. 1712–1721.
[67] Lombaert, I. M., Wierenga, P. K., Kok, T., Kampinga, H. H., de Haan, G., and Coppes, R. P., 2006, "Mobilization of Bone Marrow Stem Cells by Granulocyte Colony-Stimulating Factor Ameliorates Radiation-Induced Damage to Salivary Glands," Clin. Cancer Res., **12**(6), pp. 1804–1812.
[68] Askari, A. T., Unzek, S., Popovic, Z. B., Goldman, C. K., Forudi, F., Kiedrowski, M., Rovner, A., Ellis, S. G., Thomas, J. D., DiCorleto, P. E., Topol, E. J., and Penn, M. S., 2003, "Effect of Stromal-Cell-Derived Factor 1 on Stem-Cell Homing and Tissue Regeneration in Ischaemic Cardiomyopathy," Lancet, **362**(9385), pp. 697–703.
[69] Hattori, K., Heissig, B., Tashiro, K., Honjo, T., Tateno, M., Shieh, J. H., Hackett, N. R., Quitoriano, M. S., Crystal, R. G., Rafii, S., and Moore, M. A., 2001, "Plasma Elevation of Stromal Cell-Derived Factor-1 Induces Mobilization of Mature and Immature Hematopoietic Progenitor and Stem Cells," Blood, **97**(11), pp. 3354–3360.
[70] Asahara, T., Takahashi, T., Masuda, H., Kalka, C., Chen, D., Iwaguro, H., Inai, Y., Silver, M., and Isner, J. M., 1999, "VEGF Contributes to Postnatal Neovascularization by Mobilizing Bone Marrow-Derived Endothelial Progenitor Cells," EMBO J., **18**(14), pp. 3964–3972.
[71] Dunbar, C. E., High, K. A., Joung, J. K., Kohn, D. B., Ozawa, K., and Sadelain, M., 2018, "Gene Therapy Comes of Age," Science, **359**(6372), p. 175.
[72] Folino, A., Accomasso, L., Giachino, C., Montarolo, P. G., Losano, G., Pagliaro, P., and Rastaldo, R., 2018, "Apelin-Induced Cardioprotection Against Ischaemia/Reperfusion Injury: Roles of Epidermal Growth Factor and Src," Acta Physiol., **222**(2), p. 12924.
[73] House, S. L., Branch, K., Newman, G., Doetschman, T., and Schultz, J. J., 2005, "Cardioprotection Induced by Cardiac-Specific Overexpression of Fibroblast Growth Factor-2 Is Mediated by the MAPK Cascade," Am. J. Physiol. Heart. Circ. Physiol., **289**(5), pp. H2167–H2175.
[74] Messadi, E., Aloui, Z., Belaidi, E., Vincent, M. P., Couture-Lepetit, E., Waeckel, L., Decorps, J., Bouby, N., Gasmi, A., Karoui, H., Ovize, M., Alhenc-Gelas, F., and Richer, C., 2014, "Cardioprotective Effect of VEGF and Venom VEGF-Like Protein in Acute Myocardial Ischemia in Mice: Effect on Mitochondrial Function," J. Cardiovasc. Pharmacol., **63**(3), pp. 274–281.
[75] Nakamura, T., Mizuno, S., Matsumoto, K., Sawa, Y., Matsuda, H., and Nakamura, T., 2000, "Myocardial Protection From Ischemia/Reperfusion Injury by Endogenous and Exogenous HGF," J. Clin. Invest., **106**(12), pp. 1511–1519.
[76] Brar, B. K., Stephanou, A., Liao, Z., O'Leary, R. M., Pennica, D., Yellon, D. M., and Latchman, D. S., 2001, "Cardiotrophin-1 Can Protect Cardiac Myocytes From Injury When Added Both Prior to Simulated Ischaemia and at Reoxygenation," Cardiovasc. Res., **51**(2), pp. 265–274.
[77] Ghosh, S., Ng, L. L., Talwar, S., Squire, I. B., and Galiñanes, M., 2000, "Cardiotrophin-1 Protects the Human Myocardium From Ischemic Injury: Comparison With the First and Second Window of Protection by Ischemic Preconditioning," Cardiovasc. Res., **48**(3), pp. 440–447.
[78] Huang, C., Gu, H., Zhang, W., Manukyan, M. C., Shou, W., and Wang, M., 2011, "SDF-1/CXCR4 Mediates Acute Protection of Cardiac Function Through Myocardial STAT3 Signaling Following Global Ischemia/Reperfusion Injury," Am. J. Physiol. Heart Circ. Physiol., **301**(4), pp. H1496–H1505.
[79] Katz, M. G., Swain, J. D., Tomasulo, C. E., Sumaroka, M., Fargnoli, A., and Bridges, C. R., 2011, "Current Strategies for Myocardial Gene Delivery," J. Mol. Cell Cardiol., **50**(5), pp. 766–776.
[80] Watts, J. K., and Corey, D. R., 2012, "Gene Silencing by siRNAs and Antisense Oligonucleotides in the Laboratory and the Clinic," J. Pathol., **226**(2), pp. 365–379.
[81] Xia, H., Mao, Q., Paulson, H. L., and Davidson, B. L., 2002, "siRNA-Mediated Gene Silencing In Vitro and In Vivo," Nat. Biotech., **20**(10), pp. 1006–1010.
[82] Napoli, C., Lemieux, C., and Jorgensen, R., 1990, "Introduction of a Chimeric Chalcone Synthase Gene Into Petunia Results in Reversible co-Suppression of Homologous Genes in Trans," Plant Cell, **2**(4), pp. 279–289.
[83] van der Krol, A. R., Mur, L. A., Beld, M., Moi, J. N. M., and Stuitje, A. R., 1990, "Flavonoid Genes in Petunia: Addition of a Limited Number of Gene Copies May Lead to a Suppression of Gene Expression," Plant Cell, **2**(4), pp. 291–299.
[84] Fire, A., Xu, S., Montgomery, M. K., Kostas, S. A., Driver, S. E., and Mello, C. C., 1998, "Potent and Specific Genetic Interference by Double-Stranded RNA in Caenorhabditis elegans," Nature, **391**(6669), pp. 806–811.
[85] Novina, C. D., and Sharp, P. A., 2004, "The RNAi Revolution," Nature, **430**(6996), pp. 161–164.
[86] Horvath, P., and Barrangou, R., 2010, "CRISPR/Cas, the Immune System of Bacteria and Archaea," Science, **327**(5962), pp. 167–170.
[87] Marraffini, L. A., and Sontheimer, E. J., 2010, "CRISPR Interference: RNA-Directed Adaptive Immunity in Bacteria and Archaea," Nat. Rev. Genet., **11**(3), pp. 181–190.
[88] Deltcheva, E., Chylinski, K., Sharma, C. M., Gonzales, K., Chao, Y., Pirzada, Z. A., Eckert, M. R., Vogel, J., and Charpentier, E., 2011, "CRISPR RNA Maturation by Trans-Encoded Small RNA and Host Factor RNase III," Nature, **471**(7340), pp. 602–607.
[89] Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., and Charpentier, E., 2012, "A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity," Science, **337**(6096), pp. 816–821.
[90] Gong, C., Bongiorno, P., Martins, A., Stephanou, N. C., Zhu, H., Shuman, S., and Glickman, M. S., 2005, "Mechanism of Nonhomologous End-Joining in Mycobacteria: A Low-Fidelity Repair System Driven by Ku, Ligase D and Ligase C," Nat. Struct. Mol. Biol., **12**(4), pp. 304–312.
[91] Overballe-Petersen, S., Harms, K., Orlando, L. A., Mayar, J. V., Rasmussen, S., Dahl, T. W., Rosing, M. T., Poole, A. M., Sicheritz-Ponten, T., Brunak, S., Inselmann, S., de Vries, J., Wackernagel, W., Pybus, O. G., Nielsen, R., Johnsen, P. J., Nielsen, K. M., and Willerslev, E., 2013, "Bacterial Natural Transformation by Highly Fragmented and Damaged DNA," PNAS, **110**(49), pp. 19860–19865.
[92] Abdel-Latif, A., Bolli, R., Tleyjeh, I. M., Montori, V. M., Perin, E. C., Hornung, C. A., Zuba-Surma, E. K., Al-Mallah, M., and Dawn, B., 2007, "Adult Bone Marrow-Derived Cells for Cardiac Repair: A Systematic Review and Meta-Analysis," Arch. Intern. Med., **167**(10), pp. 989–997.
[93] Carvalho, E., Verma, P., Hourigan, K., and Banerjee, R., 2015, "Myocardial Infarction: Stem Cell Transplantation for Cardiac Regeneration," Regen. Med., **10**(8), pp. 1025–1043.
[94] Martin-Rendon, E., Brunskill, S. J., Hyde, C. J., Stanworth, S. J., Mathur, A., and Watt, S. M., 2008, "Autologous Bone Marrow Stem Cells to Treat Acute Myocardial Infarction: A Systematic Review," Eur. Heart J., **29**(15), pp. 1807–1818.
[95] Domenech, M., Polo-Corrales, L., Ramirez-Vick, J. E., and Freytes, D. O., 2016, "Tissue Engineering Strategies for Myocardial Regeneration: Acellular Versus Cellular Scaffolds?" Tissue Eng., Part B, **22**(6), pp. 438–458.
[96] Kaiser, N. J., and Coulombe, K. L. K., 2015, "Physiologically Inspired Cardiac Scaffolds for Tailored In Vivo Function and Heart Regeneration," Biomed. Mater., **10**(3), p. 034003.
[97] Mannhardt, I., Breckwoldt, K., Letuffe-Brenière, D., Schaaf, S., Schulz, H., Neuber, C., Benzin, A., Werner, T., Eder, A., Schulze, T., Klampe, B., Christ, T., Hirt, M. N., Huebner, N., Moretti, A., Eschenhagen, T., and Hansen, A., 2016, "Human Engineered Heart Tissue: Analysis of Contractile Force," Stem Cell Rep., **7**(1), pp. 29–42.
[98] Wang, Z., Lee, S. J., Cheng, H.-J., Yoo, J. J., and Atala, A., 2018, "3D Bioprinted Functional and Contractile Cardiac Tissue Constructs," Acta Biomater., **70**, pp. 48–56.