Nanotechnology-Based Drug Delivery/Treatment Methods for Ischemic Heart Diseases

Jason Brant

Mentor: Dr. Amy Navratil

Department of Zoology and Physiology

Honors Senior Capstone Project

Fall 2017

Abstract

Ischemic heart conditions are some of the most prevalent disorders in the United States. These diseases also cause a significant number of mortalities each year. Despite considerable advances in treatment of the ischemic and infarct heart, many patients still develop complications and often progress to heart failure. These outcomes are typically related to factors such as the inability to deliver medication to the affected area, lack of successful stem cell implantation, improper tissue regeneration, and additional damage caused after reperfusion to ischemic tissues. The use of nanotechnology in the treatment of the ischemic and infarct heart has shown promising results for therapeutic improvement. This review focuses on several nanotechnology-based treatments for ischemic heart diseases including simple and modified drug/stem cell delivery systems ranging from magnetic, antibody-linked, and mesoporous silica nanoparticles. Additional nanotechnology systems such as nanoscaffolds and graphene compounds assist the repair of damaged cardiac tissue, therefore, increasing treatment efficacy. Based on these technologies, future models such as multi-functional nanoparticles should be considered for further advancement in this area.

Abbreviations: CAD, coronary artery disease; ECM, extracellular matrix; GO, graphene oxide; IHD, ischemic heart disease; IR, ischemic reperfusion; MI, myocardial infarction; MSC, mesenchymal stem cell; nano-DDS, nanoparticle-mediated drug delivery system; PEG, polyethylene glycol; ROS, reactive oxygen species.

1. Introduction

Ischemic heart disease (IHD), also known as coronary artery disease (CAD), is a form of cardiovascular disease that can cause several complications including angina (chest pain),

myocardial infarction (MI), and cardiac death (Bhatia, 2010). Ischemia refers to insufficient oxygen supply to organ tissues. Atherosclerosis—a common cause of CAD—can result in the occlusion of coronary arteries—vessels that deliver blood and oxygen to heart tissue (Figure 1). This occlusion is frequently caused by lipid deposits that have hardened into plaque. If this plaque continues to develop, nearby tissues can become ischemic and die.

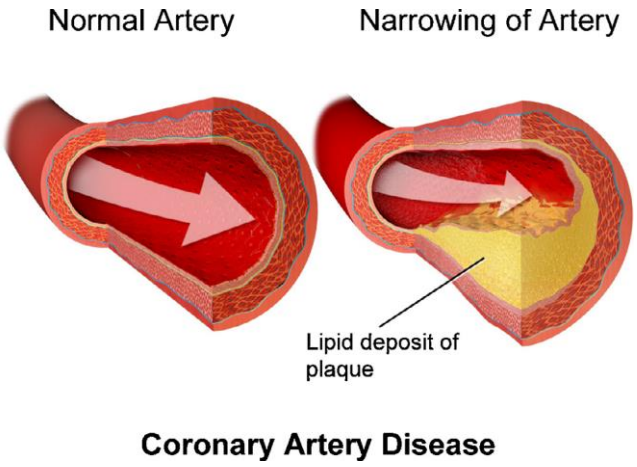

FIG. 1. An illustration of lipid accumulation in an artery. This accretion causes occlusion, which restricts blood flow to nearby tissues—leading to tissue hypoxia (Ambesh et al., 2017).

Myocardial ischemia disrupts cardiac contractile function resulting in symptoms including fatigue and angina before possibly leading to MI and cardiac cell death (Roth, 2011; Ventrelli et al., 2013). Risk factors for IHD include sedentary lifestyle, diabetes, genetic predisposition, tobacco use, unhealthy diet (composed of high salt, fat, and calories), excessive alcohol consumption, obesity, high cholesterol/blood lipids, and high blood pressure (Mendis et al., 2011).

"IHD was the leading cause of death worldwide in 2010" (Moran et al., 2014). This substantial death toll is most likely related to the characteristic features of IHD. Diseases caused by IHD such as MI, produce significant morbidity, mortality, and extensive pathologies with an overall mortality of 62.5% (Velazquez et al., 2016; Ventrelli et al., 2013). MI, being one of the

more common diseases of IHD, is responsible for every 1 out of 6 deaths in the United States (Nguyen et al., 2015). Approximately 525,000 people are predicted to develop a new MI each year and in 2013, around 15.4 million individuals were living with CAD (Ford et al., 2014). With IHD being such a prevalent concern in the United States, researchers are looking into treatment approaches with high efficacy.

Even though there have been advances in IHD treatment, some therapies can cause additional harm and many patients still progress into cardiac hypertrophy and heart failure (Evans et al., 2016). This progression could be related to the lack of adequate drug delivery to damaged tissue within a critical amount of time. Furthermore, it has been identified that surgery to repair injured cardiac tissue can be either beneficial—if there are no complications from the procedure—or damaging to the patient. For instance, coronary artery bypass surgery or open-heart surgery can result in severe central nervous system disturbances (Begum & Sharma, 2016). Nanotechnology offers considerable improvement in IHD therapy including accurate drug delivery to select tissues and successful stem cell replacement treatment for tissues damaged from MI. The objective of this research is to identify how nanotechnology can be used to improve treatment for IHD and MI through factors including targeted medication/hormone delivery and enhanced stem cell implantation.

2. Nanoparticle Drug Delivery Vectors

Drugs such as statin and erythropoietin analogs have been used to help treat or prevent acute myocardial infarction (AMI). In more serious cases, blood flow needs to be restored to certain areas of the heart. To assist ischemic reperfusion (IR) in these advanced cases, drugs such as cyclosporine are used (Matoba & Egashira, 2014). However, side-effects from too rapid

reperfusion such as the generation of reactive oxygen species (ROS) can cause unideal clinical outcomes (Evans et al., 2016). This can be related to the window of drug delivery time to the disrupted tissue. There are various nanoparticle vectors that can be used to effectively deliver medications within a critical amount of time.

Nanoparticle-mediated drug delivery systems (nano-DDSs) have been shown to have effective drug targeting through utilizing specific pathophysiological and physiological characteristics particular to specific cells or tissues (Markman et al., 2013). Nano-DDSs (ranging from 0.5-300 nm in diameter) include a wide range of vectors including micelles, liposomes, nanospheres, polymers, dendrimers, carbon nanotubes, and metallic nanoparticles (Matoba & Egashira, 2014). As seen in Figure 2, micelles and liposomes are amphipathic molecules with a single and double lipid barrier respectively, which creates a hydrophobic and hydrophilic phase. This amphipathic property allows efficient cellular uptake and the transport of both hydrophobic and hydrophilic therapeutic agents. Polymeric nanospheres can carry both hydrophilic and hydrophobic agents as well and are formed through macromolecular assembly. Dendrimers are extensively branched macromolecules synthesized through polymerization reactions emanating from a central core. This three-dimensional structure of the dendrimer allows the integration of therapeutic agents within its framework. Carbon nanotubes are pure covalently bonded carbon graphite sheets assembled in a tubular form. This structure creates a hollow inner core that allows therapeutic agents to be placed inside for transportation. Metallic nanoparticles, commonly composed of crystalline metals such as iron oxide or gold nanoparticles, have been found to have therapeutic properties through their ability to transport select agents and imaging characteristics through their external magnet responsiveness and labeling qualities. For example, gold nanoparticles can deliver drugs to select tissues after being conjugated with therapeutic

agents and targeting moieties. These particles can also simultaneously function as an imaging method by having photodynamic properties that allow the absorption of near-infrared light (Matoba & Egashira, 2014).

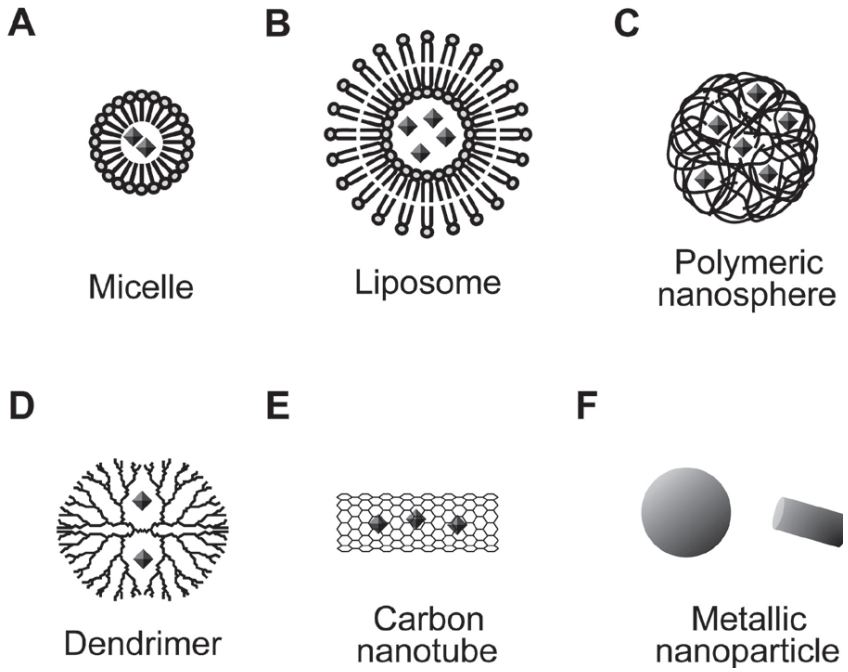

FIG. 2. An illustration of six commonly used nano-DDSs (Matoba & Egashira, 2014).

"Liposomes are the most extensively tested nano-DDSs in basic and clinical medicine with United States Food and Drug Administration approval" (Matoba & Egashira, 2014). This could be related to the unique properties that allow this nano-DDS to have noteworthy efficacy. After IR, liposome nano-DDSs are able to extravasate from the vasculature and into organs with increased permeability, which allows enhanced accumulation in the tissue (Takahama et al., 2009). Additional modification of liposomes can also affect the efficacy of this form of treatment. A study has shown that the addition of polyethylene glycol (PEG) onto the surface of liposomes both decreased cardiotoxicity and bloodstream clearance (Allen, 1994). However, liposomes should be used selectively for the intended package because data shows that

liposomes have restrictions in stability, versatility, and traceability when used for miRNA delivery (Gomes et al., 2013).

The use of basic or modified nano-DDSs can be either passive- or active-targeting based. In passive-targeting, the nano-DDS is injected intravenously or near the target tissue. This type of nano-DDS is incorporated into the cell through either specific permeability features of the injured tissue or other non-specific mechanisms. In active-targeting, a distinct molecule or targeting structure is bound to the nano-DDS such as a receptor, protein, adhesion molecule, or antibody, which allows more efficient and direct targeting to a specific location. Active-targeting nano-DDSs are particularly promising for MI since this form of treatment enhances drug delivery and myocardial IR. According to a cohort study, even though door-to-balloon time has decreased considerably, there has been no improvement in the overall mortality of MI patients (Holmes et al., 2013). This could be due to insufficient drug delivery to injured cardiomyocytes within a necessary amount of time. Since active-targeting nano-DDSs show potential for decreasing the amount of time between drug administration and treatment, mortality rates may be reduced if this form of therapy was used.

2.1 Nanoparticle Delivery of Hormones

Insulin-like growth factor-1 (IGF-1) has been identified to foster cardiac development, stimulate cardiomyocyte growth, augment cardiomyocyte survival, and enhance cardiac function *in vitro* and *in vivo* for both human and experimental models (Chang et al., 2013). However, prolonged IGF-1 overexpression in cardiomyocytes decreases cardiac function after MI (Prêle et al., 2012). These results illuminate the need for both successful delivery of IGF-1 to injured myocardium and controlled release of IGF-1. A recent study using a mouse model illustrated

how injecting polyD,L-lactide-co-glycolide (PLGA) nanoparticle copolymer loaded with IGF-1 into injured myocardium resulted in a decrease in both cellular apoptosis and infarction size, an increase in left ventricle ejection fraction, and more sustained retention and activity of IGF-1 (Chang et al., 2013). The researchers of this study identified how the PLGA nanoparticles were degraded in a slow manner, which facilitated a controlled release of IGF-1. This slow release promoted sustained treatment and prevented high levels of IGF-1 from remaining at the injection site for a prolonged period of time.

Another study by Kempen et al. used mesoporous silica nanoparticles loaded with IGF-1 to provide therapy to infarct tissues (2015). This type of nanoparticle was biodegradable from the inside out, which served as a slow release function for IGF-1. The controlled release properties of this nanoparticle allowed IGF-1 to function over an extended period of time resulting in an increase in cell survival. Ruiz-Esparza et al. have shown further support for the use of mesoporous silicon nanoparticles (2016). These researchers showed that mesoporous silicon vectors can be endocytosed and internally trafficked in cardiovascular cells without causing substantial amounts of toxicities. These vectors also tend to accumulate in injured cardiomyocytes shortly after intravenous injection, which illustrates the advantage of this form of therapy (Ruiz-Esparza et al., 2016). Since porous silicon nanoparticles are biodegradable, these vectors are ideal for use in ischemic heart treatment. Recent research suggests that porous silicon nanoparticles loaded with atrial natriuretic peptide (ANP) can increase angiogenesis (creation of new blood vessels) and tissue remodeling in cardiomyocytes (Ferreira et al., 2017). The researchers of this experiment further modified these nanoparticles by giving them a coating that provided significant long-term colloidal stability, which increased treatment effectiveness (Figure 3).

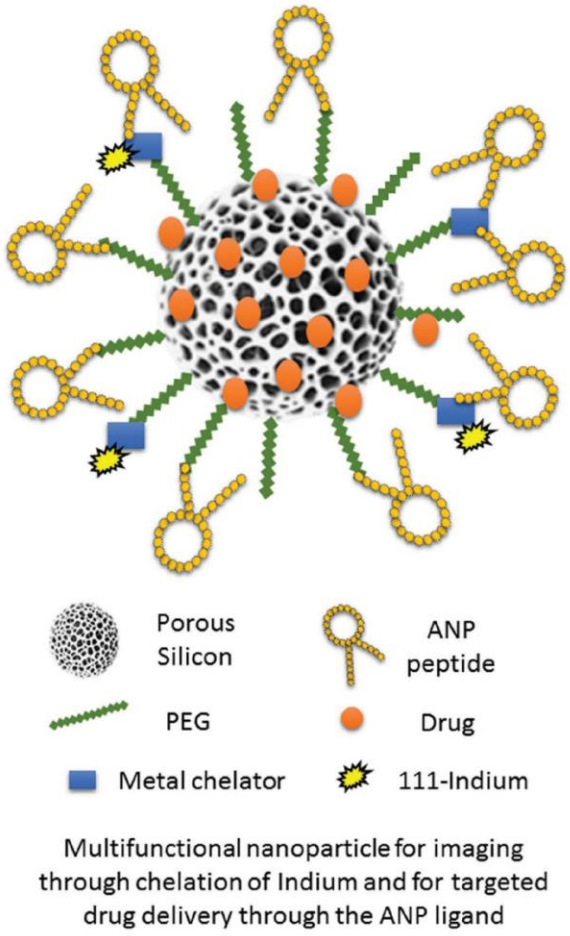

FIG. 3. Schematic representation of the porous silica nanoparticle that has additional colloidal stability due to the PEG coating. The metal chelator is used for radiolabeling/*in vivo* imaging purposes and the porous silica nanoparticle allows for superb biocompatibility and biodegradability (Ferreira et al., 2017).

Research supports the use of engineered silica nanoparticles in regenerative medicine because of their degradation properties and ease of functionalization. Despite this information, there are still some concerns about the biosafety of these nanoparticles on cellular systems. However, recent data suggests that the use of silica nanoparticles is safe for future use. A supporting study involved treatment of MI with fluorescently labeled silica nanoparticles (SiO_2-NPs) and human bone marrow-derived mesenchymal stem cells (hMSCs) in rat models (Gallina

et al., 2015). The results of this study revealed that the SiO_2-NPs exhibited compatibility and biosafety with the hMSCs showing lack of geno- and cyto-toxicity. This study also identified that lysosomal activation for SiO_2-NPs degradation was not associated with oxidative stress. Furthermore, Kempan et al. confirmed the lack of adverse effects in cells after use of silica nanoparticles in their study (2015). Kempan et al., examined cell proliferation during the first five days of growth (when silicic acid concentration was the largest) before determining that there was no change in cell proliferation, no observable cytotoxicity, and no decrease in metabolic activity when the concentration of mesoporous silica nanoparticles was at a high concentration of 250 μg/mL (2015).

Through much supporting research, the use of silica-based nanoparticles demonstrates promising future capabilities for multiple reasons including proper cellular targeting, safe biodegradation, and imaging via radiolabeling. These properties can improve treatment results because medication or stem cells can be delivered to the affected tissue efficiently while being monitored. Once delivered to the tissue, these nanoparticles are also safely degraded without cytotoxic effects—a benefit of this form of treatment.

2.2 Nanoparticle Delivery of miRNA

As previously expressed, a PLGA nanoparticle can be used to transport various items of interests to a target location. One study used this type of nanoparticle to deliver miRNA to endothelial cells in mouse models. Specific to this research, this nanoparticle contained a fluorine compound that could be tracked using a ^{19}F magnetic resonance imaging (MRI). The researchers discovered that the endothelial cells internalized the nanoparticles allowing efficient delivery of the miRNA, which increased the survival of cells exposed to hypoxia (Gomes et al.,

2013). This research illustrates the versatility of nano-DDSs since several different packages can be loaded into these nanoparticles. It also represents how a nano-DDS has to be case specific for an intended purpose. Therefore, it is important that nanotechnology use in the clinical setting should be tailored for the type of therapy and desired package.

3. Nanoparticles Attached to Microbubbles

DNA and other molecules can be packaged in nano-DDSs and attached to microbubbles (lipid bilayer structures), which are sensitive to ultrasound. The sounds waves produced by the ultrasound machine force the microbubbles close to the endothelial cells resulting in greater delivery of the cargo to the tissue (Figure 4).

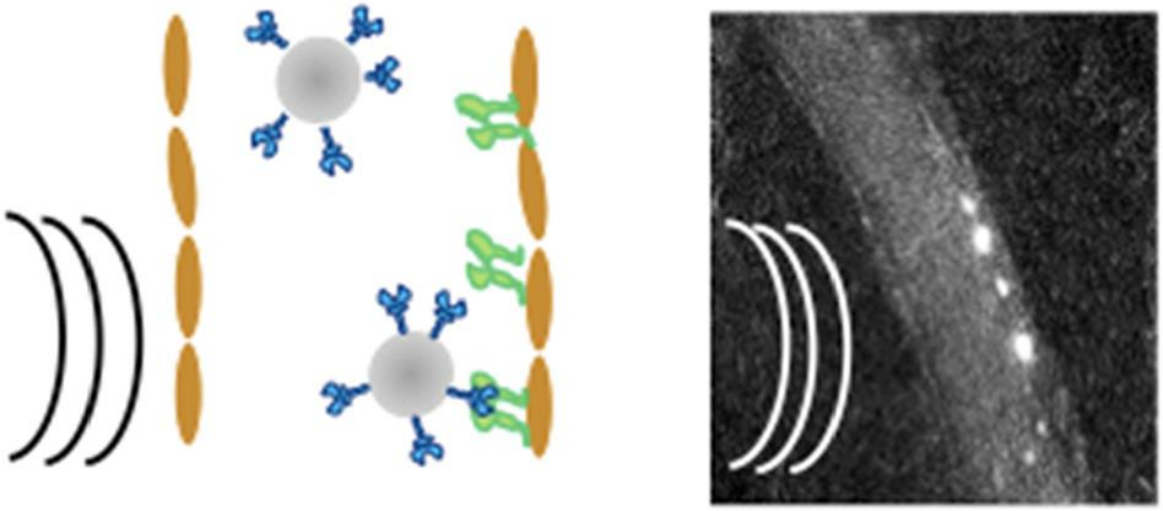

FIG. 4. Ultrasound radiation force is applied to blood vessels within select tissues. This forces the microbubbles with ligands targeted to epithelial epitopes to be pressed towards the blood vessel surface increasing interaction and cellular absorption of the package after microbubble implosion (Unger et al., 2014).

This is an effective method for both targeted package delivery and sonothrombolysis in treatment for AMI (Unger et al., 2014). Sonothrombolysis utilizes small amounts of radiation force, which can penetrate and dissolve a plaque or blood clot occluded artery as demonstrated in

animal models (Hagisawa et al., 2013). After disintegration of the plaque or lipid obstructing the artery, blood flow is restored to nearby tissue increasing survival rates for those suffering from MI (Figure 5).

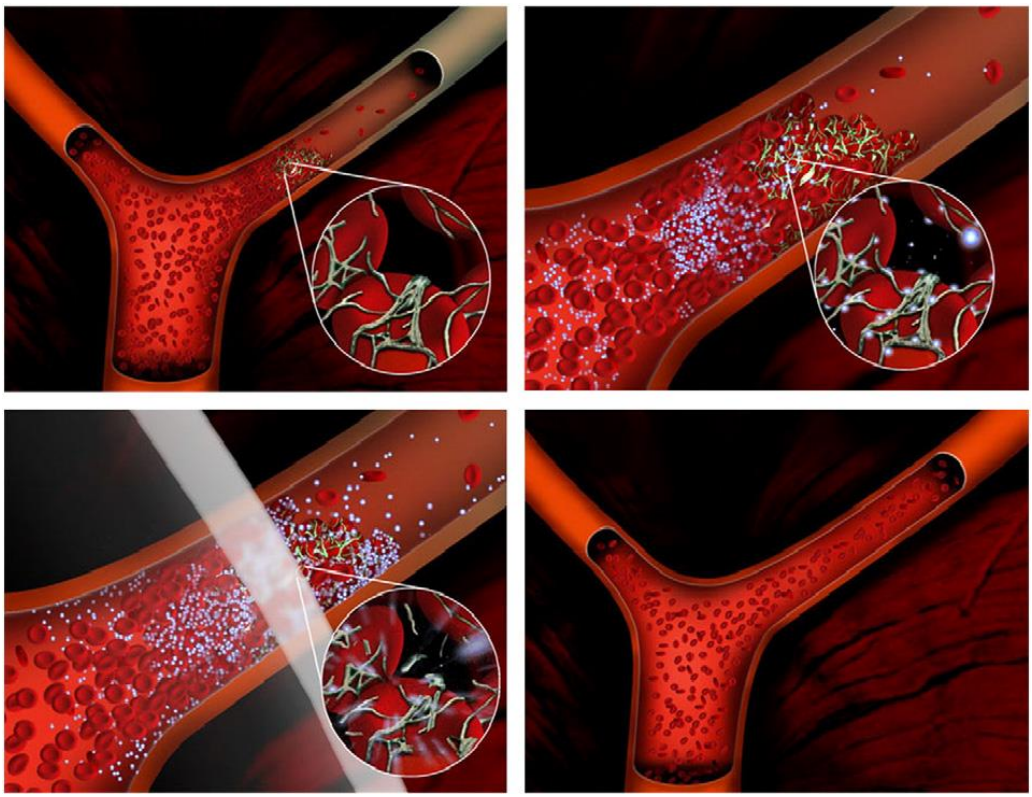

FIG. 5. Sonothrombolysis of an occluded clot using microbubbles. The top left image illustrates a vascular occlusion of proteins and erythrocytes. In the top right image, the microbubbles are administered via intravenous injection and begin to accumulate in the clot. Ultrasound is applied in the lower left image, which cavitates the microbubbles destroying the blood clot and resulting in a restoration of blood flow as depicted in the lower right image (Unger et al., 2014).

Along with sonothrombolysis, microbubbles have promising effects of delivering oxygen to the target tissue and acting as a neuroprotectant (Johnson et al., 2015; Unger et al., 2014). Additionally, ultrasound and microbubbles can be combined in a technique called ultrasound targeted microbubble destruction (UTMD), which has been used by researchers to promote cardiovascular gene and hormone delivery. Through UTMD, the microbubble—filled with gas and the package of interest (DNA, plasmid, etc.) in the center core—is selectively destroyed at

the target tissue by ultrasound waves. Fujii et al. used UTMD to delivered vascular endothelial growth factor (VEGF), genes for green fluorescent protein (GFP), and stem cell factor for treatment of MI in a mouse model (2010). Their results for the mice treated with UTMD showed GFP expression in cardiac tissue, enhanced migration of stem cells into cardiomyocytes, and improved cardiac repair when compared to control groups. This research depicts how the use of nano-DDSs paired with additional components can result in a multifactorial treatment for IHD including gene therapy, drug delivery, and improvement of CAD through sonothrombolysis.

4. Antibody-linked Nanoparticles

Linking antibodies to nanoparticles can be an efficient method for allowing a vector to reach the target location. In a study performed in rats, liposomes with an anti-cardiac troponin I (cTnI) antibody loaded with anti-miR-1 antisense oligonucleotides (AMO-1) promoted effective delivery of AMO-1 to cardiac cells (Liu et al., 2014). The antigen for the cTnI antibody, cardiac troponin I, is a marker distinctively expressed when cardiac damage (often from tissue hypoxia) occurs (Wu, 2004). Liu et al. recognized how microRNA-1 is overexpressed in ischemic cardiac tissues and that this molecule is a significant influencer of arrhythmia (2014). Therefore, delivering the AMO-1 to injured cardiomyocytes should result in reduced arrhythmia. Liu et al. discovered that this active-targeting nano-DDS not only resulted in effective delivery to damaged ischemic cells but also relieved ischemic arrhythmia and restored the depolarized resting membrane potential, therefore, improving cardiac contraction (2014).

In another recent study, a liposome carrying an anti-inflammatory therapeutic agent was conjugated with an antibody for platelet endothelial cell adhesion molecule (PECAM-1) (Howard et al., 2014). This study demonstrates how numerous types of antibodies can be

attached to the liposome nano-DDS. The antibody conjugated in this study guided the nano-DDS towards pulmonary vasculature resulting in enhanced delivery and increased therapeutic effects (Howard et al., 2014). Since PECAM-1 is expressed in vascular endothelial cells, this suggests that this active-targeting nano-DDS can be used for treatment of cardiovascular disease. Not only can an antibody targeting vascular endothelial cells be used to promote effective delivery, but the adhesion molecule itself can be used. In another study, vascular cell adhesion molecule-1 was attached to superparamagnetic iron oxide particles and through histological analyses and electron microscopy, it was determined that this nano-DDS facilitated ample accumulation of iron particles in endothelial cells (Michalska et al., 2012).

This study illustrates how using two different methods of guiding therapeutic payload can result in more accurate delivery of cargo to an intended area. It also elucidates how various proteins or signaling molecules can be attached to this form of nano-DDS to enhance specific targeting. A benefit of this ability to link multiple distinct molecules is that this enables putting more than one antibody on the surface of a liposome. Liposome nano-DDSs could be linked to antibodies targeting both macrophages and tissue cells facilitating an immune response near the injured tissue and providing therapeutic effects respectively. This unique ability for immune cells to be directed to the injured area would decrease infection and increase recovery time, therefore, improving clinical outcomes.

5. Magnetic Nanoparticles

Cardiac ischemia often causes extensive damage to cardiomyocytes, improper heart contraction, and fibrosis leading to the formation of scar tissue (Santoso & Yang, 2016). This scar tissue disrupts the ability of cardiac progenitor cells to travel to and repair the injured tissue.

To rectify this issue, pluripotent stem cells can be implanted in the injured area. However, several delivery methods have limited success most likely due to improper stem cell implantation. Stems cells transplanted with growth factor collagen matrices have been found to have poor engraftment and injecting stem cells into the injured tissue often results in less than 10% of the cells remaining at the site of injection after a few hours (Santoso & Yang, 2016). To improve this problem, superparamagnetic nanoparticles can be used.

Superparamagnetic iron oxide nanoparticles are frequently used to create a vector that is responsive to an external magnetic field. Being superparamagnetic, these iron oxide particles have orbitals with unpaired electrons, which allows them to be sensitive to a magnetic field (Beckett, 2015). This characteristic property of these vectors allows more accurate imaging via MRI and promotes more efficient cellular uptake of intended packages. Some packages that have been used range from stem cells, stents, and medication (Ambesh et al., 2017).

In a study using tissue models, anti-CD34 antibody and PEG coated Fe_3O_4 magnetic nanoparticles (Figure 6) were bound to stem cells before being directed to specific lesion sites by an external magnetic.

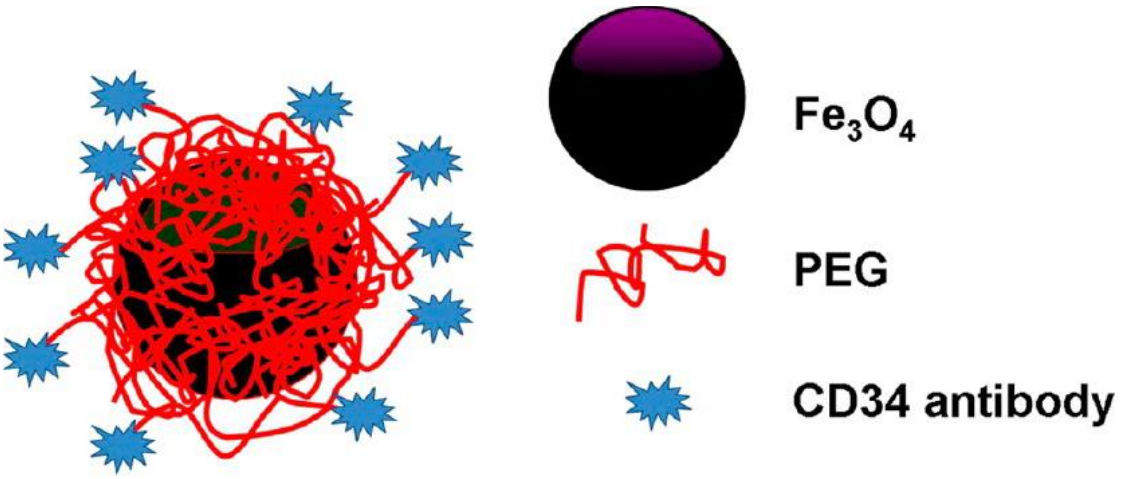

FIG. 6. The nano-DDS designed by Chen et al. (2013).

The PEG coating was used to create a hydrophilic surrounding that facilitated nanoparticle scattering and prevented protein adsorption. This research uncovered that this engineered magnetic nanoparticle was safe to use in the bloodstream and was able to effectively deliver the stem cells to the desired location (Chen et al., 2013). As shown in Figure 7, the external magnetic field supplied a force that guided the nano-DDS to the lesion site before allowing the antibody to interact with the endothelial cell and merge into the affected site initiating therapy.

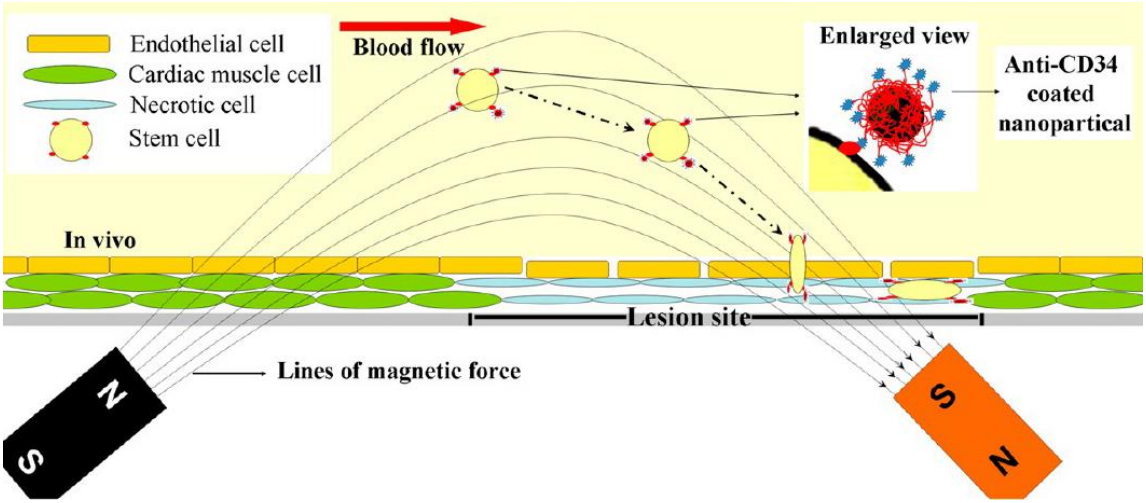

FIG. 7. A schematic depiction of the controlled direction of the PEG and CD34 antibody-coated magnetic particle to the injured myocardium (Chen et al., 2013).

What this study suggests is that this form of delivery could increase the efficacy of stem cell delivery to limited-access areas of the ischemic heart. This study also explained how these magnetic nanoparticles were able to be detected and monitored via MRI throughout treatment. An advantage of this ability to both simultaneously treat and monitor would improve treatment for patients in the clinical setting. Another study performed by Cheng et al. used magnetic targeting to guide iron core nanoparticles labeled with antibodies against antigens for both therapeutic and injured cells (Figure 8) (2014). Since an iron core was used, this nano-DDS was

also able to be detected throughout the course of therapy via MRI allowing immediate results for treatment progress.

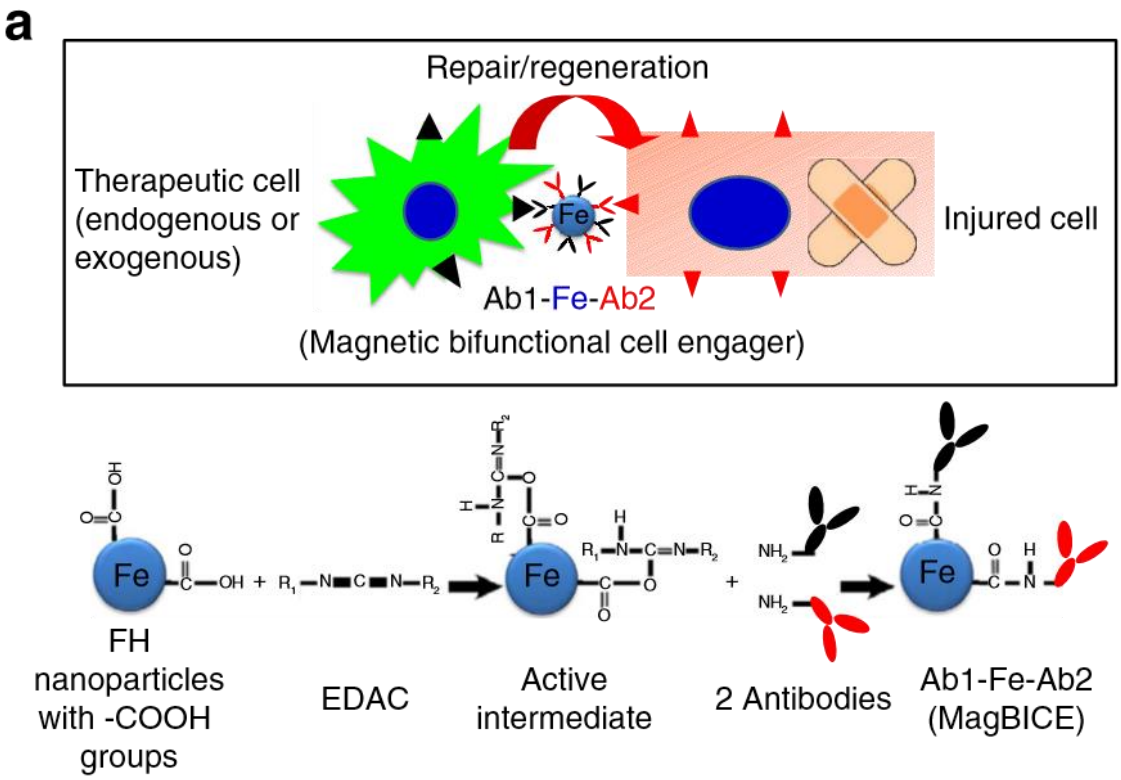

FIG. 8. Schematic of iron-antibody construct linking both therapeutic and injured cells. Through the addition of carboxylic acid functional groups on an iron core, a peptide bond can be formed with an amino acid in the heavy chain of an antibody. This allows multiple types of antibodies to be fastened to the iron core (Cheng et al., 2014).

Having antibodies linking two types of cells further guides the therapeutic stem cells to the appropriate location and has shown to have more immediate tissue repair and positive treatment outcomes (Cheng et al., 2014). What is noteworthy about this form of therapy is that these stems cells can be administered intravenously and still locate the target tissue, which provides a non-invasive treatment option for IHD.

6. Nanoscaffolding

Cardiomyocyte death induced by IHD and MI is a leading cause of heart failure and individual death. Since cardiac tissues have minimal regenerative capacity, the ability of these tissues to self-renew and return to normal cardiac function is hindered (Bergmann et al., 2009). One of the most effective ways to restore cardiac function can be attained via heart transplantation (Stehlik et al., 2012). However, besides the possible shortage of organ donors, complications of transplantation such as infections and cancer can be lethal to the patient (Fishman, 2007). The use of combined nanofibrous scaffolds and human stem cell nanotechnology offers an alternative choice of therapy to avoid both invasive surgery and additional risks that can occur post-transplantation.

Two less invasive approaches involve attaching human stem or proangiogenic cells to nano-DDSs (particularly magnetically-directed nanoparticles) or injection of these cells to the injured area. The use of stem cells to replace injured cardiomyocytes and promote effective tissue regeneration is possible. However, cellular stress is placed on these cells during isolation and detachment from their original extracellular matrix (ECM). This stress can stimulate anoikis—programmed cell death resulting after detachment from the ECM—which disrupts successful therapy potential (Tongers et al., 2014). Along with limited stem cell viability, only a small portion of transplanted cells remain at the site of injury a few hours after injection. One hour after intracoronary infusion around 2% of bone marrow-derived mononuclear cells and between 14-39% of CD34-enriched progenitor cells are still present at the site of injured cardiac tissue (Hofmann et al., 2005; Dedobbeleer et al., 2009). This issue reveals the need for establishing a structure that can attach to both stem cells and cardiac tissue to mirror a native ECM and to facilitate adequate implantation.

Certain nanoscaffold structures (fibers, matrix, etc.) have been shown help repair and maintain tissue integrity (Bhise et al., 2014). Recent research indicates that prefabricated nanofibrous scaffolds can be designed to simulate the ECM and if used in combination with stem cells, can address this challenge of cell implantation in-viability. Using the electrospinning technique, fabricated nanofibrous scaffolds have been shown to resemble native cardiac ECM. These scaffolds have also been identified to support organization and functionalities of cardiac tissues while possessing exceptional mechanical, material handling, and fiber properties (Zhao et al., 2015). One study demonstrated how using conductive electrospun PLGA scaffolds with cardiomyocytes resulted in the coupling of these cells to cardiac tissues and synchronous beating after applying an electrical impulse (Hsiao et al., 2013). This represents how nanoscaffolds can mimic a native environment experienced by cardiomyocytes, which not only facilitates implantation, but function, organization, and support as well.

Further research by Tongers et al. has shown that a peptide-based nanofiber matrix can be used as a supportive matrix for pro-angiogenic stem cells in damaged heart tissue (2014). The researchers of this study designed an amphipathic molecule composed of a hydrophobic tail, a beta-sheet domain, charged residues, and an amino acid based shortened segment of a fibronectin ECM protein, Arg-Gly-Asp-Ser peptide amphiphiles (RGDS-PA) as shown in Figure 9.

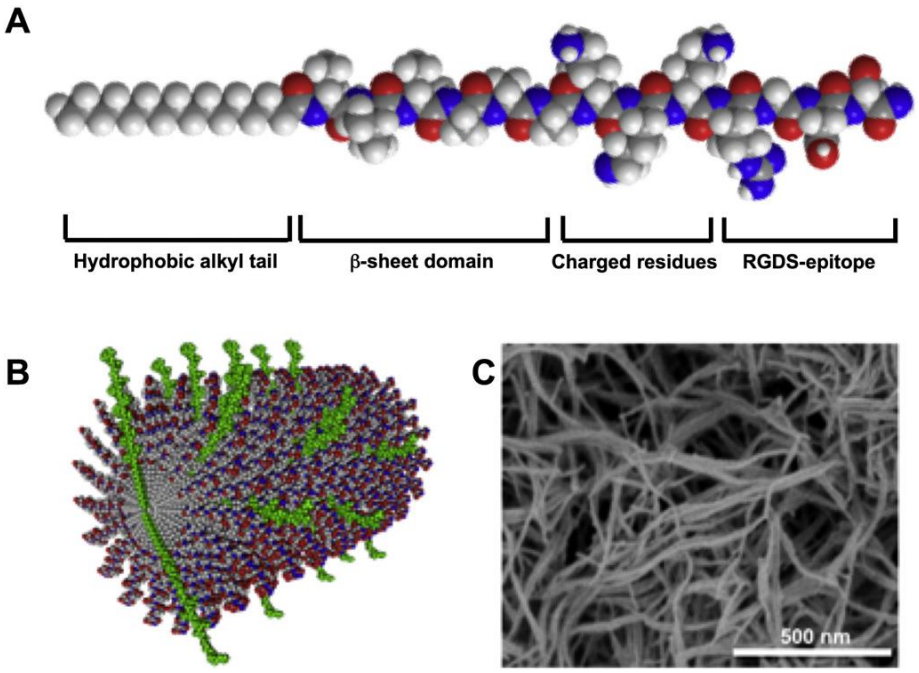

FIG. 9. (A) Molecular diagram of the RGDS-PA structure. (B) Assembly of the peptide amphiphiles into cylindrical nanofibers in an aqueous environment. The RGDS-epitope (green) section is dispersed throughout the molecule. (C) Scanning electron microscopy image of the nanofibers, which assemble into a three-dimensional nanofiber network (Tongers et al., 2014).

The chemical properties of the RGDS-PA allow it to self-assemble into cylindrical nanofibers when placed in an aqueous environment. These properties allow the formation of a hollow center that can be used to transport various packages such as stem cells, medications, and hormones. This versatility shows potential for a wide range of IHD treatment options. The RGDS-epitopes distributed on the surface of the nanofibers can simulate an ECM environment via the fibronectin-based ECM protein. These nanofibers can also interact with biopolymers and multiple cellular proteins including receptors. This permits communication of the nano-DDS with both cells and its environment enabling cellular homeostasis. Based on a mouse model, these viscous gel nanofibers facilitated new blood vessel tissue growth, which resulted in greater nearby capillary density, decreased cellular death, and increased cell regeneration and survival

(Tongers et al., 2014). Interestingly, the designed RGDS-PA construct naturally biodegrades into amino acids and lipids over a few weeks following injection showing no signs of cellular toxicity (Ghanaati et al., 2009).

7. Graphene

The use of mesenchymal stem cells (MSCs) for treatment of IHD has been found to stimulate *de novo* myocardium development, which improves the outcome of CAD (Orlic et al., 2001). It has been discovered that MSCs contribute to cardiac tissue repair via secretion of paracrine factors including cytokines VEGF and basic fibroblast growth factor, which prompt angiogenesis and subsequently IR (Kinnaird et al., 2004). Despite these promising benefits of using MSC in MI therapy, complications of successful MSC implantation such as poor engraftment and survival of MSCs can impede ideal outcomes (Lee et al., 2011). As previously elucidated, disrupted attachment of MSCs to the ECM can result in unsuccessful MSC implantation. The use of nanoscaffolds can be used to improve MSC survival and engraftment. However, a significant factor causing substandard survival rate of MSCs can be related to the production of ROS after reperfusion to the ischemic and infarcted myocardium (Angelos et al., 2006; Eltzschig et al., 2011). ROS have been recognized to inhibit fastening of MSCs to the ECM by disrupting focal contacts, which leads to anoikis and a decrease in MSC therapeutic efficacy (Park et al., 2015a). It has been hypothesized by multiple researchers that the use of graphene oxide (GO) flakes in MSC therapy for IHD can provide several advantages and increase the success rate for this form of therapy.

A two-dimensional nanomaterial composed of carbon, hydrogen, and oxygen atoms, GO is a compound that has special biomedical applications due to its exceptional amphipathic,

surface functionalizability, fluorescent quenching, electrical, and mechanical properties (Chung et al., 2013). GO has particular functional groups that allow it to adsorb ECM proteins via electrostatic, hydrogen bonding, and hydrophobic interactions, which encourages GO cellular adhesion (Shi et al., 2012). Comprehending this, Park et al. hypothesized that linking GO to MSCs would strengthen interaction between the cells and ECM even when MI hinders cell adhesion due to cardiac tissue damage (Park et al., 2015a). As illustrated in Figure 10, anoikis resulted for unmodified MSCs due to obstructed cell adhesion caused by ROS in ischemia and reperfusion injury whereas modified MSCs bound to GO prior to implantation enabled cell to ECM interactions between the MSCs and ECM-GO complex allowing MSC elusion of anoikis.

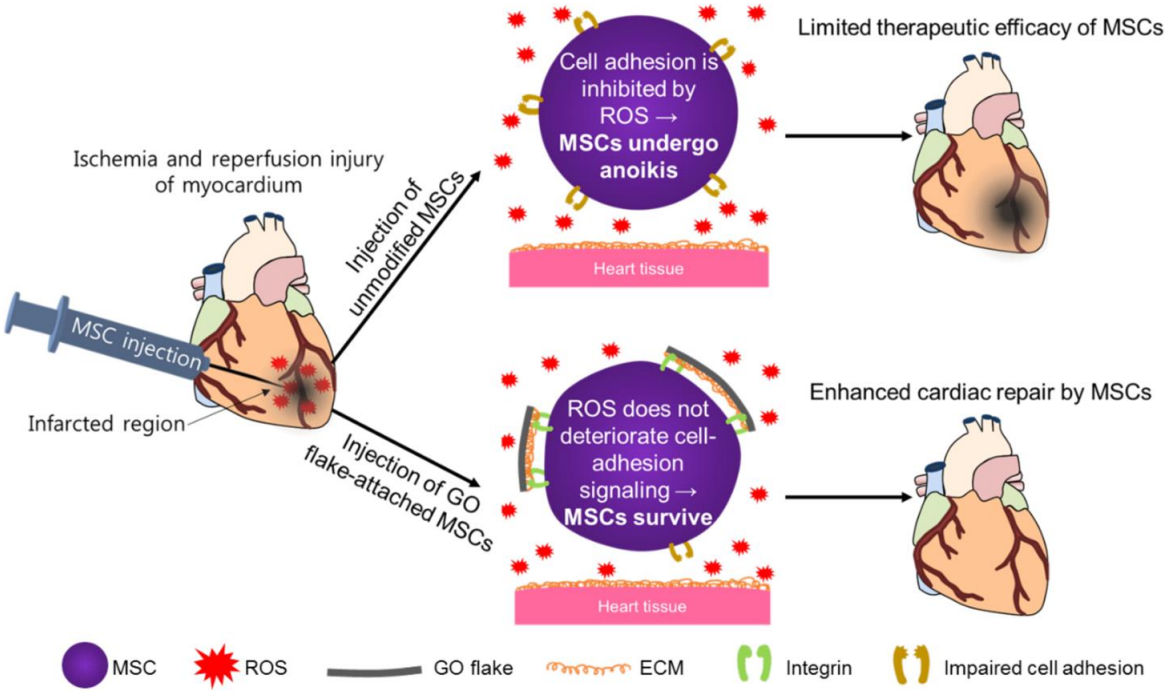

FIG. 10. Schematic of both modified and unmodified MSCs prior to implantation in the infarcted myocardium. The generation of ROS after reperfusion to injured cardiac tissue disrupts the attachment of implanted MSCs to the ECM resulting in anoikis. Adhering MSCs to GO flakes before implantation allows myocardium ECM interaction with the MSCs and the ECM adsorbed to GO flakes to effectively attach to injured cardiac tissue resulting in less anoikis and an increase in MSC therapy efficacy (Park et al., 2015a).

Park et al. discovered that survival of modified MSCs considerably exceeded that of unmodified MSCs (2015a). These researchers also confirmed that the linkage of GO flakes to MSCs prior to implantation augmented paracrine secretion from the MSCs after implantation, therefore, stimulating more immediate myocardium repair and function restoration. This study represents how using nanotechnology such as GO flakes can improve successful therapy outcomes by enhancing engraftment through anoikis avoidance and by boosting natural therapeutic function of MSCs.

Further research by Park et al. exemplifies the benefits of GO use in MSC therapy through not only enhancing ECM-MSC interactions but ameliorating other complications of MI such as improper cardiac conductive function and arrhythmia as well (2015b). MI has been identified to increase susceptibility to arrhythmia due to cardiac fibrosis and a loss of cardiomyocytes expressing gap junction proteins (De Jong et al., 2011). It has been noted that cardiac arrhythmia can be lessened via implantation of cells expressing connexin 43 (Cx43) gap junction proteins (Roell et al., 2007). Upregulated expression of Cx43 in MSCs results in an increase in both cardiac function and protective effects provided by MSCs (Hahn et al., 2008).

Park et al. recognized how a spheroid form of MSCs shows improved expression of VEGF, fibroblast growth factor-2 (FGF-2), and Cx43 (2015b). However, this form of MSCs has limited cell to ECM interactions. Therefore, Park et al. hypothesized that the combination of reduced graphene oxide flakes (RGO) with MSC spheroids would upregulate the expression of both Cx43 and paracrine factors resulting in improved MSC therapy efficacy (2015b). As aforementioned, GO has unique properties that allow it to be an excellent electro-conductor and have outstanding ECM protein adherence. Shown in Figure 11 is an illustration of the RGO flake incorporation in MSC spheroids used by Park et al. (2015b). In this diagram, the RGO flakes

attached to ECM proteins such as fibronectin (FN) facilitating cell-ECM and cell-cell interactions, which increased MSC therapeutic efficacy for MI.

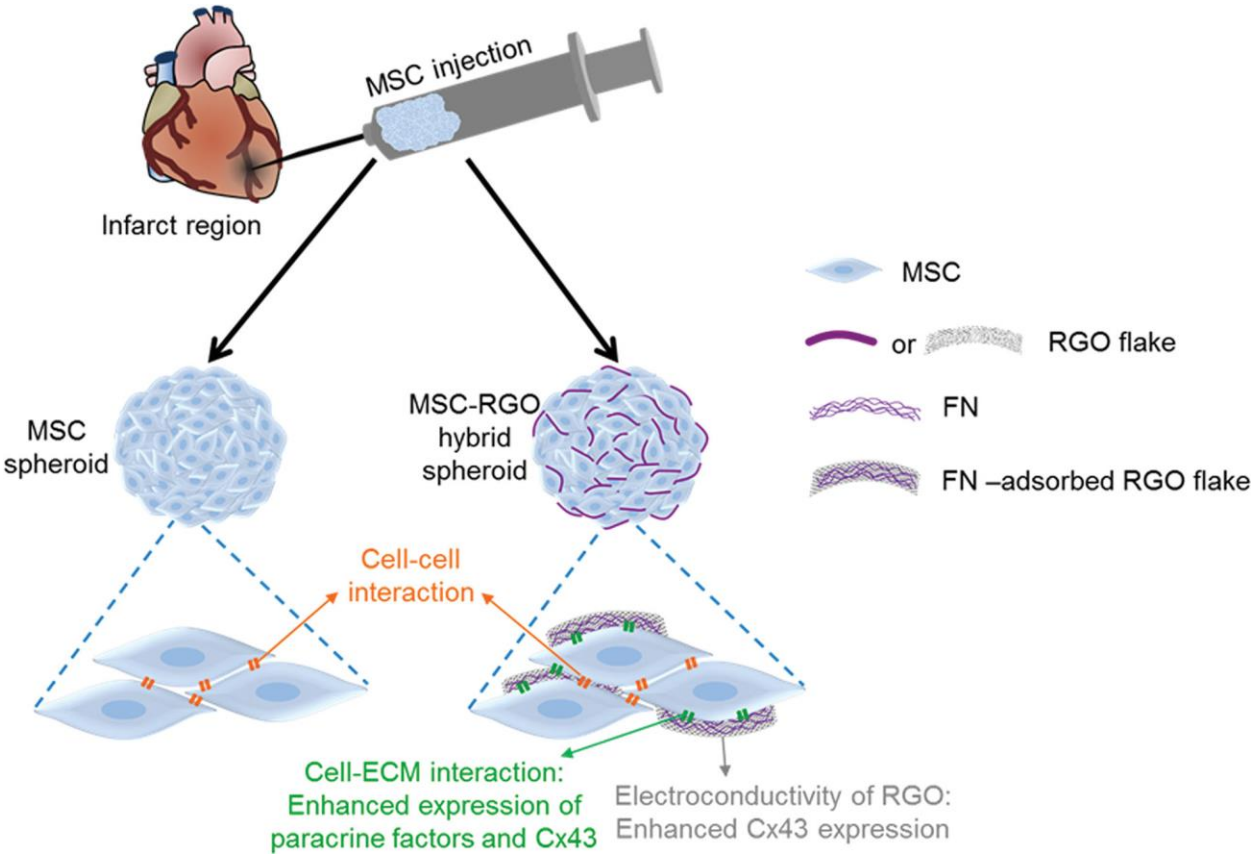

FIG. 11. Schematic representation of how RGO flakes incorporated into MSC during MSC therapy can enhance cell-ECM interactions. Without the addition of RGO flakes in MI therapy, there is still cell-cell interactions, but little cell-ECM interactions (Park et al., 2015b).

Through this study, Park et al. established that RGO demonstrated high affinity towards FN, which resulted in enhanced cell-ECM interactions, elevated paracrine factor expression, and increased cell survival (2015b). The upregulated expression of Cx43 was strongly related to the conductivity of RGO and increased paracrine factor expression. These effects led to stimulation of angiogenesis via elevated VEGF, more effective cell-to-cell communication, improved conductive cardiac function, and restoration of the injured myocardium to a native cellular state.

This study indicates how GO can provide more than one therapeutic advantage for treatment of IHD. It also demonstrates how crucial nanotechnology is for therapy efficacy.

Additional research illustrates how GO can be used with not only MSCs but incorporated into hydrogels as well. Paul et al. created a low-modulus methacrylated gelatin (GelMA) hydrogel loaded with polyethylenimine (PEI) functionalized GO nanosheets (fGO) for site-specific delivery of pro-angiogenic human vascular endothelial growth factor plasmid DNA ($pDNA_{VEGF}$) for IHD treatment (2014). PEI has been evaluated to have low cytotoxicity and when combined with GO, has been determined to be a site-specific and efficient non-viral gene delivery vector (Kim et al., 2011). Paul et al. injected this therapeutic hydrogel into infarct cardiac regions in a rat model, which caused heightened mitotic activity in endothelial cells (2014). For the fGO/GelMA rats, the researchers identified a substantial increase in cardiac tissue capillary density, reduction in scar tissue, enhanced cardiac performance verified via echocardiogram, and no remarkable differences in serum cytokine and inflammatory miRNA levels when compared to the non-fGO/GelMA rat control group. These promising results illustrate the efficacy of using GO for target-specific IHD therapy. This also suggests how using a fGO/GelMA and $pDNA_{VEGF}$ loaded hydrogel in combination with MSCs bound to GO flakes would result in an even more effective form of treatment for IHD. Combining several treatments could result in more immediate production of VEGF causing a greater angiogenesis response and more efficient tissue repair via effective MSC implantation. This would speed up recovery time for the patient, however, the possible harmful effects of too much GO is an important concern.

Despite the numerous benefits and reinforced improvement of treatment efficacy for IHD therapy using GO, the possible biological harm of GO should be addressed. One study displayed how GO could be toxic on a cellular level in a dose-dependent manner (Chang et al., 2011).

Another study demonstrated how GO flake intravenous administration in mice at a concentration of 4 mg/kg for five days caused mutations in erythrocytes and a concentration of 100 µg/mL resulted in modified gene expression for genes involved in DNA-damage control, apoptosis, and cell cycle regulation (Liu et al., 2013). Additionally, GO has been noted to elevate ROS levels in macrophages, which provokes morphological changes within the cell and eventually leads to necrosis *in vitro* (Qu et al., 2013). Therefore, it is vital to use a safe concentration and administration of GO when involving the life of a patient during treatment. Park et al. recognized the possible cytotoxic effects of GO within a biological system and decided to use a concentration of 10 µg/mL in their experiment with MSCs, which was found to cause no cytotoxic effects (2015a). In the experiment conducted by Paul et al., the GO loaded hydrogel was evaluated for cytotoxic effects through inflammation and cell proliferation assays (2014). It was determined that this GO hydrogel produced no cytotoxic effects and was compatible with rat cardiomyocytes (Paul et al., 2014). This shows promising results for the use of GO in clinical trials and possible future widespread treatment. However, the concentration for safe administration based on individual body type should be analyzed more extensively before clearing this method of therapy for clinical use.

8. Conclusion

This research has illustrated how nanotechnology can be used to advance IHD treatment. Given the progress that has been made, it should be noted that much of the presented research is in early stages of development and most applications have not made it through all stages of clinical trials. Many of the aforementioned studies were based on rat or mouse models and utilized a direct site-injection approach to the heart. In a clinical setting, a less invasive approach

such as intravenous administration should be considered to provide efficient yet compatible therapy. It is important to use the appropriate type of nanoparticle that affects treatment of IHD in positive ways, which means that the nano-DDS needs to be specifically designed for the injury and person. Including tailored design, the nanoparticles must have little immunogenicity, must not cause additional deleterious events including arrhythmia, do not result in increased inflammation, and can be either degraded or excreted once therapy is completed (Fan et al., 2010; Toh et al., 2013). Effective degradation has been illustrated by use of both mesoporous silica nanoparticles and RGDS-PA, but further research needs to be performed to analyze how many conditions these nano-DDSs can be used for.

Future studies in this field of research should address the creation of multifunctional nanoparticles similar to what Ferreira et al. designed (2017). The ability to fabricate nanoparticles that can deliver numerous types of packages and allow different forms of imaging modalities would be a considerable advancement in this field of research. Nano-DDSs have shown that stem cells and hormones can be transported to a tissue of interest. However, if multiple cargoes could be inserted into these nano-DDSs, then both new cardiomyocytes could be formed via stem cells and revascularization could be stimulated via VEGF. This application would decrease IR injury often resulting from MI therapy. As many of these systems are fine-tuned such as more precise intracellular time-release of medication/hormones and new non-toxic forms of imaging, then this field can switch from treatment to preventative medicine.

References

Allen, T. M. (1994). Long-circulating (sterically stabilized) liposomes for targeted drug delivery. *Trends in pharmacological sciences*, *15*(7), 215-220.

Ambesh, P., Campia, U., Obiagwu, C., Bansal, R., Shetty, V., Hollander, G., & Shani, J. (2017). Nanomedicine in coronary artery disease. *Indian Heart Journal*.

Angelos, M. G., Kutala, V. K., Torres, C. A., He, G., Stoner, J. D., Mohammad, M., & Kuppusamy, P. (2006). Hypoxic reperfusion of the ischemic heart and oxygen radical generation. *American Journal of Physiology-Heart and Circulatory Physiology*, *290*(1), H341-H347.

Beckett, C. (2015). Chemistry lecture for CHEM 1020: Fall 2015. University of Wyoming; Laramie, WY.

Begum, M., & Sharma, H. (2016). Scope of nanomedicine against coronary artery disease: a review. *Eur. J. Pharm. Med. Res*, *3*, 635-641.

Bergmann, O., Bhardwaj, R. D., Bernard, S., Zdunek, S., Barnabé-Heider, F., Walsh, S., ... & Jovinge, S. (2009). Evidence for cardiomyocyte renewal in humans. *Science*, *324*(5923), 98-102.

Bhatia, S. K. (2010). *Biomaterials for clinical applications*. Springer Science & Business Media.

Bhise, N. S., Ribas, J., Manoharan, V., Zhang, Y. S., Polini, A., Massa, S., ... & Khademhosseini, A. (2014). Organ-on-a-chip platforms for studying drug delivery systems. *Journal of Controlled Release*, 190, 82-93.

Chang, M. Y., Yang, Y. J., Chang, C. H., Tang, A. C., Liao, W. Y., Cheng, F. Y., ... & Hsieh, P. C. (2013). Functionalized nanoparticles provide early cardioprotection after acute myocardial infarction. *Journal of controlled release*, 170(2), 287-294.

Chang, Y., Yang, S. T., Liu, J. H., Dong, E., Wang, Y., Cao, A., ... & Wang, H. (2011). In vitro toxicity evaluation of graphene oxide on A549 cells. *Toxicology letters*, *200*(3), 201-210.

Chen, J., Huang, N., Ma, B., Maitz, M. F., Wang, J., Li, J., ... & Liu, X. (2013). Guidance of stem cells to a target destination in vivo by magnetic nanoparticles in a magnetic field. *ACS applied materials & interfaces*, 5(13), 5976-5985.

Cheng, K., Shen, D., Hensley, M. T., Middleton, R., Sun, B., Liu, W., ... & Marbán, E. (2014). Magnetic antibody-linked nanomatchmakers for therapeutic cell targeting. *Nature communications*, 5.

Chung, C., Kim, Y. K., Shin, D., Ryoo, S. R., Hong, B. H., & Min, D. H. (2013). Biomedical applications of graphene and graphene oxide. *Accounts of chemical research*, *46*(10), 2211-2224.

Dedobbeleer, C., Blocklet, D., Toungouz, M., Lambermont, M., Unger, P., Degaute, J. P., ... & Berkenboom, G. (2009). Myocardial homing and coronary endothelial function after autologous blood CD34+ progenitor cells intracoronary injection in the chronic phase of myocardial infarction. *Journal of cardiovascular pharmacology*, *53*(6), 480-485.

De Jong, S., van Veen, T. A., van Rijen, H. V., & de Bakker, J. M. (2011). Fibrosis and cardiac arrhythmias. *Journal of cardiovascular pharmacology*, *57*(6), 630-638.

Eltzschig, H. K., & Eckle, T. (2011). Ischemia and reperfusion [mdash] from mechanism to translation. *Nature medicine*, *17*(11), 1391-1401.

Evans, C. W., Iyer, K. S., & Hool, L. C. (2016). The potential for nanotechnology to improve delivery of therapy to the acute ischemic heart. *Nanomedicine*, 11(7), 817-832.

Fan, Y., Dormer, K., & Po, S. S. (2010). Autonomic Denervation Using Magnetic Nanoparticles. *Trends in cardiovascular medicine*, *20*(8), 268-272.

Ferreira, M., Ranjan, S., Kinnunen, S., Correia, A., Talman, V., Mäkilä, E., ... & Hirvonen, J. (2017). Drug-Loaded Multifunctional Nanoparticles Targeted to the Endocardial Layer of the Injured Heart Modulate Hypertrophic Signaling. *Small*, *13*(33).

Fishman, J. A. (2007). Infection in solid-organ transplant recipients. *New England Journal of Medicine*, *357*(25), 2601-2614.

Ford, E. S., Roger, V. L., Dunlay, S. M., Go, A. S., & Rosamond, W. D. (2014). Challenges of ascertaining national trends in the incidence of coronary heart disease in the United States. *Journal of the American Heart Association*, *3*(6), e001097.

Fujii, H., Li, S. H., Wu, J., Miyagi, Y., Yau, T. M., Rakowski, H., ... & Li, R. K. (2010). Repeated and targeted transfer of angiogenic plasmids into the infarcted rat heart via ultrasound targeted microbubble destruction enhances cardiac repair. *European heart journal*, *32*(16), 2075-2084.

Gallina, C., Capelôa, T., Saviozzi, S., Accomasso, L., Catalano, F., Tullio, F., ... & Giachino, C. (2015). Human mesenchymal stem cells labelled with dye-loaded amorphous silica nanoparticles: long-term biosafety, stemness preservation and traceability in the beating heart. *Journal of nanobiotechnology*, *13*(1), 77.

Ghanaati, S., Webber, M. J., Unger, R. E., Orth, C., Hulvat, J. F., Kiehna, S. E., ... & Kirkpatrick, C. J. (2009). Dynamic in vivo biocompatibility of angiogenic peptide amphiphile nanofibers. *Biomaterials*, *30*(31), 6202-6212.

Gomes, R. S., Neves, R. P. D., Cochlin, L., Lima, A., Carvalho, R., Korpisalo, P., ... & Yla?-Herttuala, S. (2013). Efficient pro-survival/angiogenic miRNA delivery by an MRI-detectable nanomaterial. *ACS nano*, 7(4), 3362-3372.

Hagisawa, K., Nishioka, T., Suzuki, R., Maruyama, K., Takase, B., Ishihara, M., ... & Luo, H. (2013). Thrombus-targeted perfluorocarbon-containing liposomal bubbles for enhancement of ultrasonic thrombolysis: in vitro and in vivo study. *Journal of Thrombosis and Haemostasis*, *11*(8), 1565-1573.

Hahn, J. Y., Cho, H. J., Kang, H. J., Kim, T. S., Kim, M. H., Chung, J. H., ... & Kim, H. S. (2008). Pre-treatment of mesenchymal stem cells with a combination of growth factors enhances gap junction formation, cytoprotective effect on cardiomyocytes, and therapeutic efficacy for myocardial infarction. *Journal of the American College of Cardiology*, *51*(9), 933-943.

Hofmann, M., Wollert, K. C., Meyer, G. P., Menke, A., Arseniev, L., Hertenstein, B., ... & Drexler, H. (2005). Monitoring of bone marrow cell homing into the infarcted human myocardium. *Circulation*, *111*(17), 2198-2202.

Holmes, D. R., Lerman, A., Moreno, P. R., King, S. B., & Sharma, S. K. (2013). Diagnosis and management of STEMI arising from plaque erosion. *JACC: Cardiovascular Imaging*, *6*(3), 290-296.

Howard, M. D., Greineder, C. F., Hood, E. D., & Muzykantov, V. R. (2014). Endothelial targeting of liposomes encapsulating SOD/catalase mimetic EUK-134 alleviates acute pulmonary inflammation. *Journal of controlled release: official journal of the Controlled Release Society*, *177*, 34-41.

Hsiao, C. W., Bai, M. Y., Chang, Y., Chung, M. F., Lee, T. Y., Wu, C. T., ... & Sung, H. W. (2013). Electrical coupling of isolated cardiomyocyte clusters grown on aligned

conductive nanofibrous meshes for their synchronized beating. *Biomaterials*, *34*(4), 1063-1072.

Johnson, J. L., Leos, R. A., Baker, A. F., & Unger, E. C. (2015). Radiosensitization of Hs-766T pancreatic tumor xenografts in mice dosed with dodecafluoropentane nano-emulsion–preliminary findings. *Journal of biomedical nanotechnology*, *11*(2), 274-281.

Kempen, P. J., Greasley, S., Parker, K. A., Campbell, J. L., Chang, H. Y., Jones, J. R., ... & Jokerst, J. V. (2015). Theranostic Mesoporous Silica Nanoparticles Biodegrade after Pro-Survival Drug Delivery and Ultrasound/Magnetic Resonance Imaging of Stem Cells. *aging*, *5*(6), 632.

Kim, H., Namgung, R., Singha, K., Oh, I. K., & Kim, W. J. (2011). Graphene oxide–polyethylenimine nanoconstruct as a gene delivery vector and bioimaging tool. *Bioconjugate chemistry*, *22*(12), 2558-2567.

Kinnaird, T., Stabile, E., Burnett, M. S., Lee, C. W., Barr, S., Fuchs, S., & Epstein, S. E. (2004). Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms. *Circulation research*, *94*(5), 678-685.

Lee, W. Y., Wei, H. J., Lin, W. W., Yeh, Y. C., Hwang, S. M., Wang, J. J., ... & Sung, H. W. (2011). Enhancement of cell retention and functional benefits in myocardial infarction using human amniotic-fluid stem-cell bodies enriched with endogenous ECM. *Biomaterials*, *32*(24), 5558-5567.

Liu, M., Li, M., Sun, S., Li, B., Du, D., Sun, J., ... & Chang, N. (2014). The use of antibody modified liposomes loaded with AMO-1 to deliver oligonucleotides to ischemic myocardium for arrhythmia therapy. *Biomaterials*, 35(11), 3697-3707.

Liu, Y., Luo, Y., Wu, J., Wang, Y., Yang, X., Yang, R., ... & Zhang, N. (2013). Graphene oxide can induce in vitro and in vivo mutagenesis. *Scientific Reports (Nature Publisher Group)*, *3*, 3469.

Markman, J. L., Rekechenetskiy, A., Holler, E., & Ljubimova, J. Y. (2013). Nanomedicine therapeutic approaches to overcome cancer drug resistance. *Advanced drug delivery reviews*, *65*(13), 1866-1879.

Matoba, T., & Egashira, K. (2014). Nanoparticle-mediated drug delivery system for cardiovascular disease. *International heart journal*, 55(4), 281-286.

Mendis, S., Puska, P., & Norrving, B. (2011). *Global atlas on cardiovascular disease prevention and control*. World Health Organization.

Michalska, M., Machtoub, L., Manthey, H. D., Bauer, E., Herold, V., Krohne, G., ... & Zernecke, A. (2012). Visualization of vascular inflammation in the atherosclerotic mouse by ultrasmall superparamagnetic iron oxide vascular cell adhesion molecule-1–specific nanoparticles. *Arteriosclerosis, thrombosis, and vascular biology*, *32*(10), 2350-2357.

Moran, A. E., Forouzanfar, M. H., Roth, G., Mensah, G. A., Ezzati, M., Flaxman, A., ... & Naghavi, M. (2014). The global burden of ischemic heart disease in 1990 and 2010: the Global Burden of Disease 2010 study. *Circulation*, CIRCULATIONAHA-113.

Nguyen, M. M., Gianneschi, N. C., & Christman, K. L. (2015). Developing injectable nanomaterials to repair the heart. *Current opinion in biotechnology*, 34, 225-231.

Orlic, D., Kajstura, J., Chimenti, S., Jakoniuk, I., Anderson, S. M., Li, B., ... & Leri, A. (2001). Bone marrow cells regenerate infarcted myocardium. *Nature*, *410*(6829), 701-705.

Park, J., Kim, B., Han, J., Oh, J., Park, S., Ryu, S., ... & Choi, D. (2015a). Graphene oxide flakes as a cellular adhesive: prevention of reactive oxygen species mediated death of implanted cells for cardiac repair. *ACS nano*, *9*(5), 4987-4999.

Park, J., Kim, Y. S., Ryu, S., Kang, W. S., Park, S., Han, J., ... & Kim, B. S. (2015b). Graphene potentiates the myocardial repair efficacy of mesenchymal stem cells by stimulating the expression of angiogenic growth factors and gap junction protein. *Advanced Functional Materials*, *25*(17), 2590-2600.

Paul, A., Hasan, A., Al Kindi, H., Gaharwar, A. K., Rao, V. T., Nikkhah, M., ... & Khademhosseini, A. (2014). Injectable graphene oxide/hydrogel-based angiogenic gene delivery system for vasculogenesis and cardiac repair. *ACS nano*, *8*(8), 8050.

Prêle, C. M., Reichelt, M. E., Mutsaers, S. E., Davies, M., Delbridge, L. M., Headrick, J. P., ... & Grounds, M. D. (2012). Insulin-like growth factor-1 overexpression in cardiomyocytes diminishes ex vivo heart functional recovery after acute ischemia. *Cardiovascular Pathology*, *21*(1), 17-27.

Qu, G., Liu, S., Zhang, S., Wang, L., Wang, X., Sun, B., ... & Jiang, G. B. (2013). Graphene oxide induces toll-like receptor 4 (TLR4)-dependent necrosis in macrophages. *ACS nano*, *7*(7), 5732-5745.

Roell, W., Lewalter, T., Sasse, P., Tallini, Y. N., Choi, B. R., Breitbach, M., ... & Von Maltzahn, J. (2007). Engraftment of connexin 43-expressing cells prevents post-infarct arrhythmia. *Nature*, *450*(7171), 819-824.

Roth, E. J. (2011). Ischemia. In *Encyclopedia of Clinical Neuropsychology* (pp. 1359-1360). Springer New York.

Ruiz-Esparza, G. U., Segura-Ibarra, V., Cordero-Reyes, A. M., Youker, K. A., Serda, R. E., Cruz-Solbes, A. S., ... & Paez-Mayorga, J. (2016). A specifically designed nanoconstruct associates, internalizes, traffics in cardiovascular cells, and accumulates in failing myocardium: a new strategy for heart failure diagnostics and therapeutics. *European journal of heart failure*, *18*(2), 169-178.

Santoso, M. R., & Yang, P. C. (2016). Magnetic nanoparticles for targeting and imaging of stem cells in myocardial infarction. *Stem cells international*, 2016.

Shi, X., Chang, H., Chen, S., Lai, C., Khademhosseini, A., & Wu, H. (2012). Regulating cellular behavior on few-layer reduced graphene oxide films with well-controlled reduction states. *Advanced Functional Materials*, *22*(4), 751-759.

Stehlik, J., Edwards, L. B., Kucheryavaya, A. Y., Benden, C., Christie, J. D., Dipchand, A. I., ... & Hertz, M. I. (2012). The Registry of the International Society for Heart and Lung Transplantation: 29th official adult heart transplant report—2012. *The Journal of heart and lung transplantation*, *31*(10), 1052-1064.

Takahama, H., Minamino, T., Asanuma, H., Fujita, M., Asai, T., Wakeno, M., ... & Asakura, M. (2009). Prolonged targeting of ischemic/reperfused myocardium by liposomal adenosine augments cardioprotection in rats. *Journal of the American College of Cardiology*, *53*(8), 709-717.

Toh, E. K. W., Chang, M. Y., & Hsieh, P. C. (2013). Research Spotlight: Functionalized nanoparticles for future cardiovascular medicine.

Tongers, J., Webber, M. J., Vaughan, E. E., Sleep, E., Renault, M. A., Roncalli, J. G., ... & Kamide, C. E. (2014). Enhanced potency of cell-based therapy for ischemic tissue repair using an injectable bioactive epitope presenting nanofiber support matrix. *Journal of molecular and cellular cardiology*, 74, 231-239.

Unger, E., Porter, T., Lindner, J., & Grayburn, P. (2014). Cardiovascular drug delivery with ultrasound and microbubbles. *Advanced drug delivery reviews*, 72, 110-126.

Velazquez, E. J., Lee, K. L., Jones, R. H., Al-Khalidi, H. R., Hill, J. A., Panza, J. A., ... & Oh, J. K. (2016). Coronary-artery bypass surgery in patients with ischemic cardiomyopathy. *New England Journal of Medicine*, *374*(16), 1511-1520.

Ventrelli, L., Ricotti, L., Menciassi, A., Mazzolai, B., & Mattoli, V. (2013). Nanoscaffolds for guided cardiac repair: the new therapeutic challenge of regenerative medicine. *Journal of Nanomaterials*, *2013*, 39.

Wu, A. H. (2004). The role of cardiac troponin in the recent redefinition of acute myocardial infarction. *Clinical Laboratory Science*, *17*(1), 50.

Zhao, G., Zhang, X., Lu, T. J., & Xu, F. (2015). Recent advances in electrospun nanofibrous scaffolds for cardiac tissue engineering. *Advanced Functional Materials*, *25*(36), 5726-5738.