Regenerative treatment of a post-remodeling acute myocardial infarct with HGF releasing mesenchymal stem cells encapsulated in OPF/GO hydrogel

Bachelor thesis

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Abstract

Acute myocardial infarcts (AMI) are responsible for over half of the global cardiovascular disease deaths. AMI survivors are prone to a lowered quality of life and a high chance of heart failure (HF). After an infarct, the cardiac repair will take place in three overlapping phases. The inflammatory phase, fibrotic phase and the remodeling phase. Resulting in fibrotic scar tissue and loss of cardiomyocytes in the infarct zone. Thereby contributing towards a decrease in cardiac function. Most therapies focus on treatment during the inflammation phase. However, disrupting the balance of the inflammatory response can lead to an increase in tissue damage. Therefore, this article focusses on a regenerative therapy post-remodeling phase when most repair processes are stabilized. The therapy uses stem cells and a hydrogel to regenerate a new and healthy myocardium. An injectable OPF/GO hydrogel can provide structural integrity and conductivity for increased stem cell survival. Together with the capability of delivering biomolecules to the infarct zone. Included are proteins periostin and TGFβ. These could possibly return homeostasis in the infarcted tissue and reduce collagen through their ability to recruit MMP and TIMP releasing fibroblasts. The OPF/GO hydrogel will carry encapsulated mesenchymal stem cells (MSCs). Due to immune tolerance and the ability to differentiate into multiple cell types needed in cardiac repair, MSCs are a promising candidate for cardiac regeneration. It is demonstrated that MSCs can differentiate into cardiomyocytes in the human body making it viable for therapy. Combined with hepatocyte growth factor (HGF), the survival rate of MSCs is increased showing an enhancement of the cardiac function. In conclusion, the concept of an OPF/GO hydrogel with encapsulated MSCs combined with fibroblast recruiting factors can regenerate myocardium and possibly degenerate the fibrotic scar tissue. Improving cardiac function and decreasing morbidity.

Table of contents

Introduction

The leading cause of global morbidity and mortality is cardiovascular disease (CVD). CVDs are responsible for 31 percent of global deaths. This translates to a death toll of 17.9 million people (World Health Organization, 2017). Half of these deaths are caused by ischemic heart disease, otherwise known as coronary heart disease (World Health Organization, 2018). Ischemia arises due to insufficient circulation of oxygen rich blood towards tissues. A sustained lack of oxygen in the heart muscle eventually leads to myocyte necrosis. This occurrence is called a heart attack or an acute myocardial infarct (AMI) (Cahill & Kharbanda, 2017). Nowadays most of the people survive the initial AMI due to advancements in medical care. The introduction of primary percutaneous coronary interventions and the addition of improved drug therapy reduced the AMI mortality rate with 15 percent in the last 30 years (Cahill & Kharbanda, 2017). However, survivors of an infarct are six times more likely to lose their life to heart failure (HF) compared to people in the same age group without an infarct (World Health Organization, 2013).

An AMI is induced through a severe restriction or even blockage of the coronary artery. This blockage in most of the cases is caused by atherosclerosis, an inflammation induced plaque build-up in the artery. When this plaque ruptures due to mechanical stress (e.g. rise in blood pressure) a blood clot can form and severely reduce the oxygenated blood flow toward the heart muscle. If the blockage is severe enough acute ischemia will occur causing myocyte death (Institute of Medicine (US), 2010). The period after the infarct (acute ischemia phase) is commonly divided in three overlapping stages.

Inflammatory phase

The inflammatory and necrotic phase takes place during the first week after an AMI. The necrosis of the cardiomyocytes induces the wound healing cascade. This cascade is triggered by cardiac mast cells that release the pro-inflammatory cytokines TNFα, IL-1β and IL-6 after ischemia (Frangogiannis et al., 1998). TNFα induces the synthesis of chemokines and adhesion molecules in inflammatory leukocytes. Secreted and activated matrix metalloproteinases (MMP's) start to degrade matrix components due to the MMP's ability to cleave collagens, gelatins and proteins (Richardson et al., 2015). Simultaneously, chemokines of the C-C and C-X-C family and cytokine IL-1β mediate the mobilization of leukocytes like macrophages (Dewald et al., 2005). Macrophages will phagocytize the degraded material. A temporary tissue consisting fibrin, fibronectin, laminin and glycosaminoglycans will form as a support structure before fibrillar collagen is upregulated and become the primary component of the scar tissue (Holmes et al., 2005). During the formation of the temporary structure IL-1 inhibits the activation of myofibroblasts until the infarct zone is cleared from the dead myocytes and extra cellular matrix (ECM) components (Saxena et al., 2013).

Fibrotic phase

After the removal of the dead cells the repair response is activated by inhibiting inflammatory signaling. Due to high levels of IL-1, the IL-1 receptor-associated kinase 3 (IRAK-3) is induced. IRAK-3, found in macrophages and fibroblasts, inhibits cytokine expression and reduces the inflammatory responsiveness in cells (Kobayashi et al., 2002). When the inflammation is suppressed, mesenchymal cells migrate towards the infarct zone. Together with already present cardiac fibroblasts the cells proliferate and differentiate into myofibroblasts (Ma et al., 2014). The migration of the future myofibroblasts is induced by the activation TGF-β and the inhibition of pro-inflammatory cytokine IL-1. During an AMI, latent TGF-β that is stored in the myocardium is activated. Infiltrated leukocytes and fibroblasts also synthesize new TGF-β (Frangogiannis, 2014). The lowered inflammatory responsiveness causes TGF-β to stimulate myofibroblast transdifferentiation. TGF-β also upregulates the synthesis of TIMPs (Tissue inhibitors of MMPs). TIMPS inhibit the previously activated MMPs and thereby stimulation the increase of collagen (Sun & Weber, 2000). The differentiated myofibroblasts

start the expression of collagen (type I, III, IV and VI) around a week post infarction. The collagen content in the infarcted zone can increase 10-fold in the following weeks (Richardson et al., 2015).

Remodeling phase

In the final phase of scar development, the infarct scar matures. This phase is called the remodeling phase and lasts for a couple of months post infarction. A small number of myocytes survived and are present in the infarcted zone. Most of the processes that formed the scar stabilize including the expression of collagen. However, there is an increase in collagen crosslinking stimulated by the expression of lysyl oxidase (cross-linking enzyme). Together with the secretion of decorin and biglycan, proteoglycans that influence the organization of collagen, the cross-linking is essential for stabilization of collagen (Richardson et al., 2015). Due to the cross-linking the strength and stiffness of the infarct is increased, interrupting efficient cardiac contraction. The metabolic activity of the infarct zone decreases with the clearance of myofibroblasts and vascular cells. Both contributing to diastolic dysfunction and heart failure due to the disruption of the cardiac ECM changing the ventricular geometry (Dobaczewski et al., 2010).

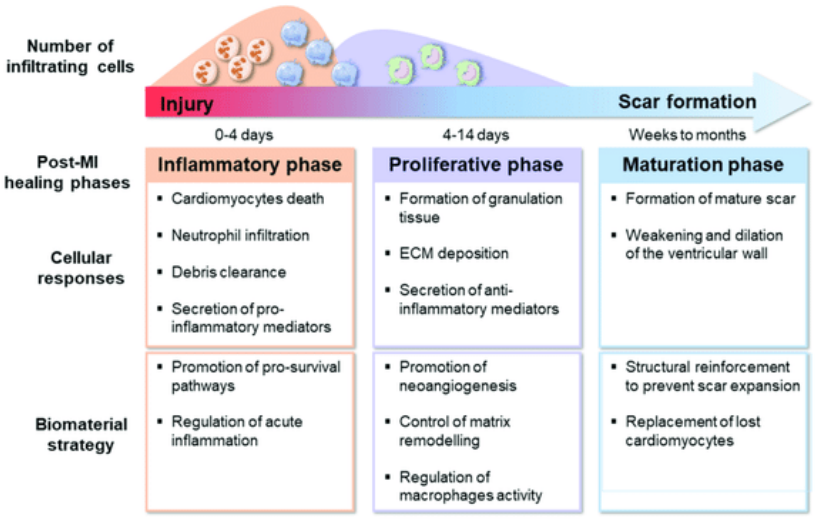

Figure 1: Cardiac response after an acute myocardial infarction. Cellular response: Displays response in inflammatory phase, fibrotic phase and remodeling phase. Biomaterial strategy: Therapeutic strategies per phase. (Ferrini et al., 2019)

Heart failure is the disability of the ventricular diastole or systole after a structural or functional cardiac disorder. HF can occur as a result of failure to restore the blood flow during the event of an AMI. This induces progressive cell death and contractile dysfunction. However, late-onset HF occurrence is also possible that due to the scar formation and ventricular remodeling (Cahill & Kharbanda, 2017). The scar tissue nevertheless prevents the ventricle wall from rupturing. But it does not have the same mechanical properties as myocardial tissue. In contrast to the myocardium it is not possible for scar tissue to contract or conduct electrical signals. It also stresses the heart muscle because there is less myocardial tissue that has to produce the same output resulting in compensatory hypertrophy (Ongstad & Gourdie, 2016).

While there are treatments for reducing the scar tissue most of them are focused on the intervention immediately after an AMI or during the first week post-AMI. After an AMI, the blood flow should be re-established to prevent further damage and eventually a smaller scar. This is realized by admitting blood clot dissolving drugs like streptokinase or urokinase. Too fully clear the clot, angioplasty is performed. A catheter with a balloon at its tip that is inflated at the location of the plaque. Causing

the artery to be expanded and the plaque to be flattened. When multiple blockages are present coronary bypass surgery is needed to restore reperfusion (Lu et al., 2015). Adequate and immediate treatment can decrease the infarct size, but scar tissue is still formed. Most of the current treatments are focused on reducing the infarct size. Targeting the inflammation response is thought to be a promising therapeutic intervention. The inhibition of pro-inflammatory signaling can prevent the cardiomyocytes from apoptosis. By inhibiting leukocyte signaling there is also a damage reduction in surviving cardiomyocytes. However, the inflammation cascade also plays an important role in the repair of the infarct. Thus, while preventing injury with anti-inflammation therapy the repair function of the cascade is also impaired. Resulting in a decrease in progenitor cells that stimulate angiogenesis in the infarct weakening the remaining cardiomyocytes. (Huang & Frangogiannis, 2018). Altering the balance of the inflammatory phase and fibrotic phase can eventually lead to an increase in tissue damage, scar formation and cell loss (Prabhu & Frangogiannis, 2016).

The research in cell-based regenerative therapies after the occurrence of an AMI has gained considerable interest. These therapies mainly focus on placing functional cardiomyocytes into the infarct zone. These functional cells are derived from multiple sources like embryonic stem cells or induced pluripotent stem cells (iPSC) as well as cardiac progenitor cells (CPC) and bone marrow-derived cells (BMCs). There is substantial disagreement which of these sources is optimal for therapy (Spath et al., 2016). Regardless, both sources can generate functional cardiomyocytes. In this article focus is laid on the application of stem cells not on their origin.

Another novel cardiac regeneration method is the usage of hydrogels. These can be used to distribute biomolecules that improve the affected cardiac tissue (e.g. VEGF, Periostin, TGF-B and Intermedin) or aid cell-based therapies. Hydrogels are semi-liquid solutions that can be used as scaffolds in tissue engineering as well as a drug delivery structure. The benefit of hydrogels is that it can be delivered to the desired tissue with a minimally invasive procedure (Peña et al., 2018).

The ultimate treatment of a post-AMI is replacement the scar tissue with a functional new myocardium. This article explores the possibility of post-AMI cardiac tissue regeneration primarily focused on cell-based regenerative therapy combined with supporting hydrogels. The therapy is focused after or during the remodeling phase since the patient needs the time to recover from the infarct. In addition, the ongoing processes stabilize during the final phase and thereby are more accurate to predict.

Injectable hydrogels to provide structural integrity and deliver biomolecules

Biomolecules and stem cells are an important factor in the regenerative treatment of an AMI. These are targeted to improving myocardial function and decreasing the influence of the scar tissue. However, the administration of these biomolecules brings difficulties such as a short half-life, nonspecific delivery and poor targeting towards the affected area (Peña et al., 2018). To overcome this problem, biomaterials that mimic the ECM are used to secure the biomolecules and stem cells against degradation. For example, by mimicking the ECM, the chance of implant-repulsion due to an immune response is reduced. Biomaterials also increase the survival rate and can improve the targeting (Truskey, 2016).

The constructs used for cardiac regeneration should not only improve cell retention but also mimic the biochemical and electromechanical function of the cardiac tissue. Additionally, it is beneficial that the biomaterial is biodegradable, biocompatible and holds matching mechanical properties as the myocardial tissue. Biocompatibility is important reduce the effect of the immune system willing to reject the material (K. Y. Ye & Black, 2011). When delivering the material to the infarcted zone it is

desired that this is done in a minimally invasive procedure. Further damage to myocardial tissue will only increase the change of HF.

There are three primary delivery methods used in cardiac regeneration. Biomaterial patches are scaffold like structures that can be fitted with cells and biomolecules. These scaffolds can be implanted on the heart wall (L. Ye et al., 2013). Another promising method are cell sheets cultured on a thermos-sensitive polymer. Due to the thermo-sensitivity it is possible to regulate the detachment of the cells. These sheets will be implanted on the heart wall (Matsuura et al., 2014). The last procedure is the injectable hydrogel, a liquid cross-linked polymer solution that transforms into a gel after a stimulus. The solution, which can contain biomolecules and cells, will be injected into the heart wall (Hasan et al., 2015).

Both procedures for biomaterial patches and cell sheets require invasive surgery. However, injectable hydrogels provide a minimally invasive procedure (catheter delivery) to achieve cardiac regeneration (Sepantafar et al., 2016). In addition to a favourable procedure, hydrogels also can be synthesized to consist needed biochemical and electromechanical functions (Peña et al., 2018). Therefore, it seems promising to focus on hydrogels in cardiac regeneration. Even though, hydrogels are lacking the biomechanical properties of cardiac tissue.

Hydrogels, as seen in *figure 2*, can be constructed with natural polymers (fibrin, collagen and chitosan), synthetic polymers (PLGA and PEG) or a combination of both. Natural hydrogels contain a high biocompatibility due to preservation of the biochemical properties of the polymers. However, they provide little control over the mechanical properties being conductivity and antioxidant abilities. In contrary, synthetic hydrogels lack biocompatibility but are more accessible to control mechanically. Furthermore, synthetic polymers are more stable and reproducible. Combining properties of both hydrogels is most likely the best option to comprise the advantages of natural and synthetic gels (Peña et al., 2018). For the cardiac regeneration, hydrogels should be used as a supportive structure to increase the survivability of the stem cells. Moreover, it should also contain biomolecules that promote cell growth and decrease the scar tissue.

During a 2012 study (Wang et al., 2012), the hydrogel OPF (oligo(poly(ethylene glycol) fumarate) combined with encapsulated mESCs significantly decreased the infarct size and collagen contents and overall improving cardiac function. OPF is a hydrogel made from fumaryl chloride and polyethylene glycol (PEG). The OPF hydrogel was encapsulated with mouse embryonic stem cells (mESC) and was injected in rats 7 days post-induced MI. The study was divided into four distinct groups, injection surgery of PBS, OPF, PBS + mESC and OPF + mESC. It was demonstrated that the OPF increased the survivability as well as the retention of the mESCs. mESCs encapsulated in OPF also inhibited ventricle remodelling after infarct improving the cardiac function. This improvement is probably induced by the structural and mechanical support that the hydrogel provides. It is remarkable that the hydrogel promotes regeneration of the infarcted tissue without the addition of cells or growth factors (OPF group). Besides the positive effects of OPF it also possesses useful properties. With thermal radical initiators, compounds that generate cations or radicals during heat exposure, added to the hydrogel it is possible to crosslink OPF at 37 degrees Celsius. Causing the hydrogel solution to solidify after exposure to body temperature (Wang et al., 2012). OPF contains a high-water content making it water soluble. It is also more biodegradable and biocompatible than natural biomaterials (Hui et al., 2013). According to a study in 2018 it is possible to create conductivity in the OPF hydrogel by adding graphene oxide nanoparticles (GO). After injection of the OPF/GO hydrogel improves conductivity between the healthy myocardium and cardiomyocytes in the infarcted scar tissue. The conductive hydrogels also promote cell adhesion, cell alignment and interaction between

cells (Zhou et al., 2018). However, the mechanisms responsible for the improvement of cardiac function after OPF/GO injection are unknown and still under investigation.

Besides functioning as a structural component, the hydrogel can also be used to deliver molecules to the infarcted area. To return the contractile function after an AMI not only the myocardium has to be restored but also the scar tissue has to be removed. Since this regenerative approach is focused on treatment during and after the scar tissue maturation, regenerated myocytes are likely to be surrounded by a higher collagen content (French & Holmes, 2019). The removal of collagen should be done carefully because the post-AMI fibrosis is a reparative and strengthening process for the heart. And interruption could cause an increased chance of heart failure (Jugdutt, 2009). After scar maturation the deposited collagen is accumulated and cross-linked, stiffening the scar structure. The insertion of a structure providing hydrogel combined with encapsulated stem cells can stimulate and contribute to the formation of a healthy myocardium on top of the scar tissue. Theoretically it could be possible to degrade the cross-linked collagen from the scar structure without the risk of post-MI rupture. Due to the regenerated myocardium that created a new supporting ECM. A mature scar predominantly consists of collagen I and III, these are the same types of collagen found in a healthy heart. However collagen levels in a post-AMI heart are increased significantly (Richardson et al., 2015). The myocardial fibrosis is linked to increased levels of MMP2 and MMP9 expressed by cardiac fibroblasts. After depositing collagen, fibroblasts leave the scar tissue or go into apoptosis (Vellaichamy et al., 2005). The increased collagen shows that MMPs do not only degrade ECM, but it can also promote production of ECM proteins by activation of TGFβ. Matrix metalloproteinases are divided in 23 enzymes in humans. Normally, MMPs remain in a low active state. However, the transcription is induced by inflammation. Apart from ECM molecule removal MMPs can also affect cell migration, growth factors, apoptosis, cytokine release and more (Nagase et al., 2006). MMP activity is regulated at multiple extents. This is done at gene expression, MMP release from the cell, enzyme activation and by specific and non-specific inhibitors. Because MMPs are part of an important signalling network in tissues, uncontrolled MMP release can be detrimental to tissues (Löffek et al., 2011). This control is in vitro regulated by the cardiac fibroblasts.

Cardiac fibroblasts (CFBs) primarily maintain the structure of the cardiac ECM but also transduce electrical and mechanical signals (Humeres & Frangogiannis, 2019). CFBs produce not only ECM molecules but also the regulatory proteins TIMPs and MMPs. These are critical to maintain ECM homeostasis (Krenning et al., 2010). However, the function of CBFs can also be changed by TIMPs and MMPs. It is shown that by cleaving collagen type I fibrosis can be triggered. A Membrane-Type MMP (MT1-MMP) can cleave and activate the inflammatory TGFβ causing new collagen production (Zavadzkas et al., 2011). Post-AMI, the homeostasis is changed since there are no fibroblast expressing MMPs or TIMPs (Fan et al., 2012). It could be possible to return these cells and restore the homeostasis and decrease the collagen deposition in the matured scar tissue. CFBs are generated from mesenchymal cells during the epithelial-mesenchymal transition. After myocardial injury CFBs can be derived from recruited bone marrow progenitor cells, monocytes and fibrocytes (Krenning et al., 2010). The transition to CFBs is induced by the proteins periostin and TGFβ. Periostin is essential in regulating the mechanical properties of the myocardium (Norris et al., 2008). TGFβ stimulates the epithelial-mesenchymal transformation and induces the transition to CFBs by activation of ALK2 (TGFβ receptors) (Olivey et al., 2006). Adding both periostin and TGFβ to the hydrogel, cardiac fibroblasts could be recruited and possibly return the homeostasis by decreasing collagen in the scar tissue.

Figure 2: Overview on the beneficial components of the OPF/GO hydrogel

In conclusion, the hydrogel is supposed to provide structure to the stem cells thereby improving survival rates. Besides the structural component, the hydrogel should also be used to deliver periostin and TGFβ to return cardiac homeostasis. By adding graphene oxide to the OPF gel, conductivity between healthy myocardium and infarct affected myocytes will be increased overall improving cardiac function as seen in *figure 2*.

Regenerating infarcted myocardium with stem cells

To increase the contractile function of a post-AMI ventricle, lost cardiomyocytes have to be replaced. Without these cardiomyocytes, the working capacity of the heart will decrease, and the morbidity of the patient will increase (Pfeffer & Braunwald, 1990). Because cardiomyocytes are not able to self-regenerate, stem cells can be used as a therapeutic intervention. In scientific literature there are found two groups focusing both on different types of stem cell therapy.

The first group of stem cells are targeted on remuscularizing the scar tissue. These stem cells are transplants of cells with the ability to form myocardium, like embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs). Furthermore, precursors from cardiomyocytes can also be used (Golpanian et al., 2016). Studies have shown that it is possible to engraft these cells and that the cells successfully differentiate into cardiomyocytes in rodents. However, the differentiation of ESCs into cardiomyocytes did not correlate with a functional improvement of the left ventricle (Riegler et al., 2015). In a non-human primate model, it is also displayed that ESC-cardiomyocytes can be produced and preserved. The cardiomyocytes displayed gradual but incomplete maturation after three months. These cells showed that a small vascular system was established between the host cells and the graft as well as a regular ECG output. But ventricular arrhythmias were seen in the graft, most likely due to asymmetry between conduction velocities between host and graft. Making it susceptible to HF (Chong et al., 2014). The remuscularization of the scar tissue seems viable but there are still questions if this model is ample for cardiac repair. Since some long term studies fail to show improvement of the cardiac function after engraftment (Golpanian et al., 2016).

The second group focuses on inducing the internal repair mechanism with the engraftment of stem cells that have less capacity to form myocytes but are used to stimulate cardiomyogenisis from host cells (Hatzistergos & Hare, 2015). In this group mesenchymal stem cells (MSCs), bone marrow mononuclear cells (BMCs) and cardiac progenitor cells (CPCs) are used to establish interactions with the host myocardium inducing vasculogenisis and cardiomyogenisis due to differentiation of MSCs (Quevedo et al., 2009). A 2010 study in swine demonstrates that MSCs derived from the bone marrow stimulate myocardial repair through cell-autonomous response and differentiation. The MSCs differentiate into cardiomyocytes directly after transplantation which contribute to an 8% increase in new myocardium. This also induces endogenous cardiac repair and a decrease in scar tissue. It should be noted that the delivery of MSCs was done 3 days post-AMI. However, there is no consensus if timing has an substantial impact on the treatment (Hatzistergos et al., 2010). Bone marrow cells engraftment in mice also show an increase in newly formed myocardium. As well as the

development of proliferating myocytes and neovascularization (Orlic et al., 2001). Compared to remuscularizing the scar tissue, this therapy leads to a lower amount of MSCs but there is a significant increase in the regeneration of the heart function.

Overall, MSCs are the most promising candidate for cardiac regeneration because they are relatively easy to isolate from a biopsy of the bone marrow or the cardiac stroma. MSCs are found in nearly every tissue, contributing towards maintenance and homeostasis. In the human body MSCs also show immune tolerance creating the option to use allogenic MSCs without tissue rejection. Furthermore, MSCs are capable to differentiate into cardiomyocytes, endothelial cells and vascular smooth muscle cells which are all important in cardiac repair. However, the efficiency of the MSCs engraftments are still low and need improvement before they are deemed feasible for treatment (White et al., 2016).

Due to the absence of the Major Histocompatibility Complex Type-II (MHC-II), there is no allogeneic tissue rejection. But MSCs also lack MHC-I making them susceptible for natural killer cells. However, studies show that there is no difference between autologous and allogenic MSCs (Rasmusson et al., 2003). This is explained due to a lower expression of receptors on the MSC membrane that naturally stimulate natural killer cells into cell lysis (Spaggiari et al., 2008). Additionally, pro-inflammatory cytokines released by the immune system are suppressed by MSCs. Antigen-presenting dendritic cells in the innate immune system are downregulated by MSCs. Dendritic cells produce tumor necrosis factor α (TNF-α), a pro-inflammatory cytokine, when MHCs are presented. However, the production of the cytokine is suppressed through the presence of MSCs due to the capability of inhibiting TNF-α producing regulatory T cells (Aggarwal & Pittenger, 2005).

Apart from the immunosuppressive properties, MSCs are also able to synthesize MMPs and TIMPs. Making them proficient in cardiac remodelling, degrading the fibrotic scar tissue and stimulate angiogenesis (Traktuev et al., 2006). By addition of factors that promote MMP expression it can be possible to reverse the maturation of the fibrotic tissue. When heme oxygenase-1 (HO-1) is presented to MSCs and injected in an infarcted myocardium, cardiac function was improved significantly. As well as an increase in peripheral blood vessels (Shu et al., 2010). Even though the MSCs were engrafted one hour after AMI, days before maturation of the scar tissue. This shows the possibility of remodelling the infarcted heart by stimulating MSCs with regenerative factors. Furthermore, CFBs are also regulated by MSCs, counteracting the fibrotic mechanism of the CFBs. Stimulating the return of homeostasis and the degradation of fibrotic tissue through the release of TIMPs and MMPs (Mias et al., 2009). The improved angiogenesis after engraftment with MSCs is due to the fact that it can also differentiate into endothelial and vascular smooth muscle cells. While MSCs also produce proangiogenic factors like VEGF and other angiogenic molecules (Merfeld-Clauss et al., 2010).

MSCs are capable of differentiate into multiple cell types like epithelial cells, osteoblasts, pulmonary cells and adipocytes. Numerous factors can be used to mediate the differentiation process. For example, addition of VEGF mediates the differentiation into osteoblasts and adipocytes (Liu et al., 2012). For the regenerative treatment of an AMI the differentiation of MSCs into cardiomyocytes is the most important. To achieve this, *ex-vivo* studies present that MSCs can be exposed to growth factors like bone morphogenetic protein-2 (BMP-2), fibroblast growth factor-4 (FGF-4) and antioxidants to form cardiomyocytes. The derived cardiomyocytes show the expression of cardiac transcription factors and form cell-cell interaction with already present myocytes (Shim et al., 2004). The addition of growth factor can be used to precondition the MSCs before implanting. However, the engraftment of MSCs *in vivo* in the infarcted zone also demonstrates differentiation into cardiomyocyte like cells without supplemental growth factors. Although this is researched in swine, it

displays that growth factors are already present around the infarcted zone (Kamihata et al., 2001). Unfortunately, when implanted in humans only a three to four percent increase in heart function is measured, most likely caused by the low survival rate and poor differentiation of the engrafted MSCs (Roura et al., 2017).

In a recent study bone marrow human-MSCs (BM-hMCs) are primed to increase survival and retention. This is done by the incorporation of genetically modified HGF releasing MSCs with BM-MSCs in a hydrogel. HGF has the property of being an antiapoptotic factor. Therefore, increasing the survival rate of BM-MSCs (Park et al., 2020). The results showed a longer survival period of the BM-MSCs and an increase in the activation of antifibrotic and angiogenic pathways through HGF. This treatment looks to be promising in treating and regenerating the infarcted heart.

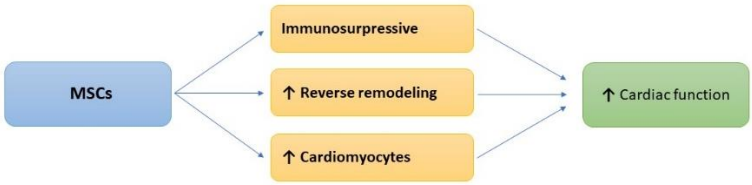

Figure 3: Overview of MSC capabilities ultimately improving cardiac function

In short, the ability to differentiate into multiple cell types like cardiomyocytes and endothelial cells is beneficial for maintaining all aspects of cardiac repair. The lack of allogenic rejection due to the absence of MHC-II is also positive. An overview of the MSCs functions is found in *figure 3.* These findings combined with the MSCs induced increase of cardiac function make them feasible for regenerative therapies. However, the efficiency of the MSCs needs an enhancement before viable.

Discussion

Therapeutic interventions after an AMI have been researched intensively. Most of the research focusses on inhibiting the inflammatory response in the inflammatory phase. This article focused on regenerative intervention post-fibrotic phase. To intervene after maturation of the scar brings advantages and disadvantages. The possibility of HF will increase when waiting for the infarct cascade to have settled. But intervening before maturation can disrupt the balance in the healing process and causing an increase in tissue damage (Prabhu & Frangogiannis, 2016). Intervening after the maturation process is therefore more viable. By combining the regeneration of cardiomyocytes together with the degradation of scarred tissue an increase in heart function can be achieved but research is needed. To accomplish this, an OPF/GO hydrogel with encapsulated MSCs should be injected on to the infarcted zone. This article displays which types of hydrogel and stem cells are the most promising to achieve this goal. Hydrogel can be used to service not only as structure for the MSCs but also to improve conductivity within the infarcted zone. In combination with CFB recruiting factors tissue homeostasis can possibly return. The MSCs show a high potential in increasing heart function and tissue regeneration. However, in vivo studies demonstrated a low survival and retention rate after implantation. By exposing the MSCs to antiapoptotic factors this rate can be increased significantly. By encapsulating the MSCs in a hydrogel the retention rate will improve as well. Together with the possibility to release growth factors through the hydrogel, a combination of both MSC and hydrogel seems unavoidable.

The OPF/GO hydrogel provides both structure and an improvement in conductivity as well as a higher biocompatibility and biodegradability. Improved conductivity is thought to have beneficial effects on the regeneration, the mechanism behind it is not yet fully understood. OPF/GO gel is only used in combination with embryonic stem cells. However, several papers show that OPF gels are compatible

with MSCs (Zhou et al., 2018). The hydrogel will cross-link and solidify after exposure to 37 degrees Celsius. Making it useful in implanting in the human body without the addition of external factors. The OPF/GO hydrogel could be the desired material for cardiac regeneration due to the high biocompatibility and biodegradability, together with the conductive properties.

Returning the lost ECM homeostasis in the infarcted zone is important. In the heart homeostasis is mediated by cardiac fibroblasts. After scar maturation the fibroblasts cease expressing both MMPs and TIMPs. By exposing MSCs to periostin and TGFβ, cardiac fibroblasts can be recruited through the epithelial-mesenchymal transition (Moustakas & Heldin, 2016). The exposure can be facilitated by releasing both periostin and TGFβ through the hydrogel. There is a lot of research done towards the effect of cardiac fibroblast induced homeostasis in a healthy myocardium. However, the influence of returning homeostasis through fibroblast in fibrotic tissue after an AMI is not fully understood. There is a possibility that fibroblasts will not reduce the fibrotic tissue resulting in a sustained compensatory hypertrophy. Nonetheless, when combined with the MSCs and the OPGF/GO hydrogel cardiac function will improve.

Implanted MSCs showed to have the most potential in developing new cardiomyocytes and regenerate myocardium post-AMI. Due to their immunosuppressive nature it is possible to use allogeneic MSCs without tissue rejection. After implanting the MSCs secrete antifibrotic and angiogenic factors increasing the viability of the tissue (Traktuev et al., 2006). These antifibrotic factors also regulate the cardiac fibroblasts making it unnecessary for external regulatory factors. Research has shown that implanted MSCs are not capable of surviving for an extended period of time in the graft (Roura et al., 2017). However, with the addition of HGF releasing MSCs in a hydrogel, the survival time can increase significantly. Improving the regeneration of the myocardium.

It is important to understand that most of the information stated results form research where is intervened directly post-AMI. It is possible that these results do not correlate if these therapeutic interventions are used when the infarcted scar tissue has matured. However, some cardiomyocytes still survive in the scar tissue (Richardson et al., 2015). Creating the impression that it is possible to still intervene and regenerate the infarcted tissue.

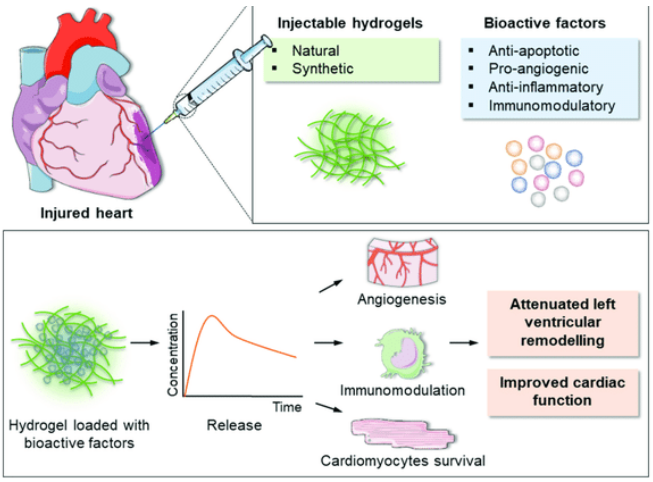

Figure 4: Schematic overview of components in injectable hydrogel. A synthetic OPF/GO gel will be used as an injectable hydrogel. Hydrogels are treated with Periostin, TGFβ and HGF as bioactive factors. Encapsulated MSCs will be present in the hydrogel (Not shown in figure). (Ferrini et al., 2019)

These findings demonstrate that the concept of an OPF/GO hydrogel combined with HGF releasing MSCs and fibroblast recruiting factors can regenerate the infarcted myocardium and decrease fibrotic tissue. Overall improving heart function and decreasing morbidity. However, many mechanisms in this concept are still unknown and need thorough research.

References

Aggarwal, S., & Pittenger, M. F. (2005). Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood*, *105*(4), 1815–1822. https://doi.org/10.1182/blood-2004-04-1559

Cahill, T. J., & Kharbanda, R. K. (2017). Heart failure after myocardial infarction in the era of primary percutaneous coronary intervention: Mechanisms, incidence and identification of patients at risk. *World Journal of Cardiology*, *9*(5), 407–415. https://doi.org/10.4330/wjc.v9.i5.407

Chong, J. J. H., Yang, X., Don, C. W., Minami, E., Liu, Y. W., Weyers, J. J., Mahoney, W. M., Van Biber, B., Cook, S. M., Palpant, N. J., Gantz, J. A., Fugate, J. A., Muskheli, V., Gough, G. M., Vogel, K. W., Astley, C. A., Hotchkiss, C. E., Baldessari, A., Pabon, L., … Murry, C. E. (2014). Human embryonic-stem-cell-derived cardiomyocytes regenerate non-human primate hearts. *Nature*, *510*(7504), 273–277. https://doi.org/10.1038/nature13233

Dewald, O., Zymek, P., Winkelmann, K., Koerting, A., Ren, G., Abou-Khamis, T., Michael, L. H., Rollins, B. J., Entman, M. L., & Frangogiannis, N. G. (2005). CCL2/monocyte chemoattractant protein-1 regulates inflammatory responses critical to healing myocardial infarcts. *Circulation Research*, *96*(8), 881–889. https://doi.org/10.1161/01.RES.0000163017.13772.3a

Dobaczewski, M., Gonzalez-Quesada, C., & Frangogiannis, N. G. (2010). The extracellular matrix as a modulator of the inflammatory and reparative response following myocardial infarction. In *Journal of Molecular and Cellular Cardiology* (Vol. 48, Issue 3, pp. 504–511). NIH Public Access. https://doi.org/10.1016/j.yjmcc.2009.07.015

Fan, D., Takawale, A., Lee, J., & Kassiri, Z. (2012). Cardiac fibroblasts, fibrosis and extracellular matrix remodeling in heart disease. In *Fibrogenesis and Tissue Repair* (Vol. 5, Issue 1, pp. 1–13). BioMed Central. https://doi.org/10.1186/1755-1536-5-15

Ferrini, A., Stevens, M. M., Sattler, S., & Rosenthal, N. (2019). Toward Regeneration of the Heart: Bioengineering Strategies for Immunomodulation. In *Frontiers in Cardiovascular Medicine* (Vol. 6). Frontiers Media S.A. https://doi.org/10.3389/fcvm.2019.00026

Frangogiannis, N. G. (2014). The inflammatory response in myocardial injury, repair, and remodelling. In *Nature Reviews Cardiology* (Vol. 11, Issue 5, pp. 255–265). Nature Publishing Group. https://doi.org/10.1038/nrcardio.2014.28

Frangogiannis, N. G., Lindsey, M. L., Michael, L. H., Youker, K. A., Bressler, R. B., Mendoza, L. H., Spengler, R. N., Smith, C. W., & Entman, M. L. (1998). Resident cardiac mast cells degranulate and release preformed TNF-α, initiating the cytokine cascade in experimental canine myocardial ischemia/reperfusion. *Circulation*, *98*(7), 699–710. https://doi.org/10.1161/01.CIR.98.7.699

French, B. A., & Holmes, J. W. (2019). Implications of scar structure and mechanics for post-infarction cardiac repair and regeneration. In *Experimental Cell Research* (Vol. 376, Issue 1, pp. 98–103). Elsevier Inc. https://doi.org/10.1016/j.yexcr.2019.01.001

Golpanian, S., Wolf, A., Hatzistergos, K. E., & Hare, J. M. (2016). Rebuilding the damaged heart: Mesenchymal stem cells, cell-based therapy, and engineered heart tissue. *Physiological Reviews*, *96*(3), 1127–1168. https://doi.org/10.1152/physrev.00019.2015

Hasan, A., Khattab, A., Islam, M. A., Hweij, K. A., Zeitouny, J., Waters, R., Sayegh, M., Hossain, M. M., & Paul, A. (2015). Injectable Hydrogels for Cardiac Tissue Repair after Myocardial Infarction. In *Advanced Science* (Vol. 2, Issue 11, p. 1500122). Wiley-VCH Verlag. https://doi.org/10.1002/advs.201500122

Hatzistergos, K. E., & Hare, J. M. (2015). Cell therapy: Targeting endogenous repair versus remuscularization. In *Circulation Research* (Vol. 117, Issue 8, pp. 659–661). Lippincott Williams

and Wilkins. https://doi.org/10.1161/CIRCRESAHA.115.307346

Hatzistergos, K. E., Quevedo, H., Oskouei, B. N., Hu, Q., Feigenbaum, G. S., Margitich, I. S., Mazhari, R., Boyle, A. J., Zambrano, J. P., Rodriguez, J. E., Dulce, R., Pattany, P. M., Valdes, D., Revilla, C., Heldman, A. W., McNiece, I., & Hare, J. M. (2010). Bone marrow mesenchymal stem cells stimulate cardiac stem cell proliferation and differentiation. *Circulation Research*, *107*(7), 913–922. https://doi.org/10.1161/CIRCRESAHA.110.222703

Holmes, J. W., Borg, T. K., & Covell, J. W. (2005). Structure and Mechanics of Healing Myocardial Infarcts. *Annual Review of Biomedical Engineering*, *7*(1), 223–253. https://doi.org/10.1146/annurev.bioeng.7.060804.100453

Huang, S., & Frangogiannis, N. G. (2018). Anti-inflammatory therapies in myocardial infarction: failures, hopes and challenges. In *British Journal of Pharmacology* (Vol. 175, Issue 9, pp. 1377–1400). John Wiley and Sons Inc. https://doi.org/10.1111/bph.14155

Hui, J. H., Ren, X., Afizah, M. H., Chian, K. S., & Mikos, A. G. (2013). Oligo[poly(ethylene glycol)fumarate] hydrogel enhances osteochondral repair in porcine femoral condyle defects knee. *Clinical Orthopaedics and Related Research*, *471*(4), 1174–1185. https://doi.org/10.1007/s11999-012-2487-0

Humeres, C., & Frangogiannis, N. G. (2019). Fibroblasts in the Infarcted, Remodeling, and Failing Heart. In *JACC: Basic to Translational Science* (Vol. 4, Issue 3, pp. 449–467). Elsevier Inc. https://doi.org/10.1016/j.jacbts.2019.02.006

Institute of Medicine (US). (2010). Cardiovascular disability: Updating the social security listings. In *Cardiovascular Disability: Updating the Social Security Listings*. National Academies Press (US). https://doi.org/10.17226/12940

Jugdutt, B. I. (2009). Limiting fibrosis after myocardial infarction. *New England Journal of Medicine*, *360*(15), 1567–1569. https://doi.org/10.1056/NEJMcibr0810801

Kamihata, H., Matsubara, H., Nishiue, T., Fujiyama, S., Tsutsumi, Y., Ozono, R., Masaki, H., Mori, Y., Iba, O., Tateishi, E., Kosaki, A., Shintani, S., Murohara, T., Imaizumi, T., & Iwasaka, T. (2001). Implantation of Bone Marrow Mononuclear Cells Into Ischemic Myocardium Enhances Collateral Perfusion and Regional Function via Side Supply of Angioblasts, Angiogenic Ligands, and Cytokines. *Circulation*, *104*(9), 1046–1052. https://doi.org/10.1161/hc3501.093817

Kobayashi, K., Hernandez, L. D., Galán, J. E., Janeway, C. A., Medzhitov, R., & Flavell, R. A. (2002). IRAK-M is a negative regulator of Toll-like receptor signaling. *Cell*, *110*(2), 191–202. https://doi.org/10.1016/S0092-8674(02)00827-9

Krenning, G., Zeisberg, E. M., & Kalluri, R. (2010). The origin of fibroblasts and mechanism of cardiac fibrosis. In *Journal of Cellular Physiology* (Vol. 225, Issue 3, pp. 631–637). NIH Public Access. https://doi.org/10.1002/jcp.22322

Liu, Y., Berendsen, A. D., Jia, S., Lotinun, S., Baron, R., Ferrara, N., & Olsen, B. R. (2012). Intracellular VEGF regulates the balance between osteoblast and adipocyte differentiation. *Journal of Clinical Investigation*, *122*(9), 3101–3113. https://doi.org/10.1172/JCI61209

Löffek, S., Schilling, O., & Franzke, C. W. (2011). Series “matrix metalloproteinases in lung health and disease” edited by J. Müller-Quernheim and O. Eickelberg number 1 in this series: Biological role of matrix metalloproteinases: A critical balance. In *European Respiratory Journal* (Vol. 38, Issue 1, pp. 191–208). European Respiratory Society. https://doi.org/10.1183/09031936.00146510

Lu, L., Liu, M., Sun, R. R., Zheng, Y., & Zhang, P. (2015). Myocardial Infarction: Symptoms and

Treatments. *Cell Biochemistry and Biophysics*, *72*(3), 865–867. https://doi.org/10.1007/s12013-015-0553-4

Ma, Y., De Castro Brás, L. E., Toba, H., Iyer, R. P., Hall, M. E., Winniford, M. D., Lange, R. A., Tyagi, S. C., & Lindsey, M. L. (2014). Myofibroblasts and the extracellular matrix network in post-myocardial infarction cardiac remodeling. In *Pflugers Archiv European Journal of Physiology* (Vol. 466, Issue 6, pp. 1113–1127). Springer Verlag. https://doi.org/10.1007/s00424-014-1463-9

Matsuura, K., Masuda, S., & Shimizu, T. (2014). Cell sheet-based cardiac tissue engineering. *Anatomical Record*, *297*(1), 65–72. https://doi.org/10.1002/ar.22834

Merfeld-Clauss, S., Gollahalli, N., March, K. L., & Traktuev, D. O. (2010). Adipose tissue progenitor cells directly interact with endothelial cells to induce vascular network formation. *Tissue Engineering - Part A*, *16*(9), 2953–2966. https://doi.org/10.1089/ten.tea.2009.0635

Mias, C., Lairez, O., Trouche, E., Roncalli, J., Calise, D., Seguelas, M. H., Ordener, C., Piercecchi-Marti, M. D., Auge, N., Salvayre, A. N., Bourin, P., Parini, A., & Cussac, D. (2009). Mesenchymal stem cells promote matrix metalloproteinase secretion by cardiac fibroblasts and reduce cardiac ventricular fibrosis after myocardial infarction. *Stem Cells*, *27*(11), 2734–2743. https://doi.org/10.1002/stem.169

Moustakas, A., & Heldin, C.-H. (2016). Mechanisms of TGFβ-Induced Epithelial–Mesenchymal Transition. *Journal of Clinical Medicine*, *5*(7), 63. https://doi.org/10.3390/jcm5070063

Nagase, H., Visse, R., & Murphy, G. (2006). Structure and function of matrix metalloproteinases and TIMPs. In *Cardiovascular Research* (Vol. 69, Issue 3, pp. 562–573). Oxford Academic. https://doi.org/10.1016/j.cardiores.2005.12.002

Norris, R. A., Borg, T. K., Butcher, J. T., Baudino, T. A., Banerjee, I., & Markwald, R. R. (2008). Neonatal and adult cardiovascular pathophysiological remodeling and repair: Developmental role of periostin. *Annals of the New York Academy of Sciences*, *1123*, 30–40. https://doi.org/10.1196/annals.1420.005

Olivey, H. E., Mundell, N. A., Austin, A. F., & Barnett, J. V. (2006). Transforming growth factor-β stimulates epithelial-mesenchymal transformation in the proepicardium. *Developmental Dynamics*, *235*(1), 50–59. https://doi.org/10.1002/dvdy.20593

Ongstad, E. L., & Gourdie, R. G. (2016). Can heart function lost to disease be regenerated by therapeutic targeting of cardiac scar tissue? In *Seminars in Cell and Developmental Biology* (Vol. 58, pp. 41–54). Academic Press. https://doi.org/10.1016/j.semcdb.2016.05.020

Orlic, D., Kajstura, J., Chimenti, S., Jakoniuk, I., Anderson, S. M., Li, B., Pickel, J., McKay, R., Nadal-Ginard, B., Bodine, D. M., Leri, A., & Anversa, P. (2001). Bone marrow cells regenerate infarcted myocardium. *Nature*, *410*(6829), 701–705. https://doi.org/10.1038/35070587

Park, B. W., Jung, S. H., Das, S., Lee, S. M., Park, J. H., Kim, H., Hwang, J. W., Lee, S., Kim, H. J., Kim, H. Y., Jung, S., Cho, D. W., Jang, J., Ban, K., & Park, H. J. (2020). In vivo priming of human mesenchymal stem cells with hepatocyte growth factor–engineered mesenchymal stem cells promotes therapeutic potential for cardiac repair. *Science Advances*, *6*(13). https://doi.org/10.1126/sciadv.aay6994

Peña, B., Laughter, M., Jett, S., Rowland, T. J., Taylor, M. R. G., Mestroni, L., & Park, D. (2018). Injectable Hydrogels for Cardiac Tissue Engineering. In *Macromolecular Bioscience* (Vol. 18, Issue 6, p. e1800079). Wiley-VCH Verlag. https://doi.org/10.1002/mabi.201800079

Pfeffer, M. A., & Braunwald, E. (1990). Ventricular remodeling after myocardial infarction: Experimental observations and clinical implications. *Circulation*, *81*(4), 1161–1172.

https://doi.org/10.1161/01.CIR.81.4.1161

Prabhu, S. D., & Frangogiannis, N. G. (2016). The biological basis for cardiac repair after myocardial infarction. In *Circulation Research* (Vol. 119, Issue 1, pp. 91–112). Lippincott Williams and Wilkins. https://doi.org/10.1161/CIRCRESAHA.116.303577

Quevedo, H. C., Hatzistergos, K. E., Oskouei, B. N., Feigenbaum, G. S., Rodriguez, J. E., Valdes, D., Pattany, P. M., Zambrano, J. P., Hu, Q., McNiece, I., Heldman, A. W., & Hare, J. M. (2009). Allogeneic mesenchymal stem cells restore cardiac function in chronic ischemic cardiomyopathy via trilineage differentiating capacity. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(33), 14022–14027. https://doi.org/10.1073/pnas.0903201106

Rasmusson, I., Ringdén, O., Sundberg, B., & Le Blanc, K. (2003). Mesenchymal stem cells inhibit the formation of cytotoxic T lymphocytes, but not activated cytotoxic T lymphocytes or natural killer cells. *Transplantation*, *76*(8), 1208–1213. https://doi.org/10.1097/01.TP.0000082540.43730.80

Richardson, W. J., Clarke, S. A., Alexander Quinn, T., & Holmes, J. W. (2015). Physiological implications of myocardial scar structure. *Comprehensive Physiology*, *5*(4), 1877–1909. https://doi.org/10.1002/cphy.c140067

Riegler, J., Tiburcy, M., Ebert, A., Tzatzalos, E., Raaz, U., Abilez, O. J., Shen, Q., Kooreman, N. G., Neofytou, E., Chen, V. C., Wang, M., Meyer, T., Tsao, P. S., Connolly, A. J., Couture, L. A., Gold, J. D., Zimmermann, W. H., & Wu, J. C. (2015). Human engineered heart muscles engraft and survive long term in a rodent myocardial infarction model. *Circulation Research*, *117*(8), 720–730. https://doi.org/10.1161/CIRCRESAHA.115.306985

Roura, S., Gálvez-Montón, C., Mirabel, C., Vives, J., & Bayes-Genis, A. (2017). Mesenchymal stem cells for cardiac repair: Are the actors ready for the clinical scenario? In *Stem Cell Research and Therapy* (Vol. 8, Issue 1). BioMed Central Ltd. https://doi.org/10.1186/s13287-017-0695-y

Saxena, A., Chen, W., Su, Y., Rai, V., Uche, O. U., Li, N., & Frangogiannis, N. G. (2013). IL-1 Induces Proinflammatory Leukocyte Infiltration and Regulates Fibroblast Phenotype in the Infarcted Myocardium. *The Journal of Immunology*, *191*(9), 4838–4848. https://doi.org/10.4049/jimmunol.1300725

Sepantafar, M., Maheronnaghsh, R., Mohammadi, H., Rajabi-Zeleti, S., Annabi, N., Aghdami, N., & Baharvand, H. (2016). Stem cells and injectable hydrogels: Synergistic therapeutics in myocardial repair. In *Biotechnology Advances* (Vol. 34, Issue 4, pp. 362–379). Elsevier Inc. https://doi.org/10.1016/j.biotechadv.2016.03.003

Shim, W. S. N., Jiang, S., Wong, P., Tan, J., Chua, Y. L., Seng Tan, Y., Sin, Y. K., Lim, C. H., Chua, T., Teh, M., Liu, T. C., & Sim, E. (2004). Ex vivo differentiation of human adult bone marrow stem cells into cardiomyocyte-like cells. *Biochemical and Biophysical Research Communications*, *324*(2), 481–488. https://doi.org/10.1016/j.bbrc.2004.09.087

Shu, T., Zeng, B., Ren, X., & Li, Y. (2010). HO-1 modified mesenchymal stem cells modulate MMPs/TIMPs system and adverse remodeling in infarcted myocardium. *Tissue and Cell*, *42*(4), 217–222. https://doi.org/10.1016/j.tice.2010.04.004

Spaggiari, G. M., Capobianco, A., Abdelrazik, H., Becchetti, F., Mingari, M. C., & Moretta, L. (2008). Mesenchymal stem cells inhibit natural killer-cell proliferation, cytotoxicity, and cytokine production: Role of indoleamine 2,3-dioxygenase and prostaglandin E2. *Blood*, *111*(3), 1327–1333. https://doi.org/10.1182/blood-2007-02-074997

Spath, N. B., Mills, N. L., & Cruden, N. L. (2016). Novel cardioprotective and regenerative therapies in

acute myocardial infarction: A review of recent and ongoing clinical trials. In *Future Cardiology* (Vol. 12, Issue 6, pp. 655–672). Future Medicine Ltd. https://doi.org/10.2217/fca-2016-0044

Sun, Y., & Weber, K. T. (2000). Infarct scar: A dynamic tissue. In *Cardiovascular Research* (Vol. 46, Issue 2, pp. 250–256). Oxford Academic. https://doi.org/10.1016/S0008-6363(00)00032-8

Traktuev, D. O., March, K. L., Tkachuk, V. A., & Parfyonova, Y. V. (2006). Adipose tissue stromal cells - Multipotent cells with therapeutic potential for stimulation of angiogenesis in tissue ischemia. In *Kardiologiya* (Vol. 46, Issue 6, pp. 53–63).

Truskey, G. A. (2016). Advancing cardiovascular tissue engineering [version 1; referees: 3 approved]. In *F1000Research* (Vol. 5). Faculty of 1000 Ltd. https://doi.org/10.12688/F1000RESEARCH.8237.1

Vellaichamy, E., Khurana, M. L., Fink, J., & Pandey, K. N. (2005). Involvement of the NF-κB/matrix metalloproteinase pathway in cardiac fibrosis of mice lacking guanylyl cyclase/natriuretic peptide receptor A. *Journal of Biological Chemistry*, *280*(19), 19230–19242. https://doi.org/10.1074/jbc.M411373200

Wang, H., Liu, Z., Li, D., Guo, X., Kasper, F. K., Duan, C., Zhou, J., Mikos, A. G., & Wang, C. (2012). Injectable biodegradable hydrogels for embryonic stem cell transplantation: Improved cardiac remodelling and function of myocardial infarction. *Journal of Cellular and Molecular Medicine*, *16*(6), 1310–1320. https://doi.org/10.1111/j.1582-4934.2011.01409.x

White, I. A., Sanina, C., Balkan, W., & Hare, J. M. (2016). Mesenchymal stem cells in cardiology. In *Methods in Molecular Biology* (Vol. 1416, pp. 55–87). Humana Press Inc. https://doi.org/10.1007/978-1-4939-3584-0_4

World Health Organization. (2013). WHO | Prevention of Recurrences of Myocardial Infarction and Stroke Study. *WHO*.

World Health Organization. (2017). *Cardiovascular-Diseases-(Cvds) @ Www.Who.Int*. http://www.who.int/en/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)

World Health Organization. (2018). the-Top-10-Causes-of-Death @ Www.Who.Int. In *The top 10 causes of death* (p. Consultado 23 de marzo de 2019). https://www.who.int/es/news-room/fact-sheets/detail/the-top-10-causes-of-death

Ye, K. Y., & Black, L. D. (2011). Strategies for tissue engineering cardiac constructs to affect functional repair following myocardial infarction. In *Journal of Cardiovascular Translational Research* (Vol. 4, Issue 5, pp. 575–591). NIH Public Access. https://doi.org/10.1007/s12265-011-9303-1

Ye, L., Zimmermann, W. H., Garry, D. J., & Zhang, J. (2013). Patching the heart: Cardiac repair from within and outside. In *Circulation Research* (Vol. 113, Issue 7, pp. 922–932). https://doi.org/10.1161/CIRCRESAHA.113.300216

Zavadzkas, J. A., Mukherjee, R., Rivers, W. T., Patel, R. K., Meyer, E. C., Black, L. E., McKinney, R. A., Marshall Oelsen, J., Stroud, R. E., & Spinale, F. G. (2011). Direct regulation of membrane type 1 matrix metalloproteinase following myocardial infarction causes changes in survival, cardiac function, and remodeling. *American Journal of Physiology - Heart and Circulatory Physiology*, *301*(4), H1656-66. https://doi.org/10.1152/ajpheart.00141.2011

Zhou, J., Yang, X., Liu, W., Wang, C., Shen, Y., Zhang, F., Zhu, H., Sun, H., Chen, J., Lam, J., Mikos, A. G., & Wang, C. (2018). Injectable OPF/graphene oxide hydrogels provide mechanical support and enhance cell electrical signaling after implantation into myocardial infarct. *Theranostics*, *8*(12), 3317–3330. https://doi.org/10.7150/thno.25504