Laura Santambrogio *Editor*

Biomaterials in Regenerative Medicine and the Immune System

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Chapter 1
Role of Mesenchymal Stem Cells, Macrophages, and Biomaterials During Myocardial Repair

Isabella Pallotta, Emily A. Wrona, Bruce Sun and Donald O. Freytes

1.1 Introduction

Myocardial infarction (MI), also more commonly known as a heart attack, is a potentially lethal condition and remains one of the leading causes of death in the USA and other industrialized nations [1]. Since heart tissue has a diminished capacity to regenerate itself, there could be a substantial reduction in heart function if sufficient damage is sustained after the infarct. One approach to help patients who have suffered an MI is to replace the cellular mass lost after the infarction with repair cells that have the capacity to heal and recover heart function to pre-infarct levels. Among the methods being investigated are the use of injected repair cells encapsulated within a hydrogel-like material or the combination of a biocompatible material seeded with repair cells [2]. One attractive repair cell candidate is the human-derived mesenchymal stem cell (MSC). Although these cells have not been shown to effectively produce functional cardiomyocytes, they have shown great potential to improve angiogenesis, reduce the rate of myocardial wall remodeling, reduce cellular death, and, as a result, improve cardiac function [3–9]. In addition to the repair capability of MSCs, their availability from the patient's bone marrow or adipose tissue makes them an ideal candidate as part of an autologous heart repair strategy.

Isabella Pallotta, Emily A. Wrona and Bruce Sun have all equally contributed to this chapter.

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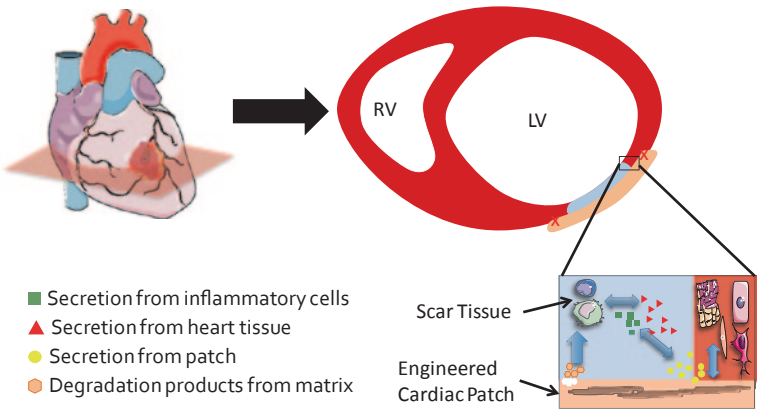

Fig. 1.1 Diagrammatic representation of the dynamic interactions between the infarcted tissue and the surrounding microenvironment composed, in part, of macrophages. Repair cells within the patch will secrete factors that may attract different subpopulations of macrophages. The matrix encapsulating the cells will also play a role in the interactions between the repair cells and the inflammatory cells *via* degradation products or direct contact. *RV* right ventricle, *LV* left ventricle. (Artwork provided by Servier Medical Arts, http://www.servier.com/Powerpoint-image-bank)

Repair cells, used alone or in combination with a biomaterial as part of an engineered cardiac patch, will inevitably be subjected to the inflammatory environment that follows the ischemic insult, as shown in Fig. 1.1 [10, 11]. This cellular environment is very dynamic and is characterized by the presence of multiple pro- and anti-inflammatory cells that appear at different phases of the remodeling process [10]. The inflammatory cells also play a crucial role during the degradation and remodeling of biomaterials [12–14]. This dynamic and critical environment is often overlooked when designing cardiac patches and could potentially dictate the success or failure of a delivered construct by inhibiting survival and/or engraftment of the cells or by modifying the natural degradation and remodeling process of the biomaterial. This chapter describes the events that follow after a myocardial infarct with emphasis on the important inflammatory events that take place during the remodeling process. This is followed by a description of MSCs as repair cells and how MSCs can be combined with biocompatible biomaterials in order to harness or modulate the inflammatory response as part of an engineered cardiac construct.

1.2 Myocardial Infarction and Inflammation

1.2.1 Myocardial Infarction

MI can be defined as damage or death of heart muscle due to deprivation of oxygen and nutrients (ischemia) by the occlusion of one of the arteries supplying blood to the heart tissue. Risk factors for MI include hypertension, cigarette smoking, diabe-

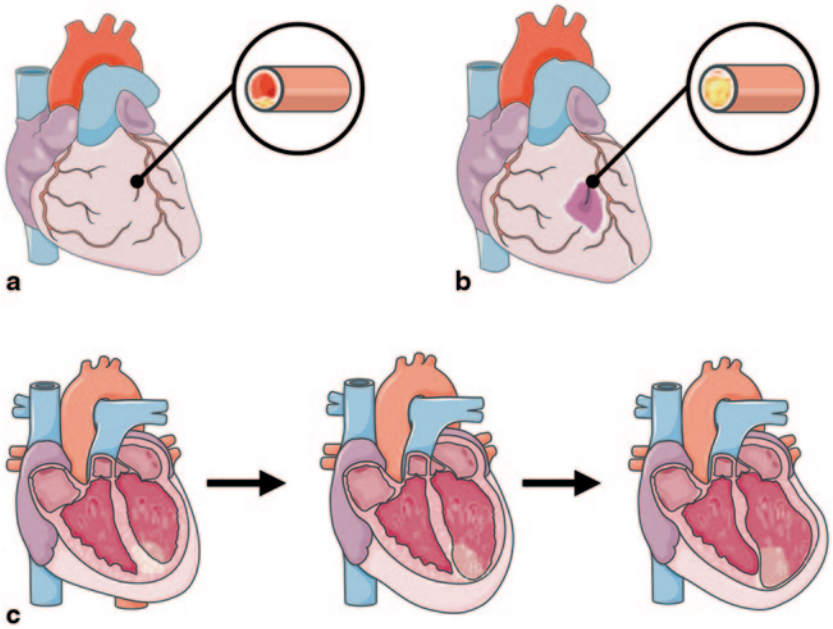

Fig. 1.2 The overall mechanism of myocardial infarction: **a** Fat and plaque build up over time and begin to clog the blood vessel. **b** Occlusion of a major coronary artery causes ischemia and necrosis of the myocardial tissue. **c** Over hours, days, and months, the loss of cardiac muscle leads to ventricular remodeling and the formation of a collagen-rich scar. How this process proceeds greatly determines the patient's prognosis and often results in decreased cardiac output. (Artwork provided by Servier Medical Arts, http://www.servier.com/Powerpoint-image-bank)

tes mellitus, and genetic hypercholesterolemia with an apparent gender bias towards men, showing a higher risk of experiencing an MI than women [15]. Although an MI can occur at any age, the gender bias decreases with age with the chances of an MI increasing equally for both men and women [15].

The overall mechanism of MI can be summarized as shown in Fig. 1.2. The first event is the occlusion of a major coronary artery. Obstruction of the coronary artery is often due to the build up of fatty materials and the rupture of this plaque causes the formation of a blood clot that can grow large enough in size to interrupt blood flow. The essential oxygen and nutrients needed to maintain the continuous muscle contraction of the heart can no longer be delivered, resulting in ischemia [15]. After a prolonged period of time, the occlusion leads to myocardial necrosis, which triggers an inflammatory response that, depending on the extent of the damage, may lead to detrimental ventricular wall remodeling. Additionally, ischemia can produce arrhythmias such as ventricular fibrillation [15]. There is some regenerative potential and necrosis prevention if blood is restored within 20 min of coronary occlusion by saving cells that have not undergone irreparable damage [15]. However, reperfusion itself may impose its own injuries since the introduction of oxygenated blood to ischemic tissue [16, 17], and the enzymes and reactive oxygen species (ROS) released from the initial inflammatory cells [18], may cause separate and secondary inflammatory responses [19].

Current approaches to engineer myocardial tissue replacements fail to address the inflammatory and healing environments after an infarct and the restoration of cardiac function to pre-infarct levels [19]. Since the level of healing achieved by the infarct can affect the overall cardiac function, new therapeutic strategies need to be explored that can restore the cellular mass lost after the infarct and create functional tissue that can restore heart function. Current strategies focus on engineering constructs that take advantage of a cellular component and scaffold material. Regardless of their success under standard culture conditions, any tissue-engineered construct will need to be screened *in vitro* for its survival within an inflammatory environment in order to increase the chances for clinical success.

1.2.2 Inflammation

Following ischemic insult, the cells of the immune system are recruited to the MI to promote an inflammatory response that helps facilitate subsequent tissue remodeling [20]. The inflammatory environment at the site of MI is not completely understood, but it is thought to be composed of sequential recruitment of different blood-derived circulating cells, as well as the activation of resident cells. Necrotic cells release signals that stimulate complement cascades, toll-like receptor (TLR)-mediated pathways, nuclear factor (NF)-κB systems, and the generation of reactive oxygen species (ROS), signaling the initiation of the inflammatory response [20]. These leukocytes are recruited *via* signals released by the necrotic tissue and travel to the infarct site by rolling and migrating across the endothelium with the help of adhesion molecules at the surface of blood vessels [21].

The first cells recruited to the site of MI are neutrophils, which begin to phagocytose cellular debris and release cytokines and proteolytic enzymes, in order to clear dead cells and necrotic tissue [16, 17]. Neutrophils also undergo apoptosis and the signals from these necrotic cells serve as the initial distress call in order to recruit peripheral blood monocytes and tissue-resident macrophages. There is evidence that monocytes are even recruited from the spleen en masse following MI [22], making them one of the most abundant cell types present within the infarcted tissue at early stages. Monocytes differentiate into macrophages *in situ* and perform multiple roles during homeostasis [23], the early stages of inflammation, and throughout the repair of damaged myocardium. Macrophages consume necrotic tissue and foreign materials, while assisting in angiogenesis and extracellular matrix (ECM) formation *via* the release of potent cytokines such as vascular endothelial growth factor-α (VEGF-α) and transforming growth factor-β (TGF-β) [16, 17, 20, 24]. For example, it has been shown that liposome-mediated monocyte depletion reduces neovascularization, myofibroblast formation and recruitment, and collagen accumulation in the area of the infarct [24]. Further understanding of how the classically and alternatively-activated macrophages work has demonstrated that these cells might exist as part of a spectrum of macrophages at multiple polarization stages constantly adjusting to the current inflammatory environment [25]. How these macrophages affect the overall healing process still needs to be investigated.

1.2.3 Macrophages and Myocardial Infarction

After an MI, there are two different surges of macrophages. There is an initial influx of pro-inflammatory (M1) macrophages, also known as classically activated macrophages, which give rise to the macrophage-mediated inflammatory response. Classical activation of M1 macrophages is associated with the release of cytokines such as interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNF-α) and is responsible for the initial degradation of biomaterials. M1 macrophages are typically polarized by lipopolysaccharide (LPS) and interferon-γ (IFN-γ) *in vitro*. This initial wave of macrophages is followed by a second wave of anti-inflammatory (M2) macrophages, also known as alternatively-activated macrophages. M2 macrophages are typically associated with tissue repair *via* the stimulation of extracellular matrix deposition and angiogenic effects (although it is still debated if M1 or M2 actually stimulate the initial angiogenic events) [20, 26, 27]. Depending on the pathway that is activated during polarization, some have cataloged M2 macrophages in subsets such as M2a, M2b, and M2c macrophages. A simplified diagram of monocyte-derived macrophages and the related cytokines is shown in Fig. 1.3. M2a refers to the macrophages originally named alternatively-activated macrophages and are typically polarized by IL-4 and IL-13. M2b macrophages are activated by immune complexes, agonists of TLRs, or IL-1R. M2c refers to macrophages activated by IL-10 or glucocorticoid hormones [28]. These subtypes of macrophages all play important but differing roles during the inflammatory response depending on the location and type of injury. The interactions of these subsets of macrophages with repair cells

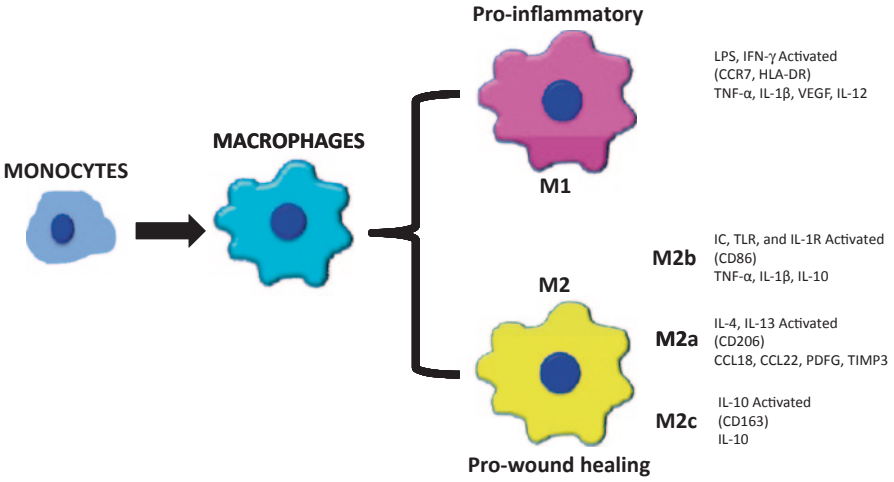

Fig. 1.3 Schematic representation of monocyte-derived macrophage subtypes M1 and M2. The main cytokines released by each subtype are shown. *LPS* lipopolysaccharide, *IFN* interferon-γ *TNF-α* tumor necrosis factor-α, *IL* interleukin, *VEGF* vascular endothelial growth factor alpha, *HLA-DR* human leukocyte antigen-DR, *M1* pro-inflammatory macrophages, *M2* pro-healing macrophages

and biomaterials are still poorly understood [26] and could have profound impact on the survival and integration of repair cells.

1.3 Mesenchymal Stem Cells in Heart Repair

1.3.1 Characteristics of MSCs

MSCs are stromal cells isolated from bone marrow or adipose tissue that have the multipotent potential to differentiate into a variety of cell types *in vitro*. Undifferentiated MSCs can potentially differentiate into different lineages of mesenchymal tissues including bone, cartilage, fat, tendon, muscle, and marrow stroma [29]. Human MSCs cultured *in vitro* appear morphologically heterogeneous and exhibit a spindle-like appearance very similar to that of fibroblast cells [30]. The isolation of MSCs from bone marrow cells *in vitro* is achieved in part thanks to their ability to adhere to tissue culture plastic. However, the use of plastic adherence also yields a heterogeneous population of cells, which includes MSCs and progenitor cells known as colony-forming units (CFUs) [7]. There are no definitive markers for isolating a pure population of MSCs. Rather, cellular purity is determined by their differentiation potential towards bone, adipose, and chondrogenic cells (thus being referred to as "multipotent mesenchymal stromal cells"). Furthermore, MSCs are characterized by the expression of markers such as CD73, SH2, SH3, CD29, CD44, CD71, CD90, CD105, CD106, CD120a, and CD124 [7, 31]. Table 1.1 summarizes the current accepted markers for the identification of MSCs.

In addition to the potential to differentiate into a variety of mesenchymal tissues, MSCs have also been described as immunoprivileged. MSCs lack the expression of histocompatibility complex II (MHC-II), along with co-stimulatory molecule expression for T cell recruitment. This characteristic is particularly beneficial in infarcted cardiac tissue, which is characterized by an intense inflammatory environment [32, 33]. The use of MSCs during preclinical heart repair studies have shown advantages over other cell types due to their potential to modulate the inflammatory

Table 1.1 Summary of potential markers for unsorted human bone marrow MSC populations

Markers	Marker potential
Stro-1	Enriches colony-forming units-fibroblasts (CFU-F) from whole bone marrow (BM)
SSEA-4	Enriches CFU-Fs from whole BM
CD146	Enriches CFU-Fs from whole BM Enriches cells with multipotency from BM-MSCs
CD271	Maintains clonogenicity and function of MSCs
GD2	High specificity for isolating BM-MSCs
CD49f	When selected for at first passage, enriches clonogenicity and differentiation
PODXL	Identifies early progenitor MSCs but decreases with high-density culture and passage

environment and improve the vascular response following an MI. There have been some initial reports suggesting that human MSCs could differentiate into cardiomyocytes and that the derived cells had potential therapeutic effects after engraftment onto healthy murine hearts [34]. Following this report, various preclinical animal studies were conducted using MSCs as a therapeutic treatment of MI models. These studies resulted in infarct size reduction, improved cardiac contractility and reduced fibrosis [6, 34, 35]. In addition to bone marrow, MSCs from different tissues can serve as potential cell sources. In animal models, MSCs were thought to differentiate into cardiomyocytes by the appearance of spontaneously contracting cells, the expression of cardiac-specific genes, and the expression of cardiac proteins [36]. However, protocols that may lead to differentiated MSCs towards cardiac cells in sufficient numbers have not been adequately described. Researchers have debated the differentiation potential of MSCs towards cardiomyocytes *in vitro* and *in vivo* and their ability to differentiate remains controversial. However, it is well accepted that MSCs play a supporting role during myocardial healing by promoting angiogenesis and serving as a reservoir of growth factors during the host tissue response [37−40].

Although the isolation of MSCs has been well documented with protocols available for clinical-grade applications, some are still cautious about their isolation and urge more characterization and understanding of the cells during culture and implantation. For example, cultures of impure heterogeneous cell population from plastic adherence isolation may need further characterization while maintaining sterility. If expansion is needed, culture medium should not contain xenogeneic ingredients such as fetal bovine serum or animal-based growth factors. Regardless, MSCs remain a very attractive cell population for the treatment of MI.

1.3.2 MSCs in Heart Repair

Even with today's advances in modern medicine and pharmacological administration, the presence of scar tissue renders the heart incapable of performing at optimal levels, resulting in decreased cardiac output. Left ventricular wall remodeling can lead to heart failure with approximately 50% of patients dying within the first 5 years [8]. MSCs have shown promising results in multiple animal studies, and in 2001 it was reported that autologous MSCs transplanted into the heart after MI reduced infarct area and elevated left ventricle (LV) function during a 3 month follow up [3, 4]. Additional studies have shown that MSC transplantation in the heart not only improved LV function, but also was shown to be safe and effective within a small study population [3, 4]. Shortly after, more *in vivo* cases of acute MI, followed by intracoronary transplantation of autologous bone marrow-derived MSCs and bone marrow-derived MSCs combined with endothelial progenitor cells, provided evidence that MSC therapy can increase myocardial viability in a safe and nonfatal manner [41, 42].

One of the first human clinical trials in the USA occurred in 2008 and consisted of 53 patients under Osiris Therapeutics, investigating the use of allogeneic MSC transplantation for acute MI [43]. The phase I clinical trial was encouraging and showed no adverse effects or infusion toxicity in immunocompetent patients. A year later, human clinical trials performed using a dose-ranging, double-blind, and placebo-controlled safety trial concluded that MSCs decreased ventricular arrhythmias, improved pulmonary function, and elevated LV ejection fraction 3 months post therapy. To date, the use of MSC therapy has been shown to be safe with a range of efficacy with many studies still ongoing for an array of cardiomyopathies.

For MSC transplantation to work efficiently and successfully, a variety of factors must be verified and implemented. These factors include the timing of delivery (e.g., post-acute MI or post ischemia), the implantation methodology, MSC characteristic, MSC survival, and also the nature of the patients' pathology. Since the timing of MSC delivery is crucial after an acute ischemic event because of inflammation and cellular necrosis, the best treatment period is prior to fibrosis. However, additional *in vivo* and *in vitro* data are necessary to determine a standardized treatment.

1.4 Interactions Between MSCs and Inflammatory Cells

As mentioned before, the injured myocardium is characterized by the invasion of pro-inflammatory (M1) and pro-healing (M2) macrophages. For this reason, MSCs, once implanted into the injured myocardium, will inevitably interact with the inflammatory cells present at the time of implantation. Because of this close coexistence, cell phenotype and behavior can be reciprocally affected and modulated. The MSC- and macrophage-released cytokines play important roles in mediating their mutual interactions. For example, M2 macrophages and their associated cytokines (e.g., IL-10, TGF-β1, TGF-β3, VEGF) can support the growth of MSCs, while M1 macrophages and their associated pro-inflammatory cytokines (e.g., IL-1β, IL-6, TNF-α and IFN-γ) can inhibit their growth [11].

MSCs have the capacity to modulate the inflammatory cells as well. In particular, MSCs show anti-inflammatory properties by promoting a more pro-healing state. When infused in induced acute myocardial infarcted animal models, MSCs seem to increase the numbers of pro-wound healing monocytes/macrophages *via* the release of IL-10. At the same time, pro-inflammatory monocytes/macrophage numbers are reduced *via* the decrease of pro-inflammatory cytokines such as IL-1β and IL-6 [44]. Additional animal studies demonstrated that MSCs injected within the infarcted myocardium allow for a shift of the infiltrated macrophage phenotype from M1 to M2. The MSCs were surrounded by arginase 1 (Arg1)-expressing (which in the mouse model, is a marker for M2) macrophages when compared to the controls [45]. The shift from a pro-inflammatory to a pro-healing phenotype can also be detected by changes in messenger RNA (mRNA) and protein expression levels. These data suggest that the modulatory action of MSCs can accelerate the

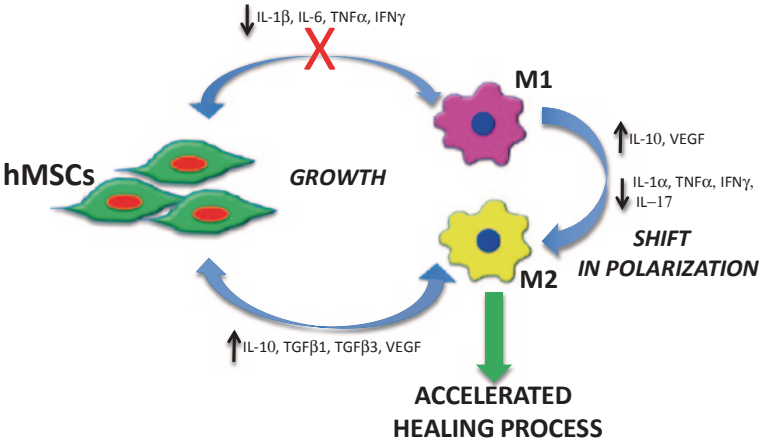

Fig. 1.4 Cross talk between MSCs and inflammatory cells. MSC growth is enhanced by M2 macrophages and inhibited by pro-inflammatory M1-derived cytokines. In turn, MSCs modulate the polarization of macrophages by promoting an M2 phenotype, while reducing an M1 phenotype. These events may result in an accelerated healing process. *hMSCs* human-derived mesenchymal stem cell, *IL* interleukin, *TNF-α* tumor necrosis factor-α, *IFN-γ* interferon-γ, *TGF-β* transforming growth factor-β, *VEGF* vascular endothelial growth factor

healing process in the injured tissue by close modulation of macrophages present at the site of injury.

Interestingly, the interaction between MSCs and macrophages is bidirectional. It has been shown that human adipose-derived mesenchymal stromal cells (hAMSCs) can alter the polarization of macrophages from M1 to M2 by increasing macrophage secretion of anti-inflammatory and angiogenic cytokines, such as IL-10 and VEGF. Moreover, these MSCs can also reduce macrophage secretion of inflammatory cytokines, such as IL-1α, TNF-α, IL-17, and IFN-γ. At the same time, macrophages were shown to upregulate the secretion of IL-4 and IL-13 by MSCs, which are known to polarize macrophages towards an M2 phenotype [46]. Figure 1.4 summarizes the possible bidirectional interactions between MSCs and macrophages.

From a tissue engineering perspective, there is a growing interest in improving the therapeutic potential of injected MSCs. For instance, there is a need to optimize the cell culture conditions before injection into the myocardium and to further improve their survival once delivered into the inflammatory environment of the infarct. Due to the reciprocal interactions between MSCs and macrophages, strategies are needed to exploit these interactions, in order to improve cellular survival and integration. For example, a promising strategy is to culture MSCs as aggregates in three-dimensional (3D) spheroids. This method has been demonstrated to increase the MSC expression of TNF-α-stimulated gene/protein 6 (TSG-6), an anti-inflammatory protein reported to be beneficial in animal models. This technique seems to suppress the inflammatory response both *in vitro* and *in vivo* when compared to MSCs cultured in the classical monolayer. Therefore, this new method of culturing

MSCs can potentially be applied to therapies in which inflammation is present at the site of implantation [47].

Another strategy to optimize the therapeutic outcome of the injected MSCs is to evaluate the source of the MSCs based on their response to the inflammatory environment. The most common source of MSCs is the bone marrow. However, adipose tissue has been recently recognized as a good alternative for its large pool of MSCs. Subcutaneous fat-derived MSCs have exhibited anti-inflammatory properties with low amounts of cytokines secretion and improved cardiac remodeling properties when compared to the right atrium and epicardial fat-derived MSCs. These data suggest that the source and preparation of MSCs may play an important role in modulating the inflammatory environment and should be considered when designing future therapies [48].

1.4.1 MSCs and Biomaterials

Although preclinical and clinical trials of MSC therapy for cardiac diseases have shown highly promising results as previously mentioned [49], some limitations still persist, including low stem cell retention in the infarcted myocardium, poor cell survival [50], and poor cell engraftment [51]. Examples of delivery routes include *via* peripheral intravenous infusion, direct surgical injection during open heart surgery, catheter-based intracoronary infusion, retrograde coronary venous infusion, or transendocardial injection [52, 53]. To overcome these limitations, the use of biomaterials represents a promising strategy to enhance the physical retention and localization of the MSCs at the site of implantation by improving engraftment and differentiation. Biomaterial-based scaffolds can act as mechanical supports that help to localize the cells at the site of injury, thus allowing them to proliferate, improve matrix deposition, and help modulate the host tissue response [53]. By tuning the material properties, the scaffolds can be engineered to control the degradation rate of the material, the diffusion rate of released growth factors, and protect encapsulated cells from the inflammatory environment. This aspect has a particular relevance to MSC transplantation therapy, since the beneficial effects of MSCs are more likely due to paracrine mechanisms *via* the release of growth factors rather than due to their ability to trans-differentiate into cardiomyocytes. Ultimately, engineered biomaterials can potentially confer protection from inflammation, which is known to be crucial during heart damage.

From a clinical point of view, in order to be suitable for regenerative medicine, a biomaterial needs to have peculiar characteristics such as biocompatibility, non-immunogenicity, non-thrombogenicity, non-cytotoxicity, and the ability to support cell differentiation and function. Based on promising results obtained in phase I clinical trials, alginate is an example of a hydrogel with potential cardiac applications, which the US Food and Drug Administration (FDA) has approved for phase II clinical trials for myocardial repair without the use of cells [54]. Engineering of cardiac constructs can take advantage of the growing number of biomaterials that are being shown safe and effective in clinical trials.

Additional characteristics that should be taken into account to improve the therapeutic use of biomaterials are material properties, such as stiffness, elasticity, porosity, and biodegradability. Investigators have characterized the behavior of MSCs cultured on polymer matrices of different stiffness and elasticity, demonstrating that these properties dramatically affect cell fate [54]. In particular, the myogenic differentiation seems to be supported by materials with an intermediate stiffness rather than very soft or hard substrates that support neurogenic and osteogenic differentiation, respectively. In another example, researchers proposed an innovative technique to switch a hydrogel-based material from a self-renewal permissive hydrogel (alginate) to a differentiation-permissive microenvironment (collagen). By fine-tuning the timing of this switch, early lineage specification can be directed, resulting in more control over cellular differentiation at the biomaterial level [56].

To date, repair cells can be delivered to the site of myocardial infarct following two major biomaterial strategies: injectable hydrogels or patches. The different formulation of the biomaterial, hydrogel or patch, will have differing mechanical properties that need to be considered. Researchers have compared hydrogels to two-dimensional (2D) sheets of biomaterials for their ability to support MSC viability and retention after being delivered to the site of MI in a rat model. The biomaterials analyzed differ in formulation and route of administration, as they include an injectable chitosan/β-glycerophosphate (GP) hydrogel, an injectable alginate hydrogel, a collagen patch, and an alginate patch [2]. All the analyzed biomaterials improved MSC viability as compared to monolayer culture, with a greater viability in the long term (6 days) when cells were encapsulated in hydrogels. This might be attributed to the ability of the gel's structure to protect the entrapped cells from oxidative stress. Twenty-four hours after transplantation, an 8-fold and a 14-fold increased MSC retention were reported when delivered *via* alginate and chitosan/β-GP gels, respectively, as compared to cells delivered in saline. Similarly, 47-fold and 59-fold increases were reported for collagen and alginate patches, respectively. Alginate is known to be nonadhesive; however, this limitation is usually overcome through functionalization with arginine–glycine–aspartic acid (RGD)-containing peptides. In addition to alginate and chitosan/β-glycerophosphate hydrogels, alpha-cyclodextrin/methoxy poly(ethylene glycol) (MPEG)–poly(ε-caprolactone) (PCL)–MPEG hydrogels have shown promising results as well. After 4 weeks post implantation, this hydrogel formulation was able to increase cell retention and vessel density in the infarcted myocardium of the injected animals. The LV ejection was improved in combination with an attenuation of the LV dilatation [55]. Similar improvement in myocardial remodeling, such as reduction in LV interior diameter at systole, increased anterior wall thickness, and increase in fractional shortening, has also been observed in experimental MI models treated with a rat-tail collagen patch [56]. More recently, human embryonic stem cell-derived MSCs, which seem to be similar to bone marrow-derived MSCs, have been reported to have beneficial effects when embedded in collagen patches and used for cardiac repair [56]. Taken together, these findings highlight the important role of the biomaterial when designing engineered cardiac constructs.

In addition to collagen and alginate, there are also promising results using decellularized sheets of human myocardium as a biomaterial-based scaffold for cell delivery [57]. One interesting example is human mesenchymal progenitor cells (MCPs) entrapped in fibrin hydrogels combined with human myocardial decellularized matrix. These hydrogel–ECM constructs were implanted onto an infarct in a nude rat model using MCPs preconditioned with TGF-β. The engineered patch led to enhanced angiogenesis and arteriogenesis through mechanisms that involved the migration of MCPs from the patch into the infarcted myocardium. Interestingly, this platform suggests that cell migration is in part regulated by altering the stromal cell-derived factor 1/C-X-C chemokine receptor type 4 (SDF-1/CXCR4) expression in MCPs. MCPs preconditioned with TGF-β, when encapsulated in the composite scaffold, showed increased CXCR4 expression and suppressed SDF-1 expression. This change in expression results in increased cell responsiveness to a gradient of SDF-1 and consequently migration into the infarcted myocardium. However, once released from the scaffold, MCPs recover the ability to release SDF-1, resulting in enhanced cell migration, vascularization, and preservation of myocardium functionality, These studies demonstrate, once again, the ability of tuning the cell behavior *via* tissue-engineering approaches that take into account the cell source, the biomaterial, and the inflammatory environment.

1.5 Conclusion

Any implanted engineered cardiac construct will come in contact with the underlying inflammatory environment. It is therefore important to have the ability to model and predict the effects of the inflammatory environment on the biomaterial and the repair cells used to create any engineered cardiac construct. It is also important to understand how the inflammatory environment will affect the degradation rate of the biomaterial and what role macrophage polarization will play during the healing process. Since engineered constructs will also have a cellular component, it is also essential that we understand the cellular interactions between the inflammatory environment and the repair cells. MSCs remain a viable candidate for targeted cellular therapies for cardiac applications and, as our understanding of the role of MSCs during myocardial healing improves, new strategies are needed to further test potential repair cells in the laboratory. One approach is to mimic important biophysical and cellular events that could have detrimental effects on an engineered cardiac construct using advanced culture systems that recapitulate these events *in vitro*. Better understanding of how engineered cardiac patches behave in an inflammatory environment *in vitro* will provide a better screening tool to predict the potential success or potential of any cardiac engineered construct.

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Chapter 2
The Role of Macrophages in the Foreign Body Response to Implanted Biomaterials

Tony Yu, Valerie J. Tutwiler and Kara Spiller

2.1 Introduction

Biomaterials are part of the solution to many unmet clinical needs, from implantable sensors to drug delivery devices and engineered tissues. However, biomaterials face an inflammatory environment upon implantation, which represents a potential obstacle to their success [1]. In this chapter, we review the consequences of the foreign body response (FBR) for biomaterial function and strategies that have been used to inhibit the FBR. We focus on the role of the macrophage, the cell at the center of the inflammatory response, as the major regulator of the FBR, and discuss implications of changing macrophage behavior on biomaterial acceptance or rejection. Finally, we discuss recent discoveries in the role of macrophage phenotype, ranging from pro-inflammatory (M1) to anti-inflammatory (M2), and the role it plays in wound healing and biomaterial vascularization and integration. We conclude with a discussion of biomaterial design strategies that have been suggested to positively interact with and potentially control macrophages in order to improve interactions between biomaterials and the inflammatory response.

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