Biomaterials: The Holy Grail for Stem Cell Therapy in the Infarcted heart

Boosting the efficacy of mesenchymal stem cell therapy for myocardial infarction patients to improve overall cardiac function

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0. Abstract

The ongoing high burden on society of cardiovascular diseases takes a total of 17.9 million lives per year globally in which a major one-third of these deaths are accounted by coronary artery diseases. This type of disease eventually leads to the occlusion of the coronary arteries of the heart, which consequently results in myocardial infarction. Following an infarction, ischemia-mediated cell death of the heart tissue results in the chronic failure of the heart due to contractile impairment mainly caused by fibrous scar formation. This type of injury is chronic damage to the heart that is not easily reversed. One current treatment is the use of mesenchymal stem cells, which sound promising in terms of their paracrine, differentiative, and immunomodulatory therapeutic potential along with other advantages such as its immune privilege and easy accessibility. However, the treatment with mesenchymal stem cells in the heart comes short in regard to their retention and survival due to the hostile ischemic microenvironment of the infarcted heart. Here, the use of biomaterials comes in handy to boost mesenchymal stem cell therapy. Biomaterials possess a wide variety of modifiable features and advantages in the context of cardiac tissue engineering. As for the enhancement of cell therapy, the biomaterial plays an important role by providing sufficient cell interactions along with creating a friendly microenvironment. This significantly promotes cell retention and survival. Thus, the use of biomaterials ensures that the therapeutic potential of mesenchymal stem cells is enhanced. The combination treatment of biomaterials and mesenchymal stem cells is already documented in several preclinical trials in which overall cardiac function was significantly more improved compared to treatment of solely these cells. Still, sufficient evidence and confirmation by human clinical trials are missing. Hence, the purpose of this study comes into play, which was to put forward the massive added benefit of biomaterials for mesenchymal stem cell therapy. Consequently, stimulating the progression of myocardial infarction treatment and thereby relieving the global burden of cardiovascular diseases in general.

Keywords cardiovascular diseases, myocardial infarction, stem cell therapy, mesenchymal stem cells, biomaterials

1. Introduction

1.1. Cardiovascular diseases

At the moment, the global burden of cardiovascular diseases (CVDs) is set as the leading cause of death worldwide. These type of diseases accounts for approximately 17.9 million deaths annually with numbers expected to increase in the future [1]. Common CVD-associated risk factors are hypertension, dyslipidemia, diabetes mellitus, smoking, and overweight (especially obesity). Currently, these risk factors are still at a critical level or increasing within the global population [2]. One of the major CVD types is classified as coronary artery disease (CAD). CAD addresses about a third of the total CVD claimed deaths and shares the same risk factors as CVDs. The pathophysiology of CAD is represented as an atherosclerosis and inflammatory disorder. This disorder is manifested by accumulated lipid levels, endothelial dysfunction, plaque built up, fibrotic formation, thrombosis, and consequently occlusion of the coronary arteries. Ultimately, leading to the obstruction of the blood flow and with it its vital components oxygen and nutrients. This sets the stage for life-threatening damaging effects for the heart tissue [3].

1.2. Myocardial infarction

The most common consequence of CAD is the manifestation of a heart attack, known as an acute myocardial infarction. Besides an acute myocardial infarction, the general term of myocardial infarction (MI) is also used in cases that are similar to the consequences of CAD. These cases are a partial coronary occlusion or a general disturbance in the blood supply and demand of the heart tissue. Such cases are to an extent less abrupt than acute myocardial infarction, though still very life-threatening in the long term. During MI, different territories of the heart tissue can be affected depending on the type of the coronary artery and the extent of the occlusion. Overall, the outcome of MI is the exhibition of ischemic cardiomyopathy. Eventually, this leads to the chronic failure of the heart tissue on either the short-, mid- or long-term [4]. Even with the current treatments, these events still lead to the death of around 4 million Europeans every year [5].

1.3. Pathophysiology

The pathogenesis of MI is typically manifested by the necrosis and apoptosis of the cardiac tissue caused by ischemia. The formation of the damage gradually progresses from the endocardial region towards the epicardial region. Affected cells in the cardiac tissue cover from cardiomyocytes to other non-cardiac cells such as vascular endothelial, smooth muscle, and nervous system cells. This particular cell death results in the filtration of immune cells in the infarcted area. With this inflammatory phenotype, myofibroblasts and endothelial cells are activated. The activation of these cells aids the repair of the damaged tissue. Though, this process results in the formation of collagenous scar tissue in the infarcted area. Eventually, this causes the remodeling and weakening of the heart chambers. As a consequence, the contractile function of the heart is impaired. Overall, the results of MI make the heart tissue prone to more complications in the future [6].

Currently, the preferred acute treatment of MI covers from medicinal drugs to reperfusion therapy. Medicinal drugs usually target hypertension and oxygen supplies to the heart [7]. Reperfusion is done by stent implantation or thrombolytic intervention, which restores the blood flow in the occluded coronary artery [4]. Though, additional injury can be caused by the abrupt metabolic and functional changes following reperfusion [8]. Still, MI injury that is done, is chronic damage for the heart that is not easily reversed and resolved, even after treatment. Thus, illustrating the urgency for a potent therapy for post-MI patients that targets the regeneration of the remodeled cardiac tissue and thereby restores the contractile function of the heart.

1.4. Stem cell therapy

One of the promising options that have arisen during the last two decades is the use of stem cell therapies as a regenerative treatment for MI caused damage. Many different stem cell types have already been used and evaluated in clinical trials in the field of MI [9]. The most important features of stem cells are their differentiative capacity (multipotency) and self-renewal potency. This allows them to give rise to new tissues whilst simultaneously maintaining the stem cell pool [10]. The mesenchymal stem cell (MSC) is one type of stem cell to be considered as a potent option for cardiac regenerative treatment. The MSC is a good option because it has strong paracrine, differentiative, and immunomodulatory properties [11, 12]. Moreover, MSC can be derived from many different organ and tissue sources such as bone marrow, adipose tissue, peripheral blood, and more [13]. This makes them an accessible and powerful option to aid the regeneration of cardiac tissue.

On the other hand, MSCs are capable to bypass the host immune system, because they do not express surface markers that are recognized by the immune cells. This makes them in a way immune-privileged stem cells [14]. In this way, MSCs derived from different persons (allogeneic) instead of MSCs derived from the same person (autologous) can be used for post-MI patients. This saves precious time in the obtainment and expansion of these cells. Thus, putting MSCs forward as an interesting and potential option for regenerative therapy for the post-MI heart.

1.5. Current issue and possible solution

As for MSC therapy, various preclinical and clinical trials have shown that treatment with MSCs caused a minimal reduction of ischemia-mediated damage and limited improvement of the overall cardiac function [15, 16]. The limitation for MSC therapy is mainly in terms of retention, survival, proliferation, and differentiation. This insufficiency of MSCs is caused by the hostile environment of the ischemic heart tissue. This environment is a result of the mechanical stress of the heart contraction and the low oxygen levels. Together with the current delivery mode of MSCs, the retention and viability of these therapeutic cells are impaired [17]. Thus, illustrating that a different approach for MSC-based therapy is needed to improve its efficacy.

At the moment, there are different strategies to improve the therapy of MSCs. An example of such a strategy is the genetic engineering of MSCs to improve their surviving capabilities. Another example is the pre-conditioning of the infarcted area with biological trophic factors to prepare for the MSC transplantation [18]. Though, one promising strategy is the combination of MSCs with biomaterials. As for biomaterials, a variety of features can be adjusted to maximize the function that it is serving [19]. In the context of this study, the use of biomaterials to improve the efficacy of MSC therapy as a regenerative treatment for MI-induced damage is evaluated. Hence, the following research question is formulated below which is set to be answered in the main body of this study. Additionally, further important information related to biomaterials and mesenchymal stem cells is provided.

What is the added benefit of biomaterials on the efficacy of mesenchymal stem cells in relation to the regenerative treatment of myocardial infarction-induced damage?

2. Biomaterials

2.1. Implication

The use of biomaterials knows its applications in the entire biomedical field for over the past 50 years where it performs or replaces a natural function [20]. As for the biomaterial, a certain range of criteria need to be met depending on the desired function that it serves. In the context of cardiac regenerative therapy, the biomaterial aims to regenerate the damaged tissue, which is also referred to as tissue engineering [21]. In particular, biomaterials may serve a variety of functions when it comes to the targeted regenerative treatment of the post-infarcted heart. In the first place, when a biomaterial is solely implanted at the infarcted site, its function is to serve as a 3D scaffold for the infarcted heart. This support guides and aids the damaged tissue for regeneration. On the other hand, when biomaterials are implanted in combination with other biological compounds, its function is to enhance these compounds. Such biological compounds are mainly cells and molecules like trophic factors with therapeutic potential. As for cells, the biomaterial serves as a reservoir to contain these cells at the infarcted site. For molecules on the other hand, the biomaterial enables the sustainable release of these factors. Consequently, the regeneration of the damaged infarcted tissue is aided in which various mechanisms play a role, depending on the used biological compound [22]. All in all, illustrating the broad function that biomaterials may serve for the post infarcted heart.

2.2. General properties

A number of criteria [22] need to be met regarding the design of the biomaterial to achieve tissue engineering regenerative effects. The first important criterium for the biomaterial is to be biocompatible The biomaterial needs to suit the particular tissue in which it is implanted and additionally for the biological compounds that are added. The cells of the targeted tissue need to be able to function, adhere and migrate normally onto and through the biomaterial. In this way, cells eventually settle and proliferate in the particular biomaterial, so that healthy tissue is regenerated. Moreover, being biocompatibility also enables the biomaterial to be accepted by the body without having it rejected by the immune system of the host due to an inflammatory reaction. Certain features of the biomaterial influence its biocompatibility, such as composition, elasticity, porosity, and viscosity [19]. The second factor that needs to be taken into account is the biodegradability of the biomaterial. Since most biomaterials are not intended to serve as permanent but as temporary constructs, they need to be degraded over time. This allows the tissue to gradually replace the biomaterial with the body's cells and extracellular matrix to ensure the full regeneration of the tissue.

The third significant variable is the mechanical properties of the biomaterial. Ideally, these properties need to match the site of the tissue in which the construct is implanted, whilst also allowing for practical handling during implantation. Still, a fine balance between mechanical strength and porosity is necessary since cell infiltration is eventually needed for the biomaterial to be a success. The fourth critical factor is the architecture of the biomaterial construct in which porosity and the material's surface play an important role. To enable cell penetration and the diffusion of nutrients and waste products into the biomaterial, a porous interconnected structure is needed. Additionally, the formation of vasculature can take place to enhance these processes. The composition of the biomaterial determines the structure of its surface. Here, certain ligands enable the interaction between the cells and the surface of the biomaterial. In this way, cellular interaction is related to the composition of the biomaterial. In the case of natural biomaterials, easy interaction is enabled. Synthetic biomaterials on the other hand, require additional molecules like the absorption of linker-proteins on the surface.

Finally, also the manufacturing of biomaterials needs to be taken into account. This is to ensure good translation from a clinical platform towards a commercially available construct. Preferably would be that the biomaterial is available off-the-shelf, so that the construct can be directly used in case it is needed.

2.3. Specific properties for heart tissue engineering

To put all the aforementioned criteria for biomaterials into the perspective of the regenerative treatment of the post-MI heart, a glance is taken at what features [23] are required. As for biocompatibility, it is in almost all biomaterials applied for the heart necessary that they are capable to bind and foster either the host or added cells. In this way, biomaterials enhance the regeneration of new cardiac tissue. Regarding biodegradability, it is not a feature that is necessarily required for a biomaterial meant for the heart. When the damaged heart requires mechanical support to prevent for instance ventricular remodeling, nondegradable patches are used to compensate contractile function and attenuate wall stress. These biomaterials are usually of synthetic origin [24]. Biomaterials in the form of hydrogels on the other hand, are able to serve as a biological activator to modulate the microenvironment. In this way, cells are recruited and vasculature formation is promoted [25]. In the case of biodegradable biomaterials, the ultimate goal is that the host's tissue replaces the applied construct to eventually ensure the regeneration of the heart tissue. Therefore, the degradation of the biomaterial should match the growth of the particular heart tissue. As for biodegradable biomaterials that serve as a carrier of cells and trophic factors, biodegradability is necessary to ensure cell retention or sustainable release of the bioactive factors [26].

In terms of the mechanical properties of the biomaterial, the desired function of the construct is considered to be the influencing factor. When aiming for a biomaterial constraint to deliver mechanical support for the heart, mechanical properties like elasticity should be similar to the compliance of the healthy heart. In this way, support at both systole and diastole is ensured. Likewise, the stiffness of the biomaterial should also be of a similar degree as the healthy heart. Stiffness may also be at a higher degree to ensure sufficient contractility. To put mechanical properties into the perspective of tissue engineering, it should be considered that the desired goal of tissue engineering is to mimic the natural extracellular matrix environment. Therefore, the mechanical properties should ideally match that of the native myocardium with a similar extracellular matrix form and composition. Finally, as for the architecture and especially porosity, it is required that the particular construct enables sufficient diffusion and transportation of nutrients and waste materials in the heart. The formation of microvasculature in the construct can overcome this hurdle. A pore size of around 100 to 300 μm would be sufficient for vascularization to take place in the cardiac tissue [27, 28].

2.4. Delivery

The delivery of biomaterials depends on the type of construct and the particular heart tissue that it is targeting for treatment. Regarding the tissue of the heart wall, it generally consists of three layers. Moving from outside to inside, these layers are respectively called epicardium, myocardium, and endocardium. The epicardium has the function to protect the heart. The myocardium consists of cardiomyocytes and fibroblasts, which carry out the contractile function. The endocardium serves its function to enclose the inside of the heart with endothelial cells [29]. Regarding the type of construct of the biomaterial, the forms of solid patches or liquid hydrogels are frequently seen. Both forms come either with or without biological compounds.

Due to the solid origin of patches, the range of application is mostly limited to surgical implantation in which the patch is sutured to the epicardial surface of the heart. In this way, the therapeutic benefit of the patch is minimized for the myocardial and endocardial regions. In addition, when aiming for a

cardiac patch with cells, the particular construct needs to be generated in vitro. This makes the production of cellular patches a time-consuming process compared to cellular hydrogels. All in all, putting the cardiac patch for tissue engineering forward as an invasive and therapeutic limiting option [30].

For hydrogels on the other hand, this limitation does not makeup. The liquid origin of the construct enables it to be used as an injectable. In this way, the particular construct can potentially be implemented at any site of the heart, either epi-, myo- or endocardial. This makes the hydrogel very suitable for tissue engineering of the heart since the region of infarction can be directly targeted. After delivery, the hydrogel solidifies into a flexible scaffold. After that, the hydrogel is able to execute its function at the particular site of the heart. On the other hand, the liquid origin of a hydrogel before injection enables it to be easily combined with biological compounds such as cells and trophic factors. Thus, putting hydrogels forward as a very advantageous and widely therapeutical applicable construct whilst also avoiding the surgical invasion [30, 31].

2.5. Composition

The composition of the biomaterial is a very important factor since all the aforementioned properties depend on it [22]. Generally, biomaterials used for cardiac tissue engineering are polymers of either natural or synthetic basis. Overall, polymers based on natural origin resemble the composition of the particular tissue that is being investigated for tissue engineering. Biomaterials of natural basis are generally of good biocompatibility and biodegradability. This prevents foreign body reactions and enables cell adhesion, proliferation, and differentiation. Consequently, making this type of biomaterial favorable for cardiac tissue engineering [32]. Moreover, natural biomaterials may also have some biological activity on their own in terms of cell recruitment and microenvironment modulation [25]. On the other hand, the natural origin also causes the biomaterial to be poor processable. This makes it hard to establish consistency in the mechanical and architectural properties of the construct.

Polymers of a synthetic basis however, do possess consistency and predictability in the production in terms of their mechanical and architectural properties. The properties of biodegradability and biocompatibility can vary for synthetic biomaterials. In the case of low biodegradable material, the replacement of the host tissue is hampered [23]. To overcome the disadvantages of either natural or synthetic biomaterials, a combination of both materials is also being used in the field of cardiac tissue engineering [33].

The most widely applied natural material used for biomaterial constructs in cardiac tissue engineering is the protein collagen. It is widely used since most cardiac extracellular matrix is comprised of collagen type I and III [34]. In this way, the biomaterial is very comparable to the natural extracellular microenvironment of the heart. This makes the biomaterial of a collagen basis highly biocompatible and biodegradable for the integration of the cardiac tissue. Moreover, the mechanical properties are easily adjustable by varying the compositions of the collagen types. In this way, the elastic and stiffness properties of the construct are influenced, so that it can be similar to the mechanical properties of the heart. Additionally, collagen constructs show low rejection by the immune system and wide acceptance by the host tissue [25]. Hence, collagenous biomaterials may also serve as a release platform for therapeutic trophic factors or as a carrier for therapeutic cells [26]. Finally, the forms in which biomaterials made from collagen come, range from cardiac patches to injectable hydrogels [37].

Other natural polymers familiar in cardia tissue engineering are the polysaccharides chitosan and alginate. Chitosan is the second most abundant polysaccharide in nature [35] and alginate is an important constituent of seaweed [36]. Again, both chitosan and alginate-based biomaterials are used in the form of patches and hydrogels [37]. For both of these materials, good biodegradability and

biocompatibility apply. As for biodegradability, it was shown that these constructs are replaced by host tissue [37, 40]. For biocompatibility, it was shown that these types of biomaterials were capable of carrying therapeutic cells and trophic factors to the infarcted heart tissue [38, 39]. Moreover, biomaterials of chitosan and alginate have the architectural property of being very porous [41]. Whereas for in particular alginate constructs, the pore size is modifiable [37]. Additionally, constructs of alginate are shown to be more capable of binding cells than chitosan constructs. This is due to the fact that alginate polysaccharides are negatively charged. Also, the formation of hydrogels is a bit more favored for alginate biomaterials since they have increased viscosity [37].

Besides collagen, also fibrin and gelatin are widely applied as natural protein polymers for cardiac targeted biomaterials. For fibrin-based materials, it applies that they are generally used in the form of hydrogels where it serves as a sort of injectable glue [42]. Therefore, it can be used as a strong scaffold for the prevention of cardiac remodeling after infarction. In this way, the contractile function is preserved [43]. On the other hand, it is also still possible to form cardiac patches based on fibrin. These patches are used for the delivery of therapeutic cells since they bind the natural fibrin [44]. As for the mechanical properties of fibrin constructs, it applies that these consist of formations of stiff fiber networks. The stiffness of these constructs depends on the overall composition [37]. For constructs based on gelatin, the most typical form is the hydrogel. This hydrogel can be either injected with or without biologicals like cells and trophic factors [25]. On the other hand, solid gelatin scaffolds in the form of biodegradable patches were also shown to perform in the ischemic heart [45]. In contrast to fibrin, gelatin constructs are shown to be more of a soft and elastic origin [37].

Commonly used synthetic polymers used in cardiac tissue engineering are the aliphatic polyesters poly lactic acid (PLA), poly glycolic acid (PGA), poly lactic-co-glycolic acid (PLGA), and poly caprolactone (PCL). PLA, PGA, and PLGA are all biodegradable constructs whereas PCL is not [37]. The products of PLA, PGA, or PLGA degradation do cause a light inflammatory reaction due to their acid origin [23]. Moreover, PLA, PGA, and PLGA biomaterials also possess the ability to alter their degradation rate by varying their composition [37]. Synthetic biomaterials like PLA, PGA, and PLGA are generally used in combination with other synthetic or natural materials to maximize the mimicking of the native myocardial microenvironment. Additionally, also biologicals like cells and trophic factors can be added to these particular types of synthetic compounds to improve its therapeutic potential in the damaged heart [26]. For PCL on the other hand, one advantage is the modification of pore size and structure. One disadvantage is the high hydrophobicity which results in low biocompatibility [37]. Overall, all different synthetic compounds (PLA, PGA, PLGA, and PCL) either come in the form of a solid patch or an injectable hydrogel [23, 25].

An overview of the aforementioned natural and synthetic biomaterials applied in the field of cardiac tissue regeneration is summarized below in Table 1 [37]. This table includes the biomaterial features biodegradability, biocompatibility, and stiffness together with the applicable forms patch and hydrogel in which the constructs can appear.

	Material	Biodegradability	Biocompatibility	Stiffness	Patch	Hydrogel
Natural	collagen	+++	+++	+	++	++
	chitosan	++	+	+	++	++
	alginate	+++	++	-	++	+++
	fibrin	+++	++	+	+	+++
	gelatin	+++	+++	-	+	+++
Synthetic	PLA	+	+	++	++	++
	PGA	++	+	++	++	++
	PLGA	++	+	+	++	++
	PCL	-	-	+++	++	++

Table 1 Overview of commonly used biomaterials in cardiac tissue regeneration [Retrieved from 37]. (- : none, + : low, ++ : medium, +++ : high)

3. Mesenchymal stem cells in combination with biomaterials

3.1. Mesenchymal stem cells

The mesenchymal stem cells (MSC) is a type of stem cell that can be derived from multiple different sources of the human body. These sources comprise the bone marrow, adipose tissue, peripheral blood, and other types of tissue [13]. MSCs are defined under the following four criteria. Firstly, MSCs must be able to adhere to plastic under standard conditions. Secondly, they must express the surface markers CD44, CD73, CD90, and CD105. Thirdly, they must not express the surface markers CD11b, CD14, CD19, CD34, CD45, CD79α, and HLA-DR. At last, MSCs must be able to differentiate into the mesenchymal lineages osteoblasts, adipocytes, and chondroblasts [46]. Additionally, also the differentiation into stroma cells, skeletal myoblasts, and endothelial cells is shown, but this is not part of the criteria [12].

Stem cells are known to be able to maintain the stem cell pool along with being able to differentiate into various cell types. This reason makes MSCs a very interesting option for the regeneration of damaged cardiac tissue [10]. Moreover, the paracrine effects of MSCs in the forms of growth factors and cytokines make the MSCs of an even higher potential option. These released biological molecules are pro-angiogenic, pro-survival, and pro-heart for the cardiac tissue environment [47]. This is especially needed after the induced damage of MI. The mechanism of action of these important paracrine factors produced by MSCs can be divided into different processes. These processes are represented below in Table 2 in which the most important growth factors and cytokines are shown [48].

On the other hand, MSCs also possess immunomodulatory effects. These effects repress the immune response and induce tissue healing in the infarcted heart [49]. Moreover, MSCs also possess the ability to be unseen by the host immune system. This is due to the lack of the co-stimulatory surface molecules CD40, CD80, CD86, and major histocompatibility complex (MHC) II, and low expression of MHC I [50]. This makes them a really promising candidate for cardiac tissue engineering since the MSC immune privilege enables the use of allogeneic over autologous transplants [14]. Hence, it is logical that MSCs are put forward as an accessible and powerful option because they possess a wide range of therapeutic mechanisms in the regeneration of the damaged infarcted heart.

Process	*Paracrine factor*
cardiac regeneration	VEGF, IGF-1, TGF-β, SDF-1, FGF-2
cardiac contractility	VEGF, FGF, FGF-2, IGF-1, TGF-β
cardiac metabolism	HIF-1α, IGF-1
anti-remodeling	MMP-2, MMP-9, IL-10, TGF-β, SDF-1, IGF-1
anti-inflammation	IL-4, IL-6, IL-10, PG-E2, HIF-1α, TGF-β
pro-survival	VEGF, IGF-1, FGF, FGF-2, TNF-α, SDF-1, EPO
angiogenesis	VEGF, IGF-1, FGF, PDGF, Ang-1, TGF-β, SDF-1, MMP-2, MMP-9

Table 2 Overview of most important paracrine factors produced by MSCs related to its cardiac protective process [Retrieved from 48]. (VEGF: vascular endothelial growth factor, IGF: insulin growth factor, TGF: transforming growth factor, SDF: stromal-cell derived factor, FGF: fibroblast growth factor, HIF: hypoxia-inducible factor, MMP: matrix metalloproteinases, IL: interleukin, PG: prostaglandin, TNF: tumor necrosis factor, EPO: erythropoietin, PDGF: platelet-derived growth factor, Ang: angiopoietin)

3.2. Interaction, retention, and survival

Despite the potential regenerative effects of MSCs in the damaged infarcted heart, limited retention and survival of the therapeutic cells hamper the efficacy of this type of therapy. The reason for this is mainly due to the harsh ischemic myocardial environment. As for the consequences of MI, the damage is especially hypoxic and inflammatory driven. In combination with the already hostile environment of a beating heart, this makes the cardiac microenvironment cell-unfriendly. These effects are mainly seen in the result of increased cell apoptosis and lack of cell adhesion to the heart tissue [17]. Regarding this problem, the use of biomaterials comes in handy. Here, especially the interaction between the MSC and the biomaterial-induced microenvironment is important [37]. As for biomaterials, the mimicking of the natural and native myocardial extracellular matrix is accomplished. This applies to both the patch and hydrogel form. Generally speaking, biomaterials provide chemical and biophysical cues for the cells in the environment [25].

As for especially natural compounds, the containment of certain inherent sequences recognizable for cells enables the cell-biomaterial interactions [30]. This also applies to the aforementioned natural polymers collagen, gelatin, fibrin, alginate, and chitosan. In this way, an improvement of the cell adhesion is reached. Consequently, a significant decrease in cell apoptosis is reached which thereby increases the overall survival of MSCs in biomaterials [51]. As for synthetic-based biomaterials on the other hand, limited cell-recognizable sequences are found for MSCs to bind on. This applies to the aforementioned synthetic polymers PLA, PGA, PLGA, and PCL. Still, these materials do possess some biocompatibility for cells in general [26]. In most cases though, a combination of synthetic and natural compounds is used to enhance the cell-biomaterial interaction. Moreover, even small peptide sequences are used to accomplish this. Overall, these strategies contribute to the enhancement of MSC survival in synthetic-based biomaterials [30].

Furthermore, the addition of a biomaterial to MSCs of either natural or synthetic origin provides the environment with an enlarged surface area in which cells can reside. This is due to the interconnected porous structure of most biomaterials. Additionally, biomaterials are also able to absorb certain protein receptors from the environment. This enables further interaction with the cell membrane and thereby increasing cell adhesion [52]. Finally, also the disturbance of the implanted MSCs is limited by the use of biomaterials. This is because biomaterials offer structural support which reduces the mechanical stress of the beating heart [25].

Overall, it could be said that the use of biomaterials lowers the hostility of ischemic myocardial tissue. In this way, a niche-like environment is established in which the MSCs can safely reside [53]. As for hydrogels in particular, it was documented in several studies that implantation of MSCs in this type of

construct significantly enhanced cell retention and survival compared to implantation of MSCs on their own [25, 54, 55, 56, 57]. It was further shown that this combination of biomaterials and MSCs improved overall cardiac function after MI. As for cardiac patches on the other hand, also the improvement of MSC retention and survival along with cardiac function was shown in several studies compared to implantation of solely MSCs [58, 59, 60, 61]. Altogether, this illustrates the fact that enhancement of cell retention and survival by the use of biomaterials elevates the therapeutic regenerative effects of MSCs for the treatment of MI.

4. Mesenchymal stem cells in biomaterials for regeneration of the heart

4.1. Preclinical and clinical studies

Over the past ten years, the regenerative capacity of MSCs on the infarcted heart without the addition of biomaterials has been examined in humans in the form of various clinical trials [62, 63, 64, 65, 66, 67]. These studies did show some advances for treating MI-induced damage. Though, optimization of MSC transplantation to the infarcted heart in terms of cell retention and survival is still crucially needed before implementation of further advanced clinical trials [68]. Thus far, there is a minimal number of performed human clinical studies, which include the combination of MSCs and biomaterials [69]. In recent years however, multiple preclinical studies performed on a range of small to large animals did document the use of MSCs applied in biomaterial constructs [70, 71, 72, 73, 74]. The next section highlights the key results of one of these recent studies. The processes of cardiac function, fibrosis, and vascularization in cardiac regeneration are highlighted. These processes are aided by the paracrine, differentiative, and immunomodulatory effects of MSCs supported by biomaterials.

4.2. Cardiac function, fibrosis, and vascularization in a preclinical example

This study analyzed the use of MSCs in a hydrogel composed of a combination of PGA and chitosan with the addition of bioglass in a MI mice model [75]. After MI induction by coronary ligation, either phosphate-buffered saline (PBS), MSCs, or MSCs in combination with the hydrogels were directly injected into the infarction zone. Four weeks after treatment, overall cardiac function was assessed by using echocardiography on the left ventricle. For the mice treated with PBS or MSCs alone, it was shown that they suffered from decreased heart function. This was due to the dilation and enlargement of the left ventricle. Treatment with MSCs alone minimally improved some of the heart function in terms of ejection fraction, fractional shortening, and overall left ventricular contractility. However, treatment of MSCs embedded in the hydrogel massively ameliorated the heart function in terms of the same parameters. These results are represented below in Figure 1.

As for fibrosis formation after four weeks, similar results were shown. The results were shown by staining as represented below in Figure 2. Here, the treatment of MSCs alone minimally reduced scar formation. MSCs loaded in the hydrogel on the other hand, significantly reduced fibrotic tissue formation. Consequently, this contributed to the improvement of cardiac function. In terms of vascularization, also the treatment of MSCs combined with biomaterials showed the highest blood vessel density in the infarcted region compared to PBS and most importantly compared to MSCs alone. These results are represented below in Figure 3.

Altogether these findings provide that a combination of MSCs and biomaterials result in a significant improvement of overall cardiac function compared to the use of solely MSCs.. These improvements are in particular seen in terms of increased contractility, decreased scar formation, and increased vascularization.

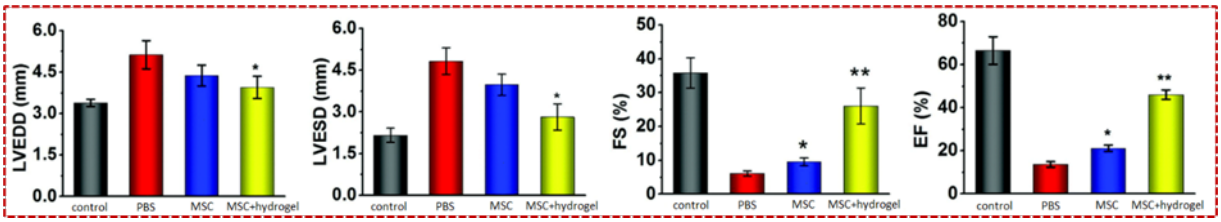

Figure 2 Echocardiographic results of the heart 4 weeks after MI induction in a mice model. Overall cardiac function was best improved for MSC + hydrogel treatment [Retrieved from 75] (LVEDD: left ventricular end-diastolic diameter, LVESD: left ventricular end-diastolic diameter, FS: fractional shortening, EF: ejection

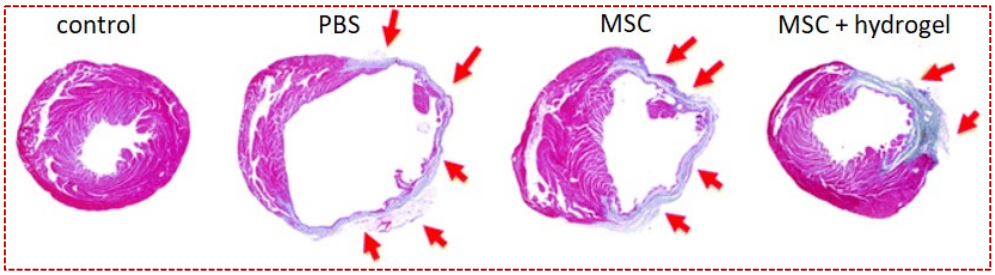

Figure 1 Imaging of fibrosis formation of heart sections 4 weeks after MI induction in a mice model wherein red arrows and blue areas indicate fibrotic tissue. Wall dilation of the left ventricle was observed in all treatment groups. MSC + hydrogel treatment showed the most reduction of fibrotic tissue formation [Retrieved from 75].

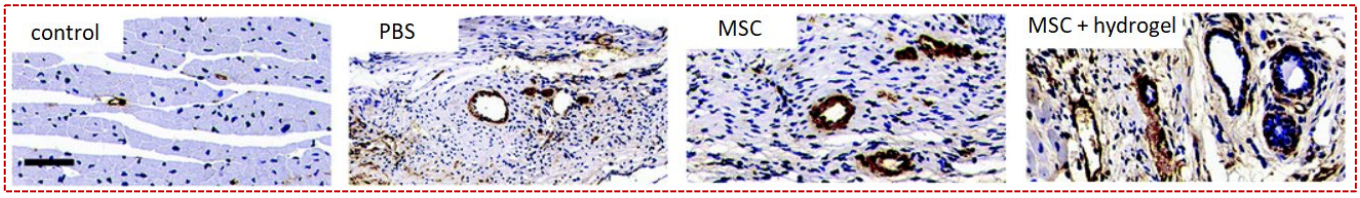

Figure 3 Imaging of vascularization of heart sections 4 weeks after MI induction in a mice model wherein black area indicates α-SMA which is a typical protein for blood vessels. Vascularization was observed for all treatment groups. MSC + hydrogel treatment showed the highest blood vessel density [Retrieved from 75].

5. Future Perspectives

5.1. New insights

Until so far, the large potential of biomaterials has been highlighted in the field of cardiac tissue engineering. It was shown that biomaterials are either used as a support for the dilated ischemic heart, as a carrier to strengthen therapeutic factors like cells and molecules, or even as an executor of both these functions. Several features of the biomaterial can be modified and tweaked to fulfill the desired goal of the biomaterial. Such features comprise biocompatibility, biodegradability, mechanical and architectural properties such as elasticity, stiffness, and porosity, and at last composition. Particular for the regeneration of the infarcted heart, a set of important criteria for the biomaterial need to be met. Overall, these include the fostering of host and therapeutic cells, the degradation at similar rates as cardiac regeneration, elasticity similar to heart compliance, and sufficient porosity for vascularization. By using natural materials either alone or in combination with other modifiable synthetic compounds, the aforementioned criteria can be achieved and further modified to maximize tissue engineering capacity. Furthermore, the superior form of injectable hydrogels over cardiac

patches ensures precise targeting of the entire infarcted region together with minimal invasiveness of the treatment.

As for MSCs, their efficacy is markedly enhanced by the support of biomaterials. This is due to the various cell-biomaterial interactions. These interactions protect MSCs from the harsh cardiac environment which results in the increased retention and survival of these cells. In this way, the great regenerative potential of MSCs in terms of immune privilege and paracrine, differentiative, and immunomodulatory effects is put at a higher level. This illustrates the ideal opportunity of creating an off-the-shelf available biomaterial in the form of a hydrogel construct that is able to give mechanical support for the heart together with the MSC carrying ability. In this way, great regenerative effects on the infarcted heart tissue are initiated in a minimally invasive way for post-MI patients.

5.2. The road ahead

Despite the wonderful sound of having ready-to-use available biomaterials with therapeutic MSCs for patients in need, still some important steps need to be taken. These steps are in terms of construct optimization [23] and the onset of clinical trials in humans. Regarding the biomaterial construct, further improvement of the mechanical properties is still necessary. This is due to the reason that no ideal composite is found yet that provides sufficient support for the heart contractility at both systole and diastole. On the other hand, manufacturing biomaterials similar to the native heart also entails a complex task due to the nonlinear elastic origin of the heart. Furthermore, balance in porosity and construct surface area is a factor that needs to be optimized. Here, some degree of porosity is needed for the formation of microvessels so that adequate diffusion is enabled by the formation. However, too porous constructs lack the ability to foster cells due to the limited surface area.

Given that the ongoing and future studies hopefully answer most of the hurdles regarding biomaterials, still one final step is necessary. This is the vital transition from preclinical towards clinical trials for full real-life implementation of MSCs loaded biomaterials in cardiac regeneration therapy. At this stage, clinical trials have shown the safety of allogeneic MSC transplants [76], hydrogels [77], and patches [78]. Furthermore, the promising results of the MSC-biomaterial combination were seen in preclinical trials. However, elucidation and confirmation in human clinical trials still need to be performed on the mid to long term of the regenerative effects of these type of constructs in the infarcted heart. Hence, this study can be used as a vehicle to illustrate the massive added benefit of biomaterials for the infarcted heart and MSC therapy. Consequently, stimulating the initiation of clinical studies for the progression of MI treatment. In this way, the ultimate goal of relieving the high globally and societal burden of CVDs is accomplished.

6. Graphical Abstract

To summarize, a general overview of this study is represented below in Figure 4. This figure illustrates the potential combination of MSCs and biomaterials for the regenerative treatment of MI. To emphasize the importance of this combination, also the current state of affairs of preclinical and clinical studies is illustrated.

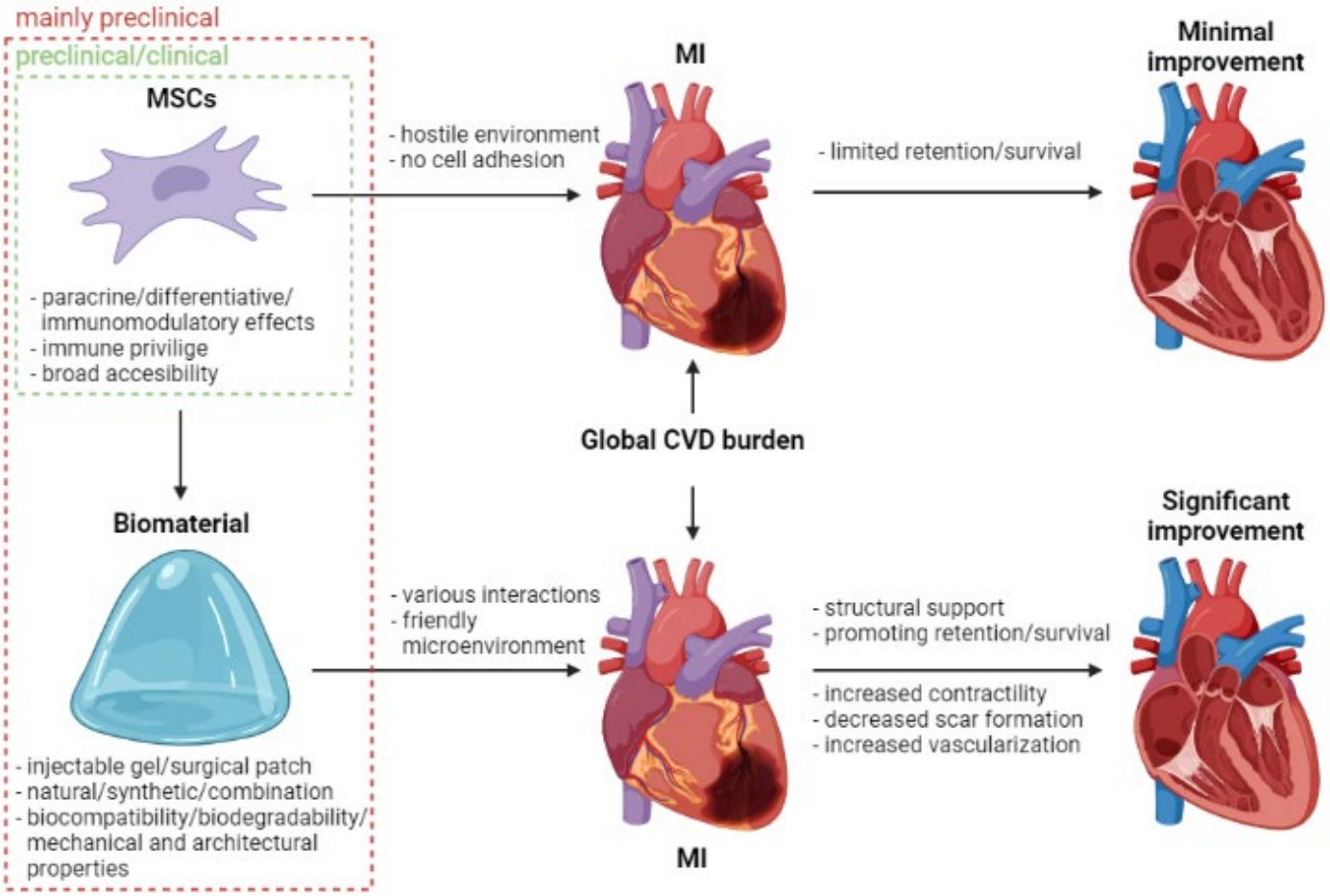

Figure 4 Graphical abstract of this study that illustrates the potential combination of MSCs and biomaterials for the regenerative treatment of MI. The current state of affairs of preclinical and clinical studies is illustrated to emphasize the necessity of this combination [Created in BioRender]. (MSC: mesenchymal stem cell, MI: myocardial infarction, CVD: cardiovascular disease)

7. References

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