Mechanotransduction of mesenchymal stem cells (MSCs) during cardiomyocytes differentiation

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ABSTRACT

Cardiac muscle cells have an innate capacity to perceive and react to mechanical strain via a mechanism known as mechanotransduction, whereby the cardiac muscle cells are intrinsically capable of sensing and responding to mechanical strain. This process occurs in the heart when mechanical inputs are converted to biochemical processes that result in myocardial structure and function changes. Mechanotransduction and its downstream effects work as compensatory mechanisms during early load adaptation. However, prolonged, and aberrant loading may cause maladaptive remodeling, resulting in altered physiological function, pathological cardiac hypertrophy, and heart failure. The rapid advancement of stem cell research has raised the hopes of both patients and clinicians. Mesenchymal progenitors have become one of the most intriguing possibilities for treating illnesses ranging from cartilage abnormalities to heart issues. Their immunomodulatory properties have also allowed for allogenic usage, besides expanding their potential for cardiomyocyte applications. In the present review, we highlighted mesenchymal stem cells (MSCs) in cardiovascular mechanotransduction, differentiation of cardiomyocytes and the use of MSCs in cardiovascular disease and tissue engineering.

1. Introduction

Recent studies revealed that high rates of morbidity and death associated with cardiovascular diseases (CVDs) cause a significant contribution to the global disease burden (Singh et al., 2016). CVDs are caused by both viral and non-infectious constituents that lead to Rheumatic heart disease and tuberculous pericarditis are among the infectious cardiovascular diseases (CVDs), whereas hypertension, myocardial infarction (MI), stroke, and peripheral artery disease are well known for non-infectious cardiovascular diseases (CVDs) for centuries. Aside from that, the cases related to CVD caused by non-infectious causes, particularly ischemic CVDs such as MI, show an increasing trend for future undertakings. MI causes the myocardial cells to become fatal and dysfunctional, most importantly, it targets ventricular remodeling resulting in additional heart failure and complications (Karantalis and Hare, 2015). Although scientific breakthroughs and improvements in surgical procedures have been made, pharmacological and surgical therapies for chronic heart disease may only postpone the course of the illness and are unable to restore the function of infarcted myocardial cells (Gao et al., 2007). Macrophages, monocytes, and neutrophils migrate to damaged tissues resulting from MI, which initiates an inflammatory response (Tavi et al., 2001). The inflammatory response enables myocyte necrosis, and a subsequent load accelerates signal transduction processes that regulate cardiac repair, which eventually leads to the formation of fibrous scar tissue that causes failure of the heart cells (Gao et al., 2007; Farzaneh et al., 2018). Heart transplantation is the sole treatment option for heart failure patients, but donor organs are in short supply, and the cost of the procedure prevents further advancement of this technique of treatment. Therefore, the utilization of stem cells as a potential therapy for heart illness appeared as a promising treatment for heart disease (White et al., 2016).

MSCs are regarded as an intriguing alternative for cell-based treatment in which they may be isolated to enhance the release of bioactive chemicals and microvesicles. Besides that, MSCs stimulate tissue repair
processes and highlight the potential to modify immunological and inflammatory cells (Epstein et al., 2017). Allogeneic cell-based therapy is fraught with the danger of immune rejection (Oztürk et al., 2021a,b). However, the absence of cell surface histocompatibility complex (HLA) class II molecules and T cell costimulatory molecules, as well as their paracrine-mediated immunomodulatory activity, confers on MSCs an immune-privileged status that eliminates the need for pharmacological immunosuppression and prevents their destruction by the host immune system (Nesselmann et al., 2008; Wang et al., 2018). As a result, MSCs may be employed safely as allografts in cardiomyopathy patients and are well tolerated in the same way as autologous MSCs (Epstein et al., 2017; Bagno et al., 2018; Miloradovic et al., 2021). As a result of their unique characteristics, such as their capacity to differentiate into cardiomyocytes cell has been shown in Figure 1, MSCs are increasingly being used in the treatment of cardiovascular diseases (Elnakish et al., 2012; Mazo et al., 2012; Oztürk et al., 2021a,b). Additionally, extracellular vesicles derived from MSCs are thought to mediate cellular functions in addition to their ability to differentiate into cardiovascular progenitor cells (Huang et al., 2013). For example, MSC-derived extracellular vesicles have been shown to increase cardiomyocyte autophagy via the AMPK/mTOR and Akt/m-TOR pathways, angiogenesis via the hypoxia-inducible factor-1 (HIF-1), and cell apoptosis and activation of the cell survival signal via the multiple microRNAs (miRNA) (Elnakish et al., 2012).

2. MSCs differentiation into cardiomyocytes

MSCs developed into cardiomyocytes, exhibiting a zonal distribution in myocardial tissue similar to cardiac myocytes upon transplantation (Kratchmarova et al., 2005; Salazar-Noratto et al., 2020). The elevated myocardial-specific marker of protein expression, such as troponin T, indicates that MSCs have differentiated into cardiomyocytes, for instance, contracting cells involves in MSCs differentiation, through the formation of cardiomyocyte feeder layers from human umbilical cord perivascular cells (Duchemin et al., 2019). In-vitro, basic fibroblast growth factor (bFGF) may increase bone marrow MSC migration and survival while retrograde (bFGF) perfusion of the coronary vein has been shown to improve MSC graft transplantation, increase phenotypic differentiation of MSCs to restore function in cardiac which in turn minimize unfavorable remodeling (Liao et al., 2012; White et al., 2016; Halim et al., 2020). Cardiomyocyte differentiation of bone marrow MSCs may also be promoted in-vitro by isolating and amplifying bone marrow MSCs with high purity in addition to excellent growth dynamics and co-stimulating them with (bFGF) and hydrocortisone. Additionally, MSCs have been genetically engineered to produce cardiomyocyte-like cells (White et al., 2016; Nguyen et al., 2019; Miloradovic et al., 2021).

The multilineage differentiation in MSCs occurs via exogenous Jagged 1 (Li et al., 2006) stimulating the Notch1 signaling pathway whereby it was found that miRNA1-2 overexpression in mouse, MSCs generated by stimulation of the Wnt/-catenin signaling pathway improved MSC differentiation into cardiomyocytes with indicating increased Nkx2.5, cTnI, and GATA4 expression and decreased cytotoxicity (Kleber and Sommer, 2004; Flaherty et al., 2012; Farzaneh et al., 2018). Many studies have indicated that beneficial effects of MSC therapy can occur due to the ease of extraction and production of bioactive chemicals and microvesicles that promote tissue regeneration and regulate immunological and inflammatory (Wang et al., 2018; Pittenger et al., 2019; Naqvi and...
McNamara, 2020). In allogeneic cell-based therapy, immune rejection plays another key role by inducing the immunomodulatory properties of MSC, which come in handy to eliminate the need for pharmacological immunosuppression and protects them from further destruction by the host immune system, whereas all this process resulted from the absence of HLA class II molecules and T cell costimulatory molecules on their surface as well as their paracrine-mediated immunomodulatory activity (Epstein et al., 2017; Bouzid et al., 2019; Halim et al., 2020).

It has been shown that MSCs may be employed safely as allografts in patients with cardiomyopathy or human autologous MSCs (Hare et al., 2017), hence, MSCs are critical in the treatment of CVD because of their unique characteristics, which include their potential to develop into cardiovascular cells, immunomodulatory activity, antifibrotic activity, and capacity for neo vasculogenesis (Koff et al., 2007; Bagno et al., 2018). Apart from their differentiation into cardiovascular progenitor cells, extracellular vesicles derived from MSCs have been shown to enhance cardiomyocyte autophagy through the AMPK/mTOR and Akt/mTOR pathways, angiogenesis via the hypoxia-inducible factor-1 (HIF-1)/Jagged pathway, and to decrease cell apoptosis and activate cell survival signaling pathway via multiple miRNAs, besides regulating cellular function (Soria-Juan et al., 2019; Miloradovic et al., 2021; Özürek et al., 2021a,b). MSCs can be differentiated into cardiomyocytes via factors such as differentiation, paracrine effects, cell-cell effects, the involvement of 5-azacytidine, and methanotransduction as described in the previous subsections. MSCs are derived from bone marrow and blood, which are characterized by their capacity for self-renewal and multilineage differentiation into the mesodermal lineage including cardiomyocytes as shown in Figure 1, were demonstrated to express the potential of MSCs can undergo myogenesis under appropriate culture conditions and in the presence of paracrine regulatory factors such as Wnt, HGF, PDGF, and Notch, which leads to cell repair strategies with cardiomyocytes.

2.1. Influence of paracrine effects during MSCs differentiation into cardiomyocytes

Most studies have demonstrated that growth factors and cytokines, such as hepatocyte growth factor (HGF), leukemia inhibitory factor (LIF), fibroblast growth factors (FGFs), platelet-derived growth factor (PDGF), mammalian homologs of Drosophila wingless (Wnts) and Notch-1 signaling pathway (Kratchmarova et al., 2005; Demirdögen et al., 2010; Zhao et al., 2016), influence the MSCs differentiation into cardiomyocytes, as illustrated in Figure 1. Recently, it has been found that cardiac muscle cells can be generated from bone marrow MSCs and the umbilical cord of Wharton’s jelly (Wang et al., 2004). Therefore, these discoveries reveal that in the presence of exogenous 5-azacytidine (5-Aza), a cytidine analog, MSCs produced cardiomyocyte-specific markers such as cardiac troponin-I and N-cadherin. The cytidine analog is implicated in the activation of phenotype-specific genes for hypomethylation, including certain cytosine in DNA (Martin-Rendon et al., 2008). On the other hand, MSCs can differentiate into muscle cells in the presence of miR1-2 in-vitro, which is well known as endogenous short non-coding RNAs (miRNAs), hence, it plays a crucial role in stem cell proliferation and differentiation (Jekushi et al., 2012), as well as in the regulation of gene expression (Saito, 1995).

Myocardial development is the primary role of miR1-2 in mammals, however, in-vitro studies have shown that it may influence cardiac myogenesis (Jekushi et al., 2012; Farzaneh et al., 2017, 2018; Halim et al., 2020). MSC markers may be identified by the expression of various surface antigens such as CD105, CD106 (Forté et al., 2006), Stro-1 and CD73 throughout a culture. However, when bone marrow mesenchymal stem cells (BMSCs) begin to differentiate into cardiomyocytes, the expression of these markers is reduced rapidly. Extensive studies revealed that cardiomyocytes are positive for beta-myosin heavy chain (beta-MHC), alpha-cardiac actin, and desmin (Costa et al., 2004) in the appropriate culture setting (Valfré Di Bonzo et al., 2008; Liao et al., 2012; Gao et al., 2014; Peng et al., 2017). In the presence of paracrine regulatory factors such as HGF, PDGF, Wnt (next subsection) and under the right growth conditions, MSCs may undergo myogenesis, leading to cardiomyocyte cell repair strategies.

2.1.1. Effects of hepatocyte growth factor (HGF)

In embryogenesis and tissue homeostasis, the HGF-Met signaling pathway is critical for a wide range of cellular activities. It has been shown that the scatter factor (HGF) has a wide range of biological impacts on early heart development, survival, migration, and proliferation or differentiation of MSCs (Costa et al., 2004). As a result of activation of HGF and its high affinity Met receptor tyrosine kinase, it similarly induces the activation of signal-transducing proteins such as extracellular signal-regulated (ERK1/2), p38 mitogen-activated (MAPK) and phosphatidylinositol-3 (P3K)/Akt kinases (Zhao et al., 2016). Another key role of HGF and Met receptor is to express cardiomyocytes specific transcription factors and structural genes which include myocyte-specific enhancer factor 2C (MEF2C), translation elongation factor EF-1 alpha (TEF1-), GATA-4, alpha myosin heavy chain (MHC), and desmin, hence they considered as an important factor in the early stages of cardiac regeneration as well as in regulating gene expression (Bouzid et al., 2019).

MSCs have been demonstrated to lose the expression of nucleostemin (NST), c-kit, and CD105 during the formation of cardiomyocytes. Interestingly, the p38-MAPK pathway involves an inhibitor known as SB203580, which has been shown to prevent HGF-induced reactions and which have been shown to suppress myogenic MSCs differentiation. It has been found that HGF therapy involving cardiomyocytes could be administered for cardiac repair, in addition, HGF is reported to play a key role in cardiomyocyte hypertrophy, moreover, Ang II is shown to increase the rate of cardiomyocytes hypertrophy (Chen et al., 2012; Liao et al., 2012). A recent experiment conducted in a culture containing placenta-derived multipotent cells with laminin releases a significant amount of HGF secretion via Avb3/Cd61 integrin, an integrin that is abundantly expressed in MSCs, reduces apoptosis and reactive oxygen species (ROS) generation in tumor necrosis factor-induced cardiomyocyte (Moghadam et al., 2016).

2.1.2. Platelet-derived growth factor (PDGF) effects

Heldin et al. (2013) described that PDGF, and its receptors regulate cell survival, particularly involving the development and growth of a cell. PDGF was also found to regulate the differentiation of murine MSCs into cardiomyocytes and may contribute to the increase in connexin43, α-MHC, and β-MHC, known as cardiomyocyte-specific markers (Kratchmarova et al., 2005). Similar studies demonstrated that PDGF-treated mouse embryonic stem cells (ES) were shown to stimulate myogenic differentiation and the number of beating embryoid bodies (Ebs) which in turn induces the molecular phenotype of cardiomyocytes (Farzaneh et al., 2018). Another study found that Platelet lysate (PL), which is generated in the human, generates several growth factors, including the PDGF which in later stages enhanced the development of rat MSCs into cardiomyocytes (Nagaya et al., 2005). PDGF seems to be an effective agent for the differentiation of murine (ES) cells and MSCs into cardiomyocytes, thus extensive studies should be involved in future studies to explore activating PDGF signaling that may stimulate human MSCs to develop into cardiomyocytes (Gao et al., 2007, 2014).

2.1.3. Effects of Wnt signaling pathway

During development, the Wnt/β-catenin dependent pathway governs several important processes (Fluherty et al., 2012; Wolke et al., 2021) including migration, polarity, patterning, organogenesis, and MSC differentiation into cardiomyocytes (Kløver and Sommer, 2004). The miR1-2, previously implicated in cardiac formation, has been demonstrated to induce Wnt/β-catenin-mediated differentiation of mouse bone marrow MSCs into cardiomyocytes, which in turn elevate myogenic regulatory factors (MRFs) (Komiya and Habas, 2008). A cytidine chemical analogue, 5-Aza, is shown to increase (GSK)-3, glycogen synthase...
kinase expression, in addition, miR-2 may then influence cardiogenesis in response to 5-Aza administration (Wan Safwani et al., 2012). Recent studies of miR-1-2 have shown to negatively regulate Hes-1 in MSCs, which may lead to the induction of cardiogenesis (Huang et al., 2013). By triggering a negative Notch signaling loop, the activation of Hes-1 suppressed the differentiation of MSCs. Cardiogenesis and embryonic heart progression rely heavily on Notch/Jagged1 signaling (Li et al., 2006), which is activated by HGF/c-Met signaling. In short, it has been indicated that Wnt signaling has a huge impact on MSCs differentiation into cardiac cell lineages (Li et al., 2006; Huang et al., 2018; Jung et al., 2020). Wnt signaling is triggered through the coupling of the Frizzled family receptors (Komiya and Habas, 2008). The expression of Wnt-associated genes in canonical Wnt signaling is regulated by cytosolic β-catenin aggregation, which stimulates its translocation into the nucleus, where it binds the TCF/lymphoid enhancer-binding factor family of TCFs to modify gene expression. Therefore, without Wnt activation, β-catenin binds to Frizzles destruction complex, which includes axin, adenomatous polyposis coli, and glycogen synthase kinase 3β (Kleber and Sommer, 2004). When β-catenin binds to its destruction complex, it is phosphorylated and targeted for degradation, preventing cytosolic accumulation.

Wnt associated with Frizzled, on the other hand, causes the axin/adenomatous polyposis coli/glycogen synthase kinase 3β destruction complex to disassemble, and cytosolic β-catenin to be stabilized, allowing for translocation into the nucleus. It changes the transcription of genes such as Brachyury and Mesp1 during mesoderm induction and IsIl1, Flk1, and Nkx2.5 during cardiac progenitor specification and differentiation after it is localized (Flaherty et al., 2012). Frizzled binding stimulates GTPases RhoA and Rac, which drive contractility and cytoskeletal rearrangement, and changes gene expression through c-Jun N-terminal kinase activation of the transcriptional regulator, activator protein 1. Noncanonical Wnt-mediated cytoskeletal rearrangement has been associated with cardiac progenitor cell migration and polarization during gastrulation and cardiac morphogenesis. Furthermore, noncanonical Wnt ligands Wnt2 and Wnt11 regulate cardiac differentiation through c-Jun N-terminal kinase/activator protein 1 (Gao et al., 2014; Hao et al., 2015; Farzaneh et al., 2018). Cell adhesions and cadherin expression (Aberle et al., 1996), which play a role in mesoderm determination and cardiac differentiation, impact the complicated Wnt signaling that orchestrates cardiogenesis (Leitolis et al., 2019). During development and heart morphogenesis, the canonical Wnt signaling pathway interacts bidirectionally with cadherin-mediated signaling to govern cell movement and adhesion (Flaherty et al., 2012). Mechanical stresses may impact cell mechanics by modifying cytoskeletal structure and contractility through modulating adhesion expression and the Wnt/β-catenin signaling cascade. Therefore, the Wingless (Wnt) pathway involves important adhesion pathways such as integrin- and cadherin-mediated signaling. Most of these pathways regulate mesoderm development, cardiac specification, and differentiation during cardiomyogenesis (Komiya and Habas, 2008). On the other hand, paracrine factor secretion permits MSCs to differentiate into cardiomyocytes, which might be exploited to treat heart disease (Ohnishi et al., 2007). Paracrine interactions may help enhance cell-based repair methods with cardiomyocytes (Farzaneh et al., 2018). In addition to paracrine regulatory factors such as Wnt, HGF, and PDGF, 5-azacytidine plays a crucial role in encouraging the differentiation of cardiomyocytes in MSCs.

2.2. Role of 5-azacytidine in the differentiation of MSCs into cardiomyocytes

Various substances may be used to encourage MSCs to transdifferentiate into cardiac cells in vitro (Oztürk et al., 2021a,b). The most common compounds utilized to stimulate cardiologyocyte development in MSCs are 5-azacytidine, BMP-2, angiotensin II, dimethyl sulfoxide, and fibroblast growth factor-4. Cardiomyocyte differentiation is induced by co-culturing MSCs with cardiomyocytes or genetic transformation (Xu et al., 2004; Martin-Rendon et al., 2008). Similarly, endothelial differentiation of MSCs may be achieved by culturing with vascular endothelial growth factor VEGF, hypoxic conditioning, or applying hemodynamic-like stresses for mechanotransduction (Gnecci et al., 2006; Markel et al., 2008; Kim et al., 2012; Zhao et al., 2016). However, neither of these differentiation procedures might provide cells that are quantitatively, morphologically, genetically, and functionally suitable for therapeutic translation (Müller et al., 2018). Trials using BM-MSCs, and rat embryonic cardiomyocytes produced cardiac-specific markers while maintaining MSC properties but lacking cardiomyocyte electrophysiological characteristics like action potential production or normal ionic (Huang et al., 2010b; Liao et al., 2012). Similarly, 5-azacytidine-treated human MSCs from the umbilical cord (UC), cord blood, and BM could not produce enough cardiomyocytes for cardiac repair (Martin-Rendon et al., 2008).

5-azacytidine is a demethylation agent which is involved in processes such as gene expression, chromatin modification, chromosomal inactivation, genomic imprinting, and endogenous gene silencing that have all been linked to DNA methylation (Sulewska et al., 2007; Zhou et al., 2009; Wan Safwani et al., 2012). DNA methylation is also essential for the maintenance of stem cells’ pluripotency and capacity for self-renewal (Sulewska et al., 2007). During hypomethylation, genes associated with pluripotency are activated, whereas genes linked with differentiation are inhibited. 5-azacytidine has been found to promote stem cells from bone marrow and adipose tissue to become cardiomyocytes (Makino et al., 1999; Burlacu et al., 2008; Rosca and Burlacu, 2011). Bone marrow stromal cells have been observed to be well associated with 5-azacytidine (Zhao et al., 2009). After being treated with 5-azacytidine, only P4 of the bone marrow-derived stem cells indicated the development of myotubes that expressed cardiac-specific markers.

A study published in 2003 found that 5-azacytidine can only be used to stimulate cardiomyogenic differentiation in cells that have been immortalized. A study published by Xu et al. (2004) found that mesenchymal stem cells generated from adult human bone marrow differentiated into cardiomyocytes. In addition, adipose tissues obtained from New Zealand white rabbits contain mesenchymal stem cells that can be converted into cardiomyocytes, and these cells begin beating spontaneously three weeks following treatment with 5-azacytidine. A 2007 study by Burlacu et al. (2008) found that 5-azacytidine promoted, rather than induced, the myogenic differentiation of bone marrow progenitor cells. On the other hand, Wan Safwani et al. (2012) found that ASCs were not able to differentiate into cardiac lineage cells following 4 weeks of induction using 5-azacytidine.

The heart has both electrical and mechanical components. Pacemaker cells in the sinus venous generate action potentials to trigger the heartbeat. This causes the contraction of cardiomyocytes, which creates the mechanical force necessary for blood to circulate throughout the body. Throughout each contractile cycle of the heart, cardiomyocytes are subjected to a range of stresses as a consequence of their natural ability to sense and respond to mechanical strain through a process known as mechano-transduction. In the heart, this process includes the transformation of mechanical impulses into biochemical processes that produce changes in the structure and function of the myocardium.

3. Cardiovascular mechanotransduction

The growth signal induced by the mechanical cues itself is amplified by the manufacture and release of transforming growth factors in heart tissue during mechanotransduction. These autocrine and paracrine substances may at first seem to be compensatory during myocyte hypertrophy (Garoffalo and Pesce, 2019) but their long-term effects are detrimental in chronic conditions. Mechanical stimuli are sensed and responded to by developing cells, tissues, and organs, giving them their distinct forms (Delaine-Smith and Reilly, 2012; Steward and Kelly, 2015). Invertebrate embryos, the heart is the first organ to begin working. In fact, to heart function, calcium excitation and contraction of the cardiomyocytes are dependent on the correct elasticity (Liu et al., 2021).
et al., 2014) mentioned that substrate stiffness affects the heart rate contraction forces as well as the cytoskeletal organization and intracellular calcium levels in cardiomyocytes (Jaalouk and Lammerding, 2009). Pulsatile fluctuations in heart internal pressure are another important element that contributes to the process of cardiogenesis (Argentati et al., 2019; Mohammed et al., 2019). In experiments that cardiac cells were grown on a decellularized heart matrix, shows broad viable cardiac muscles were formed when pulsatile perfusion was used, but thin, weak muscles were generated in an environment without perfusion (Norman et al., 2017).

Connexin expression and localization are both influenced by cyclic mechanical stretching (Liu et al., 2003). The orientation of cardiomyocytes is also transverse to the stretch axis in response to cyclic stretching. Thus, the heart’s gap junction channels may be affected by mechanical pressures, which can influence intercellular communication (Kearney et al., 2010). Cardiovascular seption and valve development may be impaired by changes in blood flow patterns (Duchemin et al., 2019). There have been various efforts reported to create cardiomyocytes from embryonic stem cells, induced pluripotent stem cells, and cardiac stem cells in recent years to discover a way to heal adult hearts after heart attacks (Kabat et al., 2020). Applying mechanical stimulation may be essential for the generation of robust cardiomyocytes from MSCs, given that the heart is an organ continually exposed to mechanical stimuli (Argentati et al., 2019).

In recent years, angiotensin AT1 receptor through Ang II has been implicated in the development of stretch-induced heart hypertrophy in several investigations, additionally, ET-1 may play a role since ET-1 in rat heart is released when endothelial cells are stretched, and it has been demonstrated to rise in the pressure (Chen et al., 2012). As a result, Ang II is released, which in turn releases ET-1. Stretching may also cause the release of Ang II. Wall stretch-induced gene expression in the ventricularis is not dependent on Ang II and ET-1, according to an AT1 receptor antagonist losartan (Grossman et al., 1975). Although Ang II is a significant element, it is not required for the load-induced hypertrophy seen in transgenic mice with the AT1 (Inger, 2003; Kolf et al., 2007; Haudenschild et al., 2009; Elnakish et al., 2012; Miloradovic et al., 2021). Mechanotransduction, on the other hand, has various pharmacological targets because of the participation of these auto and paracrine mechanisms (Tavi et al., 2001; Yamasaki, 2014; Wang et al., 2018; Duchemin et al., 2019).

4. MSCs in cardiac cell-based therapies

Regenerative medicine developed for cardiovascular diseases has become a promising approach to repairing damaged heart tissues (Chou et al., 2014). Yet, the overall repair of heart damage is still in progress due to the inability of the adult heart to promote sufficient de novo cardiomyogenesis to substitute cells lost to disease (Hao et al., 2015; Butler et al., 2017; Bagno et al., 2018; Jin et al., 2020). This is because of its intractable genetic and epigenetic state, limited cellular plasticity, and proclivity to succumb to pro-inflammatory and pro-fibrotic immune pathways (Nauta and Fibbe, 2007). Although several studies addressing various medical applications confirmed the efficacy of MSC therapy (Chou et al., 2014; Hao et al., 2015; Müller et al., 2018) the effect of MSC-based therapies fell over long-term outcome assessments compared with conventional therapies. In brief, gene and stem cell therapies are among the most promising regenerative methods in cardiovascular diseases, and experiments are currently being conducted to improve the efficacy of therapies (Tavi et al., 2001; Karantalis and Hare, 2015; Norman et al., 2017; Mohammed et al., 2019).

Currently, certain gene transfer therapies are designed mostly to downregulate and elevate the expression of immune system genes, cardiac and vascular, which are abnormally expressed in the pathologic heart (Becker et al., 2006; Hedman et al., 2003; Post et al., 2006). Most promising gene transfer therapies use ectopically expressed oncogenes to drive the growth of adult cardiomyocytes (Tsata and Beis, 2020). Recent research which is gaining popularity has shown that the transfer of lineage reprogramming gene combinations into myocardial scars occurs due to the turning of non-cardiomyocytes into beating cardiomyocyte-like cells (Mastrullo et al., 2020). Advancement in genome-engineering tools allows CRISPR/Cas system to develop the possibility of using gene therapy to permanently edit and correct disease-causing mutations in the genomes of adult cardiomyocytes, a breakthrough that has the potential to revolutionize medicine that can be employed for human benefits (Garoffolo and Pesce, 2019).

However, many preclinical and clinical investigations revealed the translation of gene therapy subjected to limitations in a clinical context. It has been demonstrated in patients with heart disease, which results in unsatisfactory efficacy based on preclinical animal models (Singh et al., 2016; Peng et al., 2017; Bagno et al., 2018; Soria-Juan et al., 2019; Khan, 2021). While direct application of cardiac therapies or gene transfer for lineage reprogramming itself has a high risk which may result in ectopic cardiomyocytes or neoplastic formation into the patient’s body followed by the recipient cell and the activity of the transferred gene is not carefully controlled (Halim et al., 2020). To resemble the applicability of the Cas system for cardiac regenerative medicine, the restriction can be identified by preclinical proof-of-concept experiments (Nguyen et al., 2019). It requires in situ genome editing of billions of cardiomyocytes at the single-cell level without expressing undesired off-target mutations and heart diseases that are genetically complex and polygenic (Norman et al., 2017; Halim et al., 2020). The highly robust, versatile capabilities of cardiac therapy utilize healthy cell transfer to damaged areas rather than isolated genes, thus, the cells exhibit their operation either directly or indirectly, whereas they directly substitute the damaged tissues with the new healthy cells, while the molecules and microvesicles secreted indirectly to activate cardiac regeneration and immune regulation via endogenous process (Mias et al., 2009).

The allogeneic stem cell transplants are obtained from adult tissues such as bone marrow, skeletal muscle, pluripotent stem cells, and heart which have been demonstrated as cell therapies, facing many difficulties in clinical translation as compared with gene therapy (Li et al., 2009). The implanted cells directly re-muscular and regenerate the damaged myocardium according to the predicted hypothesis, however, most adult cell types have limited cardiomyocyte differentiation capacity and have reduced long-term engraftment regardless of histocompatibility which resulted in immune clearance of the host myocardium (Guo et al., 2007).

Other approaches involve bona fide cardiomyogenic or regorammed cells originating from cardiac precursors and cardiomyocytes of pluripotent stem cell, has poor engraftment regardless of histocompatibility and are mostly known source of neoplasia (Valfré Di Bonzo et al., 2008; Hong et al., 2014; Razijeyeva et al., 2020) arrhythmogenesis in the host myocardium, which in later eliminates by the immune system. Direct tissue replacement and cell therapy in cardiac dysfunction showed vast effects and capability, the yield of these therapies was far beyond reaching the level of commercial demand yet. Besides, tissue replacement of damaged tissue in cardiac has huge disadvantages due to a lack of understanding of paracrine mechanisms (Gnecchi et al., 2006; Ohnishi et al., 2007; Epstein et al., 2017; Farzaneh et al., 2018) which employs endogenous repair followed by other mechanisms, secreting pro-angiogenic and pro-survival molecules and microvesicles, stimulating endogenous cardiac precursors and cardiomyocyte proliferation (Lian et al., 2010), beside modulating the immune system (Davani et al., 2003; Epstein et al., 2017; Lüger et al., 2017; Salazar-Noratto et al., 2021).

Combinatorial approaches by applying cell and gene therapies develop genetically engineered ex-vivo transplants, which provide a more comprehensive regenerative strategy than each cell type, ease maintenance and control of gene expression compared to gene therapy (Kratchmarova et al., 2005; Singh et al., 2016). Advancement in bioengineering technologies allows ex-vivo engineering of cell grafts with mixtures such as cardiomyocytes, cardiac precursors, and vascular, neuronal, and immune mesenchymal cells, which generates a
comprehensive regenerative strategy compared to each cell type alone, by expressing cardiomyogenic and non-cardiomyogenic cells (Davani et al., 2003; Huang et al., 2010a; Bagno et al., 2018; Duchemin et al., 2019; Sadeghi et al., 2019). Both cells were the two most used for heart function and regeneration (Majka et al., 2017; Masgutov et al., 2019; Salazar-Noratto et al., 2020). MSCs, which have demonstrated their potential as cell-based therapies, allow optimization in regenerative methods of cardiovascular diseases. Further understanding in application of mechanotransduction in cardiovascular engineering will aid the improvement and development of bioengineering approaches for future undertaking.

5. Application of cardiovascular tissue engineering involving mechanotransduction

Understanding how mechanical factors influence cell activity during the development of cardiovascular diseases has significant implications for cardiac tissue engineering, which is designed to replace injured tissue with a biomimetic construct (Altman et al., 2002; Nguyen et al., 2019; Tsata and Beis, 2020). Engineering cardiovascular tissue requires Endothelial cells (ECs) and Endocardial cell (EdCs) combination systems, which recognize and react to mechanical stimuli and make them generate bioengineering approaches. Furthermore, understanding the stresses exerted on the growing heart and blood vessels will aid in the development and improvement of engineered heart valves and blood vessels de novo. Despite showing vast opportunity and flexibility in becoming cell factories for tissue engineering, knowledge of the cell's mechanical and biochemical cues was far beyond reaching the level of popular demand (Halim et al., 2020).

5.1. Tissue-engineered blood vessels (TEBV)

A tubular scaffold, which consists of EdCs and smooth muscle cells, is needed to replace a natural blood vessel to create tissue-engineered blood vessels (TEBV). Another key factor involved in angiogenesis is mechanical stresses, which are necessary to manufacture engineered tissues in tissue engineering (Duchemin et al., 2019). When pluripotent stem cells (PSCs) are exposed to fluid shear stress inside embryoid bodies, these cells differentiated into embryonic stem cells (EdCs) and significantly stimulated as reported in vitro studies (Duchemin et al., 2019). Pre-vascularization of tissue-engineered vessels produced from PSCs might benefit from this applied method. The static stretching promotes parallel vessel orientation, but cyclic stretching results in diagonal vessel orientation during blood vessel formation in 3D Gelfoam scaffolds which are co-cultured with EdCs and fibroblasts (Zaragosi et al., 2006; Mias et al., 2009; Li et al., 2010). The host environment around the transplanted tissue provides an additional layer of complexity, much as in the embryo and regenerative activities of engineered tissues (Duchemin et al., 2019). In vitro has been shown to control the implanted vascular network and host tissue's orientation to facilitate transplant integration. On the other hand, in-vivo, the mechanical characteristics of the surrounding environment have been shown to influence neovascular development and remodeling in a rat bone wound healing model. The study by Boerckel et al. (2011) demonstrated that regeneration was hindered by early mechanical stress, which prevented the development of new vessels. Late mechanical stress, on the other hand, induced remodeling of the neovascular system and, ultimately, tissue regeneration (Tsata and Beis, 2020). Tissue engineering with mechanical forces provides an alternative, promising means of mass production of TEBV.

5.2. Tissue-engineered heart valves (TEHV)

Thromboembolism is caused by the replacement of faulty heart valves with mechanical heart valves that are incorporated into non-physiological flow patterns. Meanwhile, bioprosthetic valves create more physical flow patterns, however, they are often damaged after some time (Karantalis and Hare, 2015). TEHV is consequently required, particularly in younger individuals, where the valve must be fully integrated and remodeled. The personalized 3D-printed cardiovascular prosthesis is the most recent advancement in tissue engineering, allowing for the construction of TEHVs to mimic native physiological stress. There are many kinds of cells within the valve that may affect the valve significantly, but the complexity of the valve leaflet at play is still not fully understood (White et al., 2016). Adult endothelial cells may also be activated by shear stress in vitro, which allows them to shift from endothelial to mesenchymal (Davani et al., 2003; Demirdögen et al., 2010; Majka et al., 2017; Duchemin et al., 2019). Cardiogenesis and cardiac tissue engineering are all believed to play key roles in determining how MSCs sense and transmit mechanical signals, making mechanotransduction mechanisms extremely complex (Farzaneh et al., 2018; Nguyen et al., 2019).

6. Future perspectives

Mechanotransduction seems to be critical throughout heart development, from the earliest stages of cardiac mesoderm progenitors’ differentiation until the termination of myocyte hypertrophy expansion in the early years of life. Novel tools for probing cellular pressures have shown a diverse array of processes operating across many length scales which range from molecular forces to supra-cellular force patterns. As a result, physical forces cannot be seen only as fundamental switches for mechanotransduction signals, but as the primary mechanism for signal propagation between cells (Makino et al., 1999; Ogawa, 2016). Interestingly, current technological advances enable the investigation of the molecular processes by which cellular pressures modulate gene activity by regulating the translation of mechanical impulses to biochemical signals. An additional objective should be to have a better understanding of how ECM-sensing activates and translocates certain transcription factors to the cell nucleus. Indeed, it is still unknown whether chromosomal configurations can be changed in response to changes in the nucleus and its mechanical characteristics caused by changes in the ECM's physio-chemical properties (Elnakhish et al., 2012; Zhang et al., 2013; Peng et al., 2017).

To gain a better understanding of the molecular mechanisms underlying outside-in and inside-out mechanotransduction signaling pathways, integrated strategies combining super-resolution fluorescence microscopies such as stimulated emission depletion (STED), photo-activated localization microscopy (PALM), stochastic optical reconstruction microscopy (STORM), with biophysical probes and multiple protein patterning is required. Apart from these integrated imaging approaches, FRET biosensors might be used to investigate force transmission through the cytoskeleton to nuclear envelope proteins, chromatin remodeling, and mechanically driven alterations inside the nucleus (Mohammed et al., 2019). FRET between fluorophores of the same type, dubbed homo FRET, is a promising technique for visualizing and quantifying changes in protein ratios in response to force application. It is based on the signal generated when molecules labeled with an enhanced green fluorescent protein, such as G-actin, self-assemble into actin filaments (Kamkin et al., 2003; Park et al., 2012; Zhao et al., 2016; Wang et al., 2018).

Finally, a significant technological difficulty in cellular mechanotransduction is the construction of synthetic models of the stem cell niche for manipulating the stem cell microenvironment’s biophysical and biochemical features (Tavi et al., 2001). Indeed, the discoveries of mesenchymal stem cells mark a watershed moment in fundamental cell biology and the development of novel therapeutics. Understanding how mechanical stresses may influence mesenchymal stem cell function can give essential insights into the construction of artificial habitats for regenerative treatments (Norman et al., 2017). Smart stem cell environments that blend material property control stiffness, topography, and protein patterning to mimic cell-cell and cell-matrix interactions are necessary to uncover ECM signals involved in niche-like regulation of
stem cell destiny. These platforms are useful for creating 3D models of organ-level systems that can be used in the study of mechanotransduction pathways in stem cells, besides implementing a precision medicine approach by assessing significant variations in diverse patient populations (Ahanger et al., 2020).

7. Conclusion

Mechanical forces are critical for cardiovascular disease development as well as heart morphogenesis and tissue development. Despite early predictions, comprehensive dissection of molecular pathways triggered by mechanical forces has only lately been addressed. Cells can “sense” their mechanical environment and send signals to the nucleus to regulate gene expression. It is unknown how highly conserved mechanical stimuli might alter tissue regeneration in cardiovascular diseases. However, a greater knowledge of these mechanotransduction processes will lead to novel therapeutic approaches for cardiovascular diseases (Tsanta and Beis, 2020).

Preclinical research has demonstrated that MSCs therapy may improve cardiac function after myocardial infarction (Jin et al., 2020). Mesenchymal cells have caught the attention of researchers in recent years due to their potential to self-renew and undergo differentiation (Chou et al., 2014). Although we are just now beginning to understand MSC processes by which they regenerate or promote the repair of injured organs, their pleiotropic effect and paracrine effects make them promising alternatives for the treatment of cardiovascular diseases (Farranbeh et al., 2018). The existing evidence confirms the safety of the treatment involving MSCs demonstrating many beneficial effects and promoting future research. The incorporation of the most recent advancements in the area, such as in-vitro conditioning and bioengineering, would undoubtedly imply another step toward discovering an optimum therapy, especially in cardiovascular diseases. However, immunomodulatory capability and allogenic usage must be addressed first to improve the efficacy of the administration of MSCs in cardiovascular diseases.

Declarations

Author contribution statement

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