THE APPLICATION OF REGENERATIVE MEDICINE IN THE TREATMENT OF CARDIOVASCULAR DISEASES

By

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
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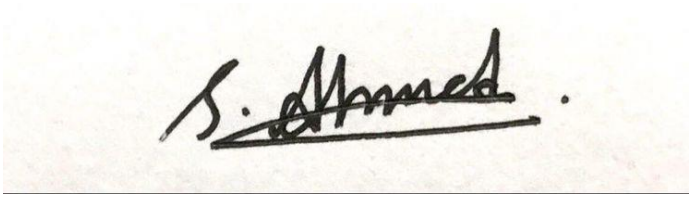

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Approval

The project titled "Application of regenerative Medicine in Cardiovascular Diseases" submitted by Saraf Nawar Ahmed (18146026) of Spring, 2018 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.).

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Ethics Statement

This project does not involve any clinical trial or human participants and no animals were used or harmed.

Abstract

Cardiovascular events are often associated with severe, irreversible damage to the myocardium and significantly high rates of morbidity. Even though current medical therapies have markedly lowered morbidity rates and improved treatment for cardiac patients, it greatly lacks the facilities to reverse the damage done to the cardiac cells. Latest advances and developments in regenerative medicine and stem cell therapy have shown great potential in overcoming this challenge. Advanced stem cell-based therapy has shown significant improvement in cardiac function in several animal models with the potential to treat both acute and chronic complications. A few cardiac regenerative therapies have also been approved in some Asian countries, and a cell based therapy has received FDA fast track status. In this paper, how stem cell therapy and tissue engineering can be incorporated into producing effective cardiac regenerative therapy to overcome the shortcomings of the conventional treatment will be elaborated.

Keywords: Regenerative medicine, Stem cell therapy, Cardiovascular disorders

Dedication

Dedicated to my parents.

Acknowledgement

I would like to express my heartfelt gratitude to my supervisors, Dr. Eva Rahman Kabir, for her unwavering support and guidance, and Dr. M. Zulfiquer Hossain, for his stimulating suggestions. My special thanks to both of them for encouraging me, proofreading my paper, and correcting my many mistakes.

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List of Acronyms

WHO	World Health Organization
CVD	Cardiovascular Disease
MI	Myocardial Infraction
CAD	Coronary Artery Disease
CM	Cardiomyocyte
HF	Heart Failure
DCM	Dilated Cardiomyopathy
NICM	Nonischemic Dilated Cardiomyopathy
AMI	Acute Myocardial Infraction
ECM	Extracellular Matrix
BMC	Bone Marrow-Derived Cells
HSC	Hematopoietic Stem Cells
MSC	Mesenchymal Stem Cells
CSC	Cardiac Stem Cells
CDC	Cardiosphere-Derived Cells
ESC	Embryonic Stem Cells
ICM	Inner Cell Mass
PSC	Pluripotent Stem Cell
hESC	Human Embryonic Stem Cells

iPSC	Induced Pluripotent Stem Cells
TERM	Tissue Engineering and Regenerative Medicine
LVEF	Left Ventricular Ejection Fraction
EF	Ejection Fraction
LAD	Left Anterior Descending Artery
PAD	Peripheral Artery Disease

Chapter 1

Introduction

1.1 What Is Regenerative Medicine?

Regenerative medicine is an interdisciplinary strategy for reconstructing, replacing, or restoring the missing parts of various organs in the body in a way that is quite close to the original structure (Arjmand et al., 2021). Regenerative medicine, stem cell therapy, and tissue engineering have emerged as an innovative biomedical field which introduces modern innovative methods that have the potential to heal and regenerate damaged tissues and organs (Pina et al., 2019). It has the ability to repair or replace tissues and organs that have been negatively affected overtime by aging, illness, or injury, and even to correct congenital deformities (Mao & Mooney, 2015).

1.2 How Does Regenerative Medicine Work?

The area of regenerative medicine comprises of a wide range of strategies, including the use of materials and de novo generated cells, as well as various combinations of these, that are used to replace missing tissue, effectively replacing it both structurally and functionally, or to contribute to tissue healing. Materials and de novo generated cells are used to replace missing tissue, effectively replacing it both structurally and functionally (Mao & Mooney, 2015). This field has been found to be promising for debilitating conditions ranging from spinal cord injury to neurological and haematological diseases, and patient-specific cell transplants.

There are numerous preclinical and clinical data that support the possibility of using regenerative medicine to treat both chronic conditions and acute complications, as well as ailments affecting a broad range of body organs and systems, such as dermal sores,

cardiovascular disorders and injuries, various cancer treatments, and many more (Mao & Mooney, 2015). Various innovative approaches that have been proposed include direct implantation of cells obtained from the patient into the areas which have the malformations, tissue-specific delivery of bioactive compounds and growth factors, biomaterials for cell-free scaffolding, and scaffolding structures with cells resembling the tissues' native extracellular matrix (Pina et al., 2019).

1.3 Applications of Regenerative Medicine in Cardiac Diseases

Cardiovascular disease is a leading cause of death around the world. According to the World Health Organization (WHO), globally, an estimated 17.9 million people died from cardiovascular diseases (CVDs) in 2019 - this represents 32 percent of all global fatalities in 2019. The majority of these fatalities (85 percent) were caused by heart attack or stroke. Societies are currently dealing with a huge and growing problem: heart failure. Unfortunately, the outlook is not good for those who suffer from the condition (Rajabzadeh et al., 2019). Most cardiovascular occurrences, such as coronary heart disease and stroke, are significantly associated with a reduction in blood supply, which results in a reduction in cellular function, organ failure, and, ultimately, death (Xie et al., 2016). Moreover, throughout the world, peripheral artery disease is a widespread illness that is a leading source of morbidity and mortality (Meng & Cooke, 2020). Numerous people with ischemic heart disease not only suffer from the acute phase of myocardial infarction (MI), but they also develop progressive heart failure as a result of the ventricular dysfunction produced by the ischemia circumstances, which is known as ischemic cardiomyopathy. Because cardiomyocytes in the adult human heart have a limited ability to regenerate, the injured myocardium is replaced by fibrotic scar tissue after a MI. The presence of scar tissue in the heart leads in a reduction in pump performance as well as a shortage in the circulatory system. Further fibrosis, myocardial loss, cardiac dysfunction,

and dilatation occur as a result of the wounded heart's remodeling process, which ultimately ends in catastrophic heart failure (Hashimoto et al., 2018). In cases of catastrophic cardiac arrests, cardiac implantation is the only possible treatment option, however, because of the imbalanced demand for donor hearts, new regenerative treatment techniques are being developed to restore lost functional ventricular muscle function (Witman & Sahara, 2018).

1.4 Rationale

Patients with irreversible end-stage organ failure continue to face a scarcity of organ donors for transplantation. The goal of tissue engineering and regenerative medicine is to discover an alternate option that will give viable remedies for people who are suffering from these conditions (Zambon et al., 2019). It is a fact that heart transplantation is not a viable option as a conventional treatment option due to a scarcity of donors throughout the world and the technical difficulties involved in the procedure (Hashimoto et al., 2018). Moreover, in the current state of the art, therapies can alleviate symptoms and reduce the progression of pathological cardiac remodeling, but they cannot address cardiac tissue repair. A thorough knowledge of the molecular pathways that underlie heart disorders is essential for the development of novel and successful treatment solutions for these conditions (Parrotta et al., 2019). The current medical treatments do not have full impact on the blood flow to the limbs. However, a unique potential exists in the field of regenerative medicine to use medicinal techniques that will allow the restoration of limb perfusion. Such approaches to stimulate angiogenesis, arteriogenesis, and vasculogenesis in order to improve limb blood flow include the use of angiogenic cytokines and cell treatments, as well as methods to improve limb blood flow (Meng & Cooke, 2020).

Chapter 2

Methodology

This review elaborates the scope of regenerative medicine in the treatment of cardiovascular diseases. After selection of the topic, hundreds of peer-reviewed research and review articles were collected from Elsevier, Frontiers, Nature, PubMed, Lancet, ScienceDirect, and Springer. More than a hundred articles were shortlisted from where relevant data and information were gathered, compiled, and referenced aptly to provide a methodical overview of cardiac regenerative medicine.

Chapter 3

The Heart

3.1 The Heart

Any animal, big or small, needs a system within their body to circulate the body fluids to all the differentiated organs and organ systems. The heart plays the role of pumping the fluids to all parts of the body, which also serves the vital function of supplying oxygen to the various extremities of the body through its rhythmic contractility (Clarks, 2015).

Heart failure is a condition that typically would affect the aged population. Recent studies carried out by Groenewegen et al., on the other hand, have suggested that the prevalence of heart failure among the young is growing (Groenewegen et al., 2020). Additionally, even though women have a substantially lower frequency of heart failure than males, but nevertheless, they account for nearly half of all instances of heart failure; heart failure with preserved ejection fraction, in particular, is more common in women than men (Christiansen et al., 2017; Conrad et al., 2018; Roger et al., 2004; Shah et al., 2009).

A precise cause of heart failure in a patient might be difficult to determine since heart failure can be considered as the chronic phase of any illness that causes impairment of the cardiac functionality. There are many different causes of heart failure, and the majority of comorbidity do not originate independently of heart failure, but rather share a set of risk factors, play a role in the pathogenesis of the disease, or operate as a sustaining factor. The majority of people with heart failure have a number of co-existing illnesses. Comorbidity is connected with increasing risk and severity of heart failure indications and is associated with a reduced quality of life as well as a poor outcome in patients with heart failure (Baldi et al., 2017; Groenewegen et al., 2020; Van Deursen et al., 2014).

There have been several nation wise cohort studies that have looked at the common risk factors for cardiac arrest, including coronary artery disease (CAD), hypertension (high blood pressure), diabetes (high blood sugar), obesity (high body weight), and tobacco smoking (high body weight). With the exception of smoking, the load of risk factors in individuals with cardiac arrest is growing with time, with hypertension, diabetes, and obesity being the most important contributors (Christiansen et al., 2017; Dunlay et al., 2009). Inflammation is also a critical factor in the occurrence of the most common kinds of heart failure (Frantz et al., 2018; Groenewegen et al., 2020).

3.2 Regenerative Capacity of the Heart

Cardiomyocytes are believed to be in a terminally differentiated stage at the time of their development. Nonetheless, the cell cycle activity and proliferative potential of cardiomyocytes fluctuate depending on the species and life stage (Hashimoto et al., 2018). The mature human heart has a limited regenerative potential, and hence regenerative medicine approaches have raised fresh expectations for the repair or replacement of damaged hearts in the past several years (Tenreiro et al., 2021). The mature human heart has a limited regenerative potential, and hence regenerative medicine approaches have raised fresh expectations for the repair or replacement of damaged hearts in the past several years (Tenreiro et al., 2021). CVDs are linked with cardiac malfunction and cardiomyocyte (CM) death, as well as the formation of fibrosis and scar tissue, and aberrant ventricular remodeling, according to their pathophysiology. Loss of cardiomyocytes is an irreversible process that is frequently followed by scar formation; these two phenomena together represent the two most serious pathological problems because cardiomyocytes loss cannot be replaced by new ones since the cardiac tissue has a very scarce regenerative capacity, and scar tissue formation or fibrosis results in heart failure (HF) or dilated cardiomyopathy (DCM) (Parrotta et al., 2019). Nonischemic dilated cardiomyopathy

(NICM) has risen to become the primary cause of advanced heart failure in the previous decade, accounting for more than half of all heart transplantations. These results imply that individuals with NICM may constitute the greatest subset of heart failure patients with a specific need for alternate treatment methods, which includes cell therapy (Vrtovec, 2018).

A case of a newborn infant who demonstrated functional cardiac recovery following a myocardial infarction implies that the human neonatal heart may have some regeneration ability (Haubner et al., 2016). A growing body of data indicates that cardiomyocyte turnover occurs in the adult animal heart, including the human heart, according to the American Heart Association (Bergmann et al., 2009). Although the turnover of cardiomyocytes is significant, it is clearly insufficient to restore the contractile function of a damaged human heart, which can lose up to 1 billion cardiomyocytes during a myocardial infraction (MI) (Laflamme & Murry, 2011). Establishing cardiac regeneration treatments is therefore an essential step in the process of restoring function to the injured heart in individuals suffering from heart disease (Hashimoto et al., 2018).

Following a myocardial infarction (MI), several experimental investigations have demonstrated that cardiac transfer of unfractionated bone marrow cells or mesenchymal stem cells (MSCs) and progenitor cells produced from bone marrow can improve functional recovery. As a result, it has been recommended that stem cells and progenitor cells produced from bone marrow be used to repair heart tissue in individuals who have had an acute myocardial infraction (AMI) (Hare et al., 2009; Lunde et al., 2006; Wollert et al., 2004). This type of regenerative treatment would provide a highly effective alternative treatment strategy to treat cardiovascular diseases (Kim et al., 2018).

Chapter 4

Modelling of Cardiac Diseases

Learning about the molecular mechanisms behind disease has enabled the discovery of targets and signaling pathways that may represent possible targets against which scientists might build novel treatment techniques (Parrotta et al., 2019). Significant progress has been made in the understanding of the molecular mechanisms behind illness, but we have made significant progress in our ability to develop viable treatments for diseases that are now untreatable due to a dearth of human cellular models for disease modeling. Human stem cell-based illness models have the benefit of allowing for a more nuanced understanding of disease processes, which in turn allows for the identification of novel treatment targets (Parrotta et al., 2019).

Successful cardiac decellularization has been demonstrated in rodent and porcine models utilizing a mix of non-ionic and ionic detergents as well as enzymes in experimental research (Ott et al., 2008; Wainwright et al., 2010). Aortic cannulation and retrograde coronary perfusion were used in the experiments. The period required to accomplish successful cardiac decellularization is dependent on the perfusion flow rate, perfusion time, tissue mass, and potency of the decellularizing agent used to create the decellularization effect. Using detergent perfusion, pig hearts were effectively decellularized, resulting in the formation of an extracellular matrix (ECM) scaffold with mechanical characteristics that were similar to those of natural myocardium in one research (Zambon et al., 2019).

When comparing the use of small animal models in cardiovascular research to the use of big animal models, there are several advantages. Small animal models have a short life span, which allows researchers to track the natural course of the illness at a faster rate than with larger animal models, which are more expensive (Rajabzadeh et al., 2019).

There are several advantages of small animal models in cell-based heart failure therapy, such as maintaining lower maintenance costs, being simpler to handle and house, having a shorter gestation period and lifetime, and being appropriate for proof-of concept and "high-throughput" investigations are all advantages. In contrast to other arrhythmias, ischemia-reperfusion caused arrhythmias are rare and easy to correct when they do occur, making them ideal for genetic selection and the development of transgenic strains (Camacho et al., 2016). However, there are many disadvantages of using small animal models for modelling CVDs, they are as follows: the small animal models are phylogeneticallly distant from humans, the pathophysiology of the illness may not be translatable to humans, they may produce differing response and sensitivity to medications, and they are not ideal for use in chronic investigations (Camacho et al., 2016). Regardless of the fact that small animal models are useful for studying human diseases, large animal models are the more efficient alternative (Rajabzadeh et al., 2019).

Large animal models have, on the other hand, several advantages, including the fact that they are the most physiologically and clinically similar to humans, they enable chronic investigations to be conducted, cardiac mechanisms and responses can be evaluated in the intact mammal, they are able to respond to all of the methods and analysis performed in humans, and that their cardiac system more closely resembles to the human heart (Camacho et al., 2016). However, large animal models have a number of disadvantages, that include greater maintenance expenses, being more difficult to handle and maintain (require complex infrastructures and skilled workers), and having a longer gestation period and lifetime. Moreover, arrhythmias induced by ischemia reperfusion is more common in canine and porcine cardiovascular models, making them less appropriate than small animals for genetic selection and generation of transgenic strains, as well as less suited for the development of spontaneous illness models as compared to small animals (Camacho et al., 2016).

Chapter 5

Cell-based Therapy Options for Cardiac Treatment/Repair

Cell-based treatments have been proposed as a means of alleviating some of the obstacles that limit and prevent cardiovascular regeneration from taking place. The ultimate goal of cell-based treatments is to regenerate areas of injured myocardium with engrafted, functioning cells that recover lost cardiovascular function, allowing adequate oxygenated blood and vital nutrients to be delivered to all of the body's major organs. Despite the numerous operational, economic, and ethical obstacles that stand in the way of such a medical accomplishment, the field continues to advance as a result of the mutual cooperation and collaboration between stem cell scientists, who work in studying and investigating the unique mechanisms of cardiovascular repair and regeneration, and healthcare practitioners in cardiology and regenerative medicine, among others (Witman & Sahara, 2018). In the meanwhile, medical device firms are still searching for the perfect patch, that must optimally be biocompatible, thromboresistant, impermeable, and immune to infections, as well as not calcify or shrink with time (Pavy et al., 2018). A large amount of information regarding the use of stem cell therapy in regenerative medicine is available, and its application continues to be promising.

5.1 Stem Cells Used for Cardiac Regeneration

Isolating and maintaining basic cardiac cells of human, whether healthy or disease-ridden, in a culture for the length of time necessary to conduct an investigation can be difficult (Parrotta et al., 2019). Among patients with acute myocardial infarction, it is critical to restore reperfusion to the infarct-related coronary artery as soon as possible in order to recover ischemic myocardium and keep the infarct size as small as possible. Even though percutaneous transluminal coronary angioplasty with stent implantation is the preferred method

for restoring cardiovascular flow, delaying procedure results in the loss of cardiomyocytes and the development of heart failure, which is a leading cause of abiding cardiac disease and mortality in the general population. Upon the death of viable myocardium, a harmful process of left ventricular remodeling begins that results in chamber inflation and contractile failure in a large proportion of the population. When it comes to repairing injured myocardium, stem cell therapy has surfaced as a fresh treatment option to explore (Kim et al., 2018). Candidates for the initial stem cell - based trial included skeletal myoblasts, which were thought to generate cardiac contraction, as well as bone marrow-derived cells and mesenchymal stem cells, both of which exhibited cardiogenic potential when cultured in vitro (Hashimoto et al., 2018).

5.2 Skeletal Myoblasts

In the beginning, skeletal myoblasts were utilized to remuscularize the wounded cardiac muscles and reestablish its contractile function. When transplanted, experimental skeletal myoblasts survived and developed into the myogenic stream in various animal models, and the therapy increased the ejection fraction in subjects with both ischemic and non-ischaemic cardiomyopathies (Al Attar et al., 2003; Durrani et al., 2010; Marelli et al., 1992; Taylor et al., 1998). In the earlier clinical studies, skeletal myoblasts were found to have beneficial outcomes, with patients suffering from ischemic cardiomyopathy reporting better cardiac function after receiving the cells (Menasché et al., 2008; Povsic et al., 2011). However, lengthy follow-up investigations have failed to demonstrate any positive impacts of this use. Furthermore, unfavorable consequences such as arrhythmogenesis occurred as a result of the failure of skeletal myoblasts to merge electromechanically with neighboring cardiomyocytes, as previously stated (Fouts et al., 2006). As a result of these disappointing results, the use of skeletal myoblasts in additional clinical investigations was ruled out (Hashimoto et al., 2018).

5.3 Bone Marrow-Derived Stem Cells

Bone marrow-derived cells (BMCs), which are primarily composed of hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs), have the ability to develop into mesoderm and ectoderm tissues when exposed to the suitable circumstances (Ai et al., 2017). BMCs have become a popular source of cells for tissue engineering because of their safety and convenience, as well as the ease with which they may be cultured in vitro and replanted after being harvested (Duke & Taylor, 2013). BMC transplantation has been proven to be effective in migrating and populating diseased cardiac tissue, which can be generated into various cells that aid improve myocardial contractility, stimulate angiogenesis, and avoid unfavorable remodeling of ventricles in a number of studies (Ali-Hassan-Sayegh et al., 2015; Sampson et al., 2013). This approach has been used in the treatment of various cardiac discomfort, heart failure, myocardial infraction, and other cardiac conditions (C. Wang et al., 2019).

Several clinical trials have demonstrated that transplanting BMC is safe and practical in the therapy of cardiomyopathy, whereas others have been inconclusive or are still being conducted (Golpanian et al., 2015; Perin et al., 2015). Researchers found that BMCs enhance cardiac function, boost physical activity tolerance, and minimize malignant arrhythmias and death, according to several studies. Other studies have showed that BMC transplantation did not halt development of the disease or lower death rates. In fact, some studies have shown that it has the potential to cause problems such as arrhythmias, embolism, myocardial infraction, and tumors, among others (Martino et al., 2010). The lack of consistency of these study results could be due to the limited sample group, the origin of the cardiomyopathy, the ailment of the illness, the category of cell utilized for transplantation, the multitude and pathway of cellular therapy, the duration of treatment and follow-up (C. Wang et al., 2019). The potential efficacy of cardiac regeneration treatments that are based on bone marrow-derived mononuclear cells

is still up in the air, and no definitive conclusion has been made on the subject as it still undergoes thorough research (Hashimoto et al., 2018).

According to a study carried out by Wang et. al in 2018, when compared to normal treatment, BMC therapy for cardiomyopathy appears safer and practical, and it does not result in significant impairment to heart function or patient survival. Cell treatment led to improved left ventricular function as well as alleviation of left ventricular remodeling, with the positive impact lasting for a long period of time. Patients' survival, lifespan, and wellbeing were improved as a result of improving cardiovascular function. According to their subgroup analysis, an intracoronary infusion of bone marrow derived MSCs at a larger dose may result in increased therapeutic success in some patients. Further, appropriately conducted trials with much more accurate and patient-centered results would be necessary in order to verify these findings (C. Wang et al., 2019).

5.4 Cardiac Stem Cells

The mature human heart has always been seen as a post-mitotic structure with little ability for self-rejuvenation. However, recent evidence suggests otherwise with the discovery that the heart has capability of undergoing cardiomyocyte turnover at a limited level (Müller et al., 2018). The term "cardiac stem cells" (CSCs) refers to indigenous cells of the heart that have the ability to proliferate, self-renew, and differentiate into many cell types. Cardiomyocytes, smooth muscle cells, and endothelial cells are the three primary cardiac cell types that can be differentiated or derived from CSCs (Messina et al., 2004).

Over the past decade, it has been discovered that there are several types of cardiac stem cells and progenitors, including cardiosphere-derived cells (CDCs), stem cell antigen (Sca-1+), insulin gene enhancer protein (Isl1+), and c-Kit+ cells (Laugwitz et al., 2005; Matsuura et al.,

2004; Tallini et al., 2009). Animal models with better myocardial function were created using cardiac adult cells, which have been employed in several investigations (Johnston et al., 2009; L. Wang et al., 2017). In 2019, Wang et al have demonstrated that recurrent administrations of CDCs obtained from newborns can dramatically enhance cardiac activities in dilated cardiomyopathy (DCM) rat models as opposed to the use of adult CDCs in a rat model of DCM (S. Wang et al., 2019). When injected into infracted hearts, CSCs have demonstrated a limited ability to mend compromised heart tissue by both vivo differentiation and cell fusion with existing host cardiac myocytes (Parrotta et al., 2019).

As such, preclinical studies in animal models have revealed some positive effects of cardiac-derived cell transplantation in specific instances. Clinical studies, on the other hand, were launched with just a rudimentary knowledge about how these cells assist during cardiac regeneration (Hashimoto et al., 2018). The insufficient effectiveness of adult stem cells and progenitors in stem cell-based cardiac treatment is partly influenced by the significant technological obstacles associated with their extraction from respective tissues of origin as well as the absence of optimal growth conditions for these cells (Parrotta et al., 2019).

5.5 Embryonic Stem Cells

Embryonic stem cells (ESCs) originate from the inner cell mass (ICM) of the blastocyst. These ESCs later differentiate into the cell types of the three germ layers - endoderm, mesoderm, and ectoderm. They are pluripotent in nature, which means they can differentiate and proliferate to give rise to virtually any and all types of cells whether in an in vitro or in vivo setting (Parrotta et al., 2019). So far, several in situ techniques have been developed to stimulate cardiomyogenic division and maturation from ESCs, yet the production of purified and functional cardiomyocytes in sufficient quantities is still a difficult task. Animal tests in the initial phases of development revealed that the cardiovascular environment was necessary to

induce the conversion of implanted ESC into cardiac tissue capable of replacing damaged tissue of the host. Additional thorough in vivo tests, on the other hand, indicated that teratoma development might occur following intramyocardial injection of undifferentiated heterogenous ESCs. To this end, novel methodologies for the identification, production, and refinement of ESC-derived cardiomyocytes have been developed with the goal of enhancing the current state of knowledge. This is in line with much more advanced pre-clinical research, which have demonstrated that ESC-derived cardiac cells are competent of electromagnetically integrating into the myocardium of the host and thus have a positive impact on the procedure of cardiac remodeling, decreasing the formation of scar tissue, and enhance cardiac function without the risk of forming teratomas in both smaller and bigger animal models (Müller et al., 2018).

The practical use of ESCs and related derivatives in the treatment of cardiovascular diseases is controversial, despite the favorable results of several Phase I and II clinical studies. This is due to the fact that the use of ESCs raises a number of ethical dilemmas. Moreover, their clinical translation is also hampered by the possibility of immune rejection as well as chances of genetic unpredictability and instability, and tumorigenic possibilities (Müller et al., 2018).

Due to the advantageous properties of pluripotent stem cells (PSCs) and embryonic stem cells (ESCs), they are considered as an extremely valuable and irreplaceable tool for a wide range of medical applications, including the study of the initial stages developmental biology, models for various diseases, carrying out screening and toxicity testing for various drugs, different applications in regenerative medicine and cell transplantation (De Angelis et al., 2019; Singh et al., 2015; Tanaka et al., 2015; Zhu & Huangfu, 2013). Since their discovery in 1998, human embryonic stem cells (hESCs) have progressed swiftly and steadily in terms of their potential, thriving more and more as time goes on (JA et al., 1998). Despite being extremely intriguing,

the use of human embryonic stem cells (ESCs) is limited by a number of constraints: (i) their refinement necessitates the obliteration of the embryo, resulting in substantial ethical and moral controversies; (ii) even though there is the possibility of generating mutant ESC lines to stimulate particular disease causing mutations, they frequently fail to adequately reconstruct the pathogenicity seen in patient populations; and lastly, (iii) their prospective utilization as cellular therapy is impeded by the threat of immune reaction and, as a result, withdrawal owing to their allogenic character, which makes them unsuitable for use in clinical trials (Ghosh et al., 2016; Halevy & Urbach, 2014; Parrotta et al., 2019).

5.6 Induced Pluripotent Stem Cells

Pluripotent stem cells (PSCs) are uniform cells, yet to differentiate, with the capacity to self-rejuvenate indefinitely and generate derivatives of all the three embryonic germ layers (namely endoderm, mesoderm, and ectoderm), as well as cells capable of regenerating a whole life form, regardless if it is in vitro or in vivo (Parrotta et al., 2019). Because of their theoretically limitless proliferative ability and capacity to develop into numerous types of cell lines, pluripotent stem cells are considered to be attractive cellular resources for regenerative medicine for a variety of intractable disorders, including diabetes and cardiac disorders (Takahashi et al., 2007).

The use of induced pluripotent stem cells (iPSCs) in particular, whether autologous or allogeneic, is predicted to reduce immunogenic rejections following transplantation of cells or tissues. This has been examined in several mammalian allogeneic transplantation models as well as in clinical research (Morizane et al., 2017; Murata et al., 2020; Sugita et al., 2016).

It was the breakthrough in 2007 that human cells lines can be effectively reconfigured to form induced pluripotent stem cells that marked a pivotal point in the field of stem cell biology.

These cells have since surfaced as an exceptional new channel for overcoming the many drawbacks associated with the use of animal models and human embryonic stem cells. In the beginning, induced pluripotent stem cells (iPSCs) were created by enhanced expression of four critical transcriptional factors into fibroblasts via a virus, the transcriptional factors are c-MYC, KLF4, SOX2, and OCT4 (Takahashi et al., 2007). In the following years, several researchers reported the creation of induced pluripotent stem cells (iPSCs) from other somatic cells and employing a new and unique reprogramming mixture (Yu et al., 2007). Induced pluripotent stem cells have two key benefits as compared to mutant ESCs: (i) there are no ethical concerns related to the formation and utilization of iPSCs because they are generated from somatic cell lines; and, (ii) they retain the genetic and epigenetic accounts of the people from whom they were obtained, whereas mutated ESCs do not (Parrotta et al., 2019).

While patient-specific iPSC-derived cells, unlike ESCs, were expected to provide major advantages, such as the absence of ethical concerns and the inability to induce an immunological response - unfortunately, several studies have shown genomic unreliability in iPSCs, which were either caused by already existing abnormalities within the original mature cells or by mutations arising during the reprogramming procedure and the culture period (Müller et al., 2018; Takahashi et al., 2007; Yoshihara et al., 2017; Yu et al., 2007). An important immunologic advantage of iPSCs in stem cell therapy is their ability to generate autologous iPSCs that are immunologically equivalent to that of the host and utilized for transplant therapy. Unfortunately, establishing autologous iPSCs from each individual patient approved for therapeutic application would be expensive and lengthy in duration, impeding the therapy's standard (Murata et al., 2020). In recent times, by the utilization of cocktails of molecules, chemically induced iPSCs have been developed. Despite taking all these factors into consideration along with the major advances, human iPSC-derived cells are yet to be

utilized in clinical trials in the treating a multitude of cardiovascular disorders. However, they are viable potential candidates for this process (Müller et al., 2018).

Chapter 6

Reprogramming of Cells to Form Functional Cardiomyocytes

In recent advances, it was discovered that four transcription factors proved to be adequate to convert mouse fibroblasts into undifferentiated and immature pluripotent stem cells without the need for additional factors. The four transcription factors are c-MYC, KLF4, SOX2, and OCT4. Pluripotent cell lines that are highly patient-specific could be generated without using somatic cell transfer if defined factors were used in the reprogramming of human cells. However, the discovery that c-MYC activation promotes the apoptosis and maturation of human embryonic stem cells implies that a set of circumstances omitting this gene may be necessary to reprogram these cells in the human body (Yu et al., 2007).

To increase the effectiveness of cardiac reprogramming, several techniques have been employed, including the inclusion of growth factors and miRNAs to the reprogramming cocktails, as well as the altering of existing signal transduction pathways (Hashimoto et al., 2018).

With recent advancements in cellular programming, the manipulation of fibroblasts to directly form cardiomyocytes, without the necessity of undergoing an intermediate stage has been made possible and it is called trans-differentiation (Parrotta et al., 2019). However, regardless of the achievements medical researchers should proceed with care when conducting clinical trials regarding in vivo direct reprogramming for cardiomyocyte transdifferentiation. This is because the efficiency of direct cardiomyocyte reprogramming has limited efficiency (ranging from 0% to 10%) owing to the lack of agreement regarding the markers and phenotypes (Lalit et al., 2016; Miyamoto et al., 2018). Moreover, the physiological and

biochemical differences in humans and rodents, the models for the latter cannot be accurately translated into human models (Chen et al., 2018).

It is more difficult to do directly carry out cardiac reprogramming in case of human cells as compared to for mouse cells because human cells have a much lower reprogramming rate and need an extended period of time to develop cardiomyocyte features. In line with other cellular reprogramming techniques, this increasing complexity is also seen here, such as with reprogramming human iPSC which also requires much greater difficulty than reprogramming to form mouse iPSCs despite the cocktail used for reprogramming containing the identical factors (N. Yang et al., 2011). Two critical factors which may contribute to this difference in complexity for reprogramming the cells in humans and mice are possibly the increased epigenetic barrier and the extended development time in humans as compared to mice (Hashimoto et al., 2018; Mohamed et al., 2017; Zhou & Huang, 2011).

Chapter 7

Use of 3D in Regenerative Medicine

The objective of achieving cell engraftment following cardiac injection is still a long way off. The viability of cells which are administered is jeopardized by the existence of scar sections that are less expandable, therefore tissue engineering breakthroughs are now focused on the creation of cardiac tissues that can be grafted onto the sections that have been infracted. Tissue engineering strives to create 3-dimensional cardiac tissue with a sophisticated ECM composition akin to the human heart's original structure. Various extracellular matrix (ECM) materials, including as collagen, fibrin, or Matrigel are actively being researched (Dattola et al., 2019). Using 3D human heart structures derived from human iPSCs and endothelial cells, researchers were able to repair substantial cardiovascular deformities in hearts of guinea pigs. In pathological remodeling the researchers observed a significant reduction followed by increased growth of myocytes, greater angiogenesis, as well as higher electrical coupling within the heart after implanting the constructs (Weinberger et al., 2016).

In recent time, cellular matrixes and smooth muscle cells have been generated from human iPSCs which were used to construct a fibronectin scaffold (Gao et al., 2018). While research is currently ongoing to determine the best mix of scaffolding technology and cell/tissue types for a given application, measures to ensure the health and viability of cells that have been transplanted, the promotion of neo-angiogenesis, and the minimization of apoptosis must be further explored (Parrotta et al., 2019). Most recently, the strategies employed in tissue engineering and regenerative medicine (TERM) have been primarily focused on building three-dimensional porous scaffolds as well as hydrogels, which have resulted in biocompatible constructions with controlled rate of degradation and porosity that is necessary

for effective transfer of nutrient, vital gases, and regulatory factors (Pina et al., 2019). In contrast to the animal models currently utilized in pre-clinical investigations, 3D models of human tissue of illness may allow more effective testing of regenerative medicine techniques in human physiology. Increased disease model accuracy may enhance the effectiveness of regenerative medicine treatments and speed up the transition of promising techniques to the field (Mao & Mooney, 2015).

Chapter 8

Limitations of Regenerative Medicine in Cardiac Cell Therapy

Even though the use of cellular treatments to increase perfusion has exhibited success in preclinical research and small clinical trials, however significantly bigger randomized clinical trials were largely unsuccessful. Inadequate understanding of the right dose, frequency, administration technique, and angiogenic mechanism or cellular treatments may be to blame for the lack of benefits observed (Jonsson et al., 2012; Meng & Cooke, 2020; Moazzami et al., 2011).

The emergence of promising pre-clinical research demonstrating considerable stem cell-mediated cardiovascular regeneration opened the stage for clinical application to occur as quickly as possible. Nevertheless, evidence from human trials are conflicting, with the majority of findings indicating only minor to no medicinal stem cell benefits in the long term (Afzal et al., 2015; Gyöngyösi et al., 2015; Jeevanantham et al., 2012; Müller et al., 2018). A possible cause could be that therapeutic components are not delivered to the target areas in adequate quantities, resulting in the failure to achieve the therapeutic thresholds required for the regeneration of the cardiomyocytes when the findings are applied to medical settings. Biomaterials that may perform as a matrix to promote survival of the graft and functionality, shield therapeutic elements from degradation, and function as a depot to provide therapeutic factors over an extended period of time has gained interest as a potential solution to this problem. In this way, delivery methods that are biomaterial-based serve as a scaffold or framework for improving the therapeutic benefits of cellular and non-cellular treatment approaches in the clinical setting (Chow et al., 2017; Hashimoto et al., 2018; Oduk et al., 2018; Pascual-Gil et al., 2015; Seif-Naraghi et al., 2013; L. L. Wang et al., 2017). On the other hand,

3D printed tissue-engineered myocardial patches have shown to improve treatment outcomes as well as increasing the frequency of the delivery of the stem cell therapy, which also produced positive results (Hashimoto et al., 2018; Riegler et al., 2015; Tokita et al., 2016).

A further obstacle on the path to practical use of stem cell and other regenerative treatments is the limits of existing animal models of disease. The significance of animal models in translational medicine is undeniably crucial; yet, variations across species, such as differences in the genetic foundation of pathology as well as variances in physiology and anatomy, may explain for translational failures (Milani-Nejad & Janssen, 2014). To put it simply, as of yet there is no universally suitable animal model for preclinical investigations presently offered, and an accurate model must be adopted for each purpose and application of regenerative medicine (Hashimoto et al., 2018).

Moreover, a significantly higher number of clinical studies have been carried out on patients with ischemic heart failure where promising beneficial outcomes were established in the early stages but in larger trials the similar outcome was not produced. Even though the actual reason behind the inconsistent outcome has not been fully identified, a possible explanation could be that in spite of producing improvements in the activity of the myocardium, the therapy had no effect whatsoever on the advancement of atherosclerosis and this could be a major limitation in bringing about the desired therapeutic effect in patients suffering from ischemic heart failure (Vrtovec, 2018).

Chapter 9

Cardiac Products Available Regenerative in the Market

9.1 CardioCel

A tissue-engineered bovine pericardial patch first introduced at the Royal Brompton Hospital in London in 2014, CardioCel VR has been shown to have a beneficial impact in terms of avoiding inflammatory responses as well as calcification in the heart (Brizard et al., 2014; W. Neethling et al., 2014; W. M. L. Neethling et al., 2013). Initially the patch goes through decellularization, followed by which it is crosslinked with minute quantities of glutaraldehyde and then it is decontaminated to eliminate any remnants of glutaraldehyde remaining on the patch surface. Lastly, the sheet is disinfected and maintained in a glutaraldehyde-free condition to ensure that it remains effective. As a result, the patch is fully prepared as a result it does not necessitate any additional cleaning (Pavy et al., 2018).

In a 2 year study conducted by Pavy et al., with regard to lower pressure zones, the patch performed well, with no stenosis resulting from thickness or calcification being seen. Under high pressure, however, they observed initial transplant failure due to an enormous intimal response - a finding that, for this kind of patch, had not been reported in the past. In newborns, their findings demonstrate that the patch becomes mostly stenotic following widening of the aortic arch, something they believe is due to a discrepancy between native aorta's and CarioCel patch's elasticity when subjected to circulatory pressure, according to the researchers. Circulation of blood causes stress on the walls of the aorta, that can result in a intimal hypertrophic reaction, that can develop an aortic stenosis of severe intensity if left untreated (Pavy et al., 2018).

It is vital to note that the creation of the CardioCel patch represents a significant step forward in the prevention of premature calcification in younger patients, especially those who are vulnerable to problems caused by calcification of the patch itself (Bourguignon et al., 2015). But the research they conducted has indicated that the CardioCel technology can develop tough outer neointimal growth, in neonates and babies this can result in blockage of the aortic position (Pavy et al., 2018).

9.2 Cellgram-AMI

Cellgram-AMI is an autologous bone marrow MSCs derived stem cell therapy administered in case of acute myocardial infraction, and it has been marketed in South Korea since 2011 (Childs et al., 2020). Damage caused by myocardial infarction was the therapeutic target for this treatment, and the therapeutic endpoint examined was the left ventricular ejection fraction (LVEF) that was measured six months after the initiation of the therapy. An improvement of 6 percent was observed in the ejection fraction (EF) after just one dosage of the drug (Childs et al., 2020; H. Yang, 2011).

The mechanism by which Cellgram AMI works can be divided into two parts: the direct effect and the paracrine effect, they each represent the regenerative effect on the cardiac muscles and microenvironment of the tissue respectively. Cellgram - AMI®, the BM-MSCs administered into patient populations in this investigation, has been certified by the manufacturer for their ability to give rise to cardiac tissue by differentiation and generate the paracrine effect of releasing growth factors and cytokines that induced angiogenesis in the cardiovascular system, as demonstrated in this work (Kim et al., 2018).

They discovered that the injection of BM- MSC into the coronary artery that has been affected by the infraction located at the left anterior descending artery (LAD) greatly enhanced the regeneration of worldwide LVEF 7 months after the initiation of the therapy. After 12 months

of follow-up, the recovery of the LVEF was persistently seen, moreover the infusion of the BM-MSs has been well tolerated with no significant side effects (Kim et al., 2018).

9.3 Ixmyelocel-T (Vericel)

Ixmyelocel-T is an improved multicellular treatment that is derived from autologous bone marrow mononuclear cells. A past studies have indicated that ixmyelocel-T treatment has the capacity to reduce the release of inflammation causing cytokines in response to several stimuli as well as the ability to effectively eliminate cells that are apoptotic. This subgroup of cells has the ability to play a role in the regeneration of tissues (Ledford, Murphy, et al., 2013; Ledford, Zeigler, et al., 2013; Powell et al., 2012). According to the findings of the two case studies included in the meta-analysis conducted by Wang et al., distribution of ixmyelocel-T in populations with dilated ischemic cardiomyopathy did not show a significant impact on the left ventricular ejection fraction, but the results demonstrated a substantial reduction in the risk of a serious adverse cardiovascular incident (Henry et al., 2014; Patel et al., 2016). This finding may pave the way for a novel treatment strategy for cardiovascular diseases. However, additional research must be carried out to determine the effectiveness of this therapy (C. Wang et al., 2019).

Chapter 10

Future Prospects

According to emerging research, patient features such as diagnosis, presence of comorbidities, the use or need of co-medications, and the cumulative risk factors associated with cardiovascular disorders all have a significant impact on the therapeutic success of the stem cell therapy administration (Delewi et al., 2014; Steinhoff, Nesteruk, Wolfien, Kundt, et al., 2017; Zwetsloot et al., 2016). Moreover, an extended interpretation and understanding of the ailment as well as the working mechanism of the endogenous reparative process of the cardiovascular system and how it interacts with the administered stem cells is crucial (Steinhoff, Nesteruk, Wolfien, Große, et al., 2017). In light of these findings, the adoption of individualized cardiovascular stem cell treatments, whereby the choice of the stem cell origin, necessary customization, as well as administration are all influenced by the specific features of the patient, is highly recommended (Becher et al., 2011). As a result, future research should emphasize on the establishment of particular respondent reviews and even more so on the discovery of biomarkers that can be used for the prognosis in order to recognize patient populations that will favor the most from specific stem cell therapies (Fernández-Avilés et al., 2017; Madonna et al., 2016). Thus, advanced standardization of research methodologies and designs, as well as the development of a worldwide extensive database which provides access to all for the purpose of registering and publishing of studies and trials both clinical and preclinical, will significantly increase the comparability, accessibility, and reliability of the data acquired as a result of these studies (Fernández-Avilés et al., 2017; Jansen et al., 2014; Müller et al., 2018; Trindade et al., 2017).

In general, there is a significant positive impact on the results of regenerative medicine therapies after generating a pro-regeneration setting inside the body of the patient. In order to achieve this objective, deeper and more advanced understanding of the function of the immune system in regeneration, as well as technologies with the ability to modulate immune response as per need, would be beneficial. Improved knowledge regarding how the patient's age, current state of disease, and such other factors impact regeneration is also vital for improving the area of cardiac regenerative medicine in the coming years (Eming et al., 2014; Mao & Mooney, 2015; Oh et al., 2014; Scales & Huffnagle, 2013).

Further studies must be conducted to establish efficacious regenerative strategies to treat peripheral artery disease (PAD) which would combine therapies that include the scope for arteriogenesis with angiogenesis. This is primarily because only the angiogenic expansion of the vascular system is not sufficient to alleviate the obstructions from the cardiac vessels (Meng & Cooke, 2020).

Patch material is recurrently used in surgical methods in order to accomplish a full recovery. Therefore, patch rejection may be a contributing factor for the need of reoperations. As a result, countless tissue engineers are currently on the lookout for the ideal patch material that has some specific features that include immunity towards infections, high durability, resistance to shrinkage or calcification, high biocompatibility, and results that are predictable (Pavy et al., 2018). It will be necessary to develop methods that allow completely vascularized patches to be effectively merged anatomically with cardiac vessels of the host at the moment of transplantation, hence increasing the likelihood of survival of the graft in large designed transplant tissues (Mao & Mooney, 2015).

Chapter 11

Conclusion

Recent breakthroughs and encouraging preclinical results in the area of cardiac regeneration surely offer reason to be hopeful about the development of novel and successful regenerative treatments for the damaged heart. Although the outcomes of prior human heart regenerative clinical trials have been unsatisfactory, scientists must recognize and work to resolve the restrictions of preclinical investigations regarding cardiac regeneration, as well as the challenges associated with clinical translation. In addition, the formation of the human heart is a complicated and essentially spatially and temporally choreographed process that requires careful attention to detail. Because of this, it is likely that mending the human heart will involve a mix of several different treatment techniques (Hashimoto et al., 2018). In order to comprehend how this technology may be converted into meaningful clinical use through improvement of critical processes like differentiation as well as purification stages, researchers must first fully grasp how it works. More often than not, the breakthroughs that provide the finest therapeutic value also bring the most severe hurdles and this is also the case for the field of regenerative medicine and stem cell therapy for the cardiovascular system (Parrotta et al., 2019).

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