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Cardiac Regeneration: A Promising Future for Tissue Engineering and Cardiac Repair

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ABSTRACT: Cardiovascular diseases are the leading cause of human death worldwide. These diseases have detrimental effects on the heart, damaging the tissue and disrupting healthy heart function. Cardiac functioning begins to dissipate as a result of cardiac cell death and subsequent damage to the structures of the heart. Cardiac regeneration is an evolving field in cardiac therapy that aims to rebuild damaged cardiac tissue that may have previously been beyond repair. Understanding how cardiac cells function within the heart is crucial in studying tissue engineering and cardiac regeneration. With the loss of cardiac cells following cardiovascular disease, the ability to extract healthy cardiac cells for tissue engineering becomes greatly limited. Thus, tissue cultures and cellular reprogramming become crucial methods for in vitro expansion of healthy cells. Understanding regeneration in the heart, cell sources necessary for cardiac regeneration, and the existing models that have been implemented for cardiac regeneration are crucial in advancing its study and improving methods to take cardiac regeneration to clinical trials. Imaging techniques to model patient-specific structures of the heart will ultimately help map damaged tissue, assisting with the entire regeneration process. Investigating the limitations of these techniques is equally important to improving cardiac regeneration, with the possibility to save countless lives.

INTRODUCTION

For many decades, cardiovascular-related diseases (CVDs) have been the leading cause of death globally as well as a major contributor to disabilities.[1] Despite advancements in medical care that have resulted in a decline in age-standardized CVD deaths occurring globally, the total CVD global deaths have continued to increase over the last three decades, and previous progress in counteracting the mortality effects of CVDs has become uneven and begun to stall.[2] The risk of exposure to CVDs has become increasing prevalent, with changes in the ecological and geological environment, modifications in lifestyle, and shifts in social policies contributing to CVD risk.[3] As a result, there has been great concern about heart repair and treatment.

CVDs have been understood to irreversibly damage cardiomyocytes, the cells in the heart responsible for the contractile functions of the heart. This leads to the formation of scar tissue, ultimately damaging the structure and function of the heart.[4] The adult human heart has limited regenerative capacity and so regenerative medicine therapies have shed new hopes in repairing or even replacing damaged hearts.[5,6] The advancements in the field of tissue engineering, particularly concerning cell reprograming, the development of functional biomaterials, and the fabrication of biomimetic cardiac tissue, have gained traction in providing possible

avenues for tissue regeneration applications within the heart. Biomaterial-based approaches have gained increasing attention for cardiac tissue engineering and regeneration after contributing to improved cardiac function, promoting angiogenesis, and diminishing severe immune responses following clinical trials on animals.[7]

Nevertheless, creating fully effective functional cardiac tissue in vitro is still a challenge in the field of tissue engineering, with the regeneration of an entire human organ in vitro, specifically the heart, being difficult in terms of specific chamber orientation and electrical function.[8] Despite this, implementations of tissue engineering to reconstruct cardiac tissue have reported better contractile function from the heart, and cardiac progenitor cells, responsible for contributing to cardiac tissue regeneration, have been reported to give rise to cardiomyocytes in vitro and in vivo after transplantation and to enhance cardiac function post-infarction.[9]

This review will highlight the progress made in the field of cardiac tissue engineering regarding regenerative tissue applications on the heart, with a particular focus on cell reprogramming in the context of tissue engineering, growing these cells in various culture media, and the applications of these cells within the cardiac tissue engineering process. An introduction to the cardiac system, along with the current status on regeneration and cell sources necessary to engineer cardiac tissue, and the experimental applications of working with the heart, including imaging techniques and the fabrication of biomimetic cardiac tissue, will also be discussed.

CARDIAC CELLS

Introduction to Cells

Cells are fundamental building blocks within an organism's body, present in all living organisms. They are responsible for carrying out all the vital functions, and they ultimately play a crucial role in organ function. In the process of regeneration, organisms have been shown to produce new cells when replacing an amputated, damaged structure.[10] Therefore, it remains crucial to produce and introduce new cells to the body when implementing regeneration after a particular body part or organ has faced severe damage. With cells being a crucial factor necessary for regeneration, it is crucial to produce the cells that predominantly make up the composition of the heart: cardiomyocytes.[11]

Cardiomyocytes are the striated, branched cardiac muscles found in the heart that are responsible for contracting together synchronously to enable the heart to work as a pump.[12] Following cardiac injury, a substantial number of cardiomyocytes are lost, and the heart forms a fibrotic scar to serve as patch where the cardiac muscle cells previously resided. However, the scar tissue does not maintain the ability to effectively contract.[13] In the event of a heart disease, one also observes cardiomyocyte cell death.[14] Therefore, combating the effects of CVDs and enhancing the possibilities of cardiac tissue regeneration can be made a possibility by exploring areas of cell reprogramming concerning cardiomyocytes, understanding how to culture these cells so that they can be reapplied within the human heart, and uncovering other cell sources necessary for cardiac tissue engineering.

Cell Culture

Cells occupy a crucial role in the functioning of organs, and damage to organs, such as CVDs, often results in the cells within that organ being damaged. As a result, the understanding and induction of cells to be introduced into the body becomes extremely important, particularly when discussing regeneration. However, to understand the cells within the body that are necessary for regenerative purposes and develop techniques to increase the number of required cells, these cells need to be extracted in vitro and grown outside the body. This is achieved through cell cultures.

Cell cultures are laboratory methods that enable the growth of eukaryotic or prokaryotic cells in physiological conditions. They enable the study of human health and diseases through the extraction of infected human cells, which can then be examined in a sterile, cultured environment. Utilizing the practice of cell cultures working with the homogeneity of clonal cell populations, specific cell types, and well-defined culture systems removes any interfering genetic or environmental confounding variables, thereby allowing data generation with high reproducibility, which may not otherwise be consistent when examining and working with an entire organ.[15] This then enables a possible avenue for inducing a high number of cardiomyocytes required for cardiac regeneration.

Among the various culture techniques available, two well-established models are 3D and 2D culture systems. 2D systems are the traditional monolayer cultures that are straightforward, cost-effective, and convenient for observation. However, 2D cultures fall short in simulating the in vivo morphology of the extra cellular matrix (ECM), and cells in these 2D cultures are compelled to adapt to their complex biological behaviors. These complications have resulted in a turn to 3D systems that better mimic the physiological context of the ECM.[16] 3D culture systems are more complex systems than 2D culture systems, as they have been developed to mimic in vivo conditions, and therefore provide a good alternative for the in vitro imitation of the human heart tissue. Generally divided into scaffold-based and scaffold-free, 3D heart tissue models allow cells to organize themselves into a 3D structure resembling the human myocardial cell organization. A major advantage of 3D cultures is that their more accurate imitation of the internal human environment allows researchers to better replicate in vitro cardiac tissue morphological, biochemical, and mechanical features.[17]

In the cardiovascular field, 3D culture systems are further differentiated into two categories; they either contain a scaffold matrix, generally a hydrogel, mixed with and populated by cells to form a strip shaped micro-tissue between attachment sites, or they are smaller cellular spheroids formed by self-assembly without scaffold proteins. An increasing number of studies have used a mix or co-culture several cell types, such as rodent and human induced pluripotent stem cell-derived cardiomyocytes.[18] In fact, larger tissue formats like multilayered cell sheets, re-cellularized hearts, or large biomaterial patches, which are too expensive and slow in manufacturing for drug testing, are developed for regenerative medicine, leading to advancements in issue and cardiac regeneration.[19]

Cell Reprogramming

Cellular reprogramming is the process by which cells can be reliably changed from one tissue type to another, enabling novel approaches to more deeply investigate the fundamental basis of cell identity.[20] The process of cellular reprogramming is not simply limited to a technique, but rather an application that can be used within the human body for regenerative purposes. The importance of maintaining high quantities of cardiomyocytes following CVDs remains a problem, and the issues extends to the fact that cardiomyocytes have little capability of proliferation after birth.[21] Through the process of trans differentiation, the conversion of one cell to another, cells that are abundant in the body can be reprogrammed into desired cell phenotypes to restore tissue function in damaged areas.[22] This process presents a possible avenue for further investigation into reprogramming the abundant cells in the body into the necessary cardiomyocytes, which may be scarce.

Several approaches for cellular reprogramming concerning the induction of cardiomyocytes have been investigated, and have proved successful in cardiomyocyte production, indicating a promising avenue towards cardiac tissue regeneration. Firstly, through combinatorial expression of two cardiac transcription factors, Gata4 and Tbx5, and a cardiac-specific subunit BAF chromatin-remodeling complex, Baf60c, the direct differentiation and conversion of mouse mesoderm into beating cardiomyocytes can occur.[23] Secondly, following experimentation using a combinatorial strategy, it was identified that a combination of microRNAs was capable of inducing direct cellular reprogramming of fibroblasts into cardiomyocyte-like cells in vitro.[24] Thirdly, by treating human fibroblasts with a combination of nine compounds, termed 9C, cardiomyocyte-like cells can be generated that uniformly contract and resemble human cardiomyocytes in their epigenetic properties.[25] Lastly, the introduction of three cardiogenic transcription factors: Gata4, Mef2c, and Tbx5 (GMT) can induce the direct reprogramming of cardiac fibroblasts into cardiomyocytes, and this discovery of the direct cardiac reprogramming factor GMT has also enabled the induction of cardiomyocytes inside the body.[26]

THE HEART AND ITS REGENERATIVE CAPABILITIES

Introducing the System of the Heart

The cardiovascular system is a crucial system within the body, composed of the heart, blood vessels, and blood. The cardiovascular system is designed to ensure the continuous survival of all cells within the body, which it achieves by maintaining the intermediate chemical environment of each cell in the body.[27] The heart remains at the center of the cardiovascular system, responsible for pumping blood through the pulmonary and systemic systems of the body. The cardiovascular system, in turn, serves as a body-wide network of vessels that transports nutrients, respiratory gases, metabolic waste, and hormones, distributes and dissipates heat, and assists in defending the body against disease.[28] The cardiovascular system, and ultimately the heart, plays a crucial role in the healthy function of the human body.

Adult hearts fail to regenerate following injury and CVDs, and this failure to regenerate myocardium is a crucial contributor to heart failure and ultimately death worldwide.[29]

Therefore, complications that occur within the cardiovascular system and affect the heart ultimately have a detrimental effect on the rest of the body, making the applications of regenerative medicine surrounding cardiac tissue more pressing.

Regeneration Within the Heart

Currently, experimentation in heart regeneration has been restricted to mouse model hearts. In a particular experiment, fetal rodent hearts that had undergone a specific genetic ablation were able to restore around 50% of the lost cardiomyocyte mass indicating that the embryonic environment and the transcriptional state of embryonic cardiomyocytes facilitate cardiomyocyte cell cycle re-entry and repopulation of the heart, an important discovery in understanding the regenerative potential from the embryo and the change thereafter following the first few weeks of life.[29] The field of heart regeneration has entered a period of extraordinary progress in the understanding of endogenous heart regeneration, stem cell differentiation for exogenous cell therapy, and cell-delivery methods.

However, the regenerative capacity expressed by adult individuals, human in particular, is not entirely favorable to generate cardiac tissue necessary following CVDs and injury. Although the heart retains the ability to regenerate in some lower vertebrates, adult mammals do not retain the capacity to replenish the heart with enough cardiomyocytes to restore function following injury. Therefore, the concept of heart and cardiac regeneration does not refer to a single area of study but rather encompasses the advancements and novel discoveries in fields including native cardiomyocyte renewal, cell therapy, direct reprogramming, and tissue engineering approaches to rebuild damaged heart tissue.[30]

Multiple approaches have been explored to promote and possibly achieve regeneration within the heart. Firstly, there are cell-free therapies for cardiac repair, where recombinant DNA, RNA-based, or protein therapeutics have been used in regenerative medicine. In myocardial infarction (MI) models, the injection of particular cardioactive growth factors, such as Neuregulin1, has induced sustained improvement in myocardial function.[31] Furthermore, the utilization of the cardiomyogenic factor Follistatin Like 1 can stimulate the recovery of contractile function and limit fibrosis following MI injury.[32] Secondly, cell-based therapies have been proposed as a promising approach for treating advanced heart failure and repairing damaged myocardial tissue. Thirdly, adult stem cell transplantations have been a possible avenue for cardiac regeneration. Experimental evidence suggests that adult stem cells, such as bone marrow cells, bone marrow purified hematopoietic stem cells, and bone marrow purified mesenchymal stem cells, can differentiate into cardiomyocytes. Lastly, pluripotent stem cell-based therapies have opened another path for cardiac regenerative possibilities. Specifically, it has been understood that human embryonic stem cells can differentiate into multiple cell types and thus have great therapeutic potential in regenerative medicine.[33]

Cell Sources

Cell sources for cardiac tissue engineering and regeneration refer to the variety of cell types that can be used in cardiac tissue engineering constructs. These cell types include cardiomyocytes, often derived from

induced pluripotent stem cells or isolated rodent hearts, cardiovascular cells, fibroblasts, various progenitor cells, stem cells, and spheroids.[34] Embryonic stem cells, as a type of stem cell, can be collected from the inner mass cells of blastocytes and can differentiate into cell types of all germ layers.[35] Through experimentation, cardio-intrusive cues were used to differentiate embryonic stem cells to cardiac progenitor cells and embed them in a fibrin patch, which in turn improved cardiac function. Induced pluripotent stem cells are widely used in regenerative medicine, and they are often obtained from a patient's somatic cells. These cells are similar to embryonic stem cells and have been used to regenerate the heart in a variety of animal studies. Cardiac stem cells, which can be acquired from the heart, are extremely useful for cardiac regenerative purposes, as they have the potential to self-renew and can differentiate into different cell types, such as vascular smooth muscle cells, cardiomyocytes, and endothelial cells.[36] Lastly, cardiac progenitor cells are defined by having self-renewing, clonogenic properties, as well as multipotent differentiation capabilities both in vivo and in vitro, which are desirable for promoting cardiac regeneration.[37]

At the core of the heart, however, lies cardiomyocyte-rich tissue to allow for rhythmic pump contractions. These cells appear to be the most prevalent cardiac cell population in the heart, and the need to acquire these cells is extremely important for regenerative applications to succeed.[6] Discovering sources to acquire cardiomyocyte cells is crucial to engineer cardiac tissue, and although there are existing limitations around human tissue availability, human adult cardiomyocytes can be isolated during myocardial biopsies.[38] However, this process needs to be done at quickly because following isolation, cardiomyocytes undergo a profound structural and functional remodeling, leading to cell differentiation and loss of viability.[39]

REGENERATIVE MODELS AND METHODS

Existing Models

There has been increasing demand for new therapeutic models, and such models are necessary to advance the understanding of the biological mechanisms of cardiac tissue regeneration, and test therapeutic approaches to regenerate tissue and restore cardiac function. In vitro model systems allow researchers to study biological systems while still maintaining a high level of control over the experimental parameters.[40] Such in vitro models include single cell models, 2D culture models, 3D culture models, coculture models, microfluidic system models, tissue models, whole heart models, and lastly cell sources as discussed in the previous section.

Examining the electromechanical properties of single cardiomyocytes has been crucial to the understanding of cardiac physiology. The electrophysiology of a single cardiomyocyte can be evaluated using the patch clamp technique, which can characterize the phenotype of CMs differentiated from embryonic stem cells.[41,42] Cardiomyocytes grown in 2D culture systems can elucidate molecular signaling pathways, assess the cardiotoxicity of a particular drug, and evaluate gene therapy approaches.[43,44,45] 3D models have been used as scaffolds to mimic the natural extracellular matrix, making the models extremely close to human myocardium, serving as models of healthy

human heart physiology.[40]

Cocultures systems enable a variety of growing cell types to be cultured together, which in turn stimulates the cellular communication that would normally occur between cells of different types.[46] The coculture of human embryonic stem cells with human umbilical-vein endothelial cells and mouse fibroblasts resulted in the formation of vascular networks resembling capillaries, and the coculture enhanced cardiomyocyte proliferation.[47] Microfluidic techniques make use of soft lithography techniques and have been constructed to study the electrophysiology of single cardiomyocytes in microfabricated patch-clamp devices or cell-to-cell signaling between pairs of cardiomyocytes.[48,49] These systems also make use of microfabrication techniques that provide advanced structure from well-defined scaffolds, which can improve drug discovery and better mimic heart physiology. Lastly, examining the whole heart of an organism outside its body allows for the evaluation of cardiac function ex vivo.

In vivo model systems provide a more accurate understanding of the complex behaviors of the heart within a living organism. In vivo model systems include noncardiac regenerative models and cardiac regenerative models. Noncardiac animal models, and noncardiac mammalian regeneration, can provided possible clues to cardiac regenerative biology using methods that are more practical because of lower cost and fewer technical and logistical challenges, and then can provide insight into potential mechanisms of cardiac regeneration. Cardiac regenerative models, on the other hand, focus on examining small and large animal mammalian models that have been studied extensively with the intention of discovering therapeutic interventions to effectively regenerate the heart and cardiac tissue following injury and CVDs.[40]

Advanced Optical Imaging Techniques

Optical imaging uses light and special properties of photons to obtain detailed images of organs, tissue, cells, and possibly even molecules.[50] Optical imaging is a non-invasive imaging technique that functions based on the absorption, scattering, and fluorescence properties of the incident particle. Optical imaging provides depth-resolved, high-resolution images of tissue microstructures in real time by measuring the interference between the light backscattered from the sample tissue and the light reflected from a reference. The use of optical imaging techniques can help understand a patient's heart structure, which can help prepare for procedures by identifying structures to avoid and locating areas for treatment and repair. The imaging plays a crucial role in early diagnosis of CVDs, as well as in monitoring and providing guidance for cardiac procedures. Due to the high resolution, fast imaging speeds, and non-invasive procedure obtained from optical imaging, this technique has seen increased clinical use over the past few decades.[51]

Molecular imaging, in particular optical imaging, has advanced in a way that allows for the characterization of biological processes at the cellular and subcellular levels, which could ultimately play a crucial role in the examination of cardiac tissue to understand their biological processes and whether tissue may be damaged or not.[52] Current methods of therapy for cardiac regeneration, including stem-cell therapy,

have been promising as discussed, but the challenges of such therapies are dependent on cell survival of the introduced cells, possible implantation difficulties due to adverse mechanical forces, and possible hypoxia However, the use of advanced optical imaging techniques has emerged as a novel approach to track engraftment, the formation of healthy blood cells following a transplant, as well as track the survival and rejection of transplanted tissue, which is crucial in understanding the efficacy of procedures conducted for cardiac regeneration, making this technique very valuable.[53,54]

Emerging Models to Constructure Cardiac Tissue

The majority of 3D cardiac models, until now, have been fabricated to be flat or small structures, which can limit volumetric expansion and functional maturation. The need to establish a model that could overcome these limitations of scale, maturity, and functionality of human cardiac tissues has been fabricated by integrating native heart-like cellular and extracellular components and dynamic flow. This new 3D human cardiac tissue model was successfully validated for various biomedical applications, including cardiotoxicity prediction, cardiac disease modeling, and regenerative therapy.[55] Together, this 3D tissue model is extremely beneficial in imaging the heart and its cardiac tissue with respect to identity and predicting the presence of cardiac disease, as well as applying this model for cardiac regeneration.

With the current shortage of heart donors available and the urgent need for new sources of cardiac regeneration medicine, tissue engineering using bioactive materials and 3D bioprinting to create microscale cardiac tissue has shown great advantages and promise in the field of cardiac regenerative therapy. 3D bioprinting focuses on the tight fitting of a sufficient number of functional cardiomyocytes with a feasible scaffold on a small scale.[56]

3D bioprinting has ultimately emerged as an approach for in vitro generation of functional cardiac tissue for drug screening and cardiac regenerative therapy. The use of 3D bioprinted cardiac patches, fabricated by human coronary artery endothelial cells, collagen microprinting, and an alginate matrix, resulted in high levels of cell proliferation and differentiation, which are crucial for regeneration of cardiac tissue. 3D bioprinting is a layer-by-layer additive technology that precisely deposits biomaterials and active cells in accordance with a certain spatial pattern that has high resolution simulation of the heart.[57]

Using a combination of different bioprinting approaches, a variety of cardiac constructs, including cartilage and a heart valve, have been successfully constructed, which is crucial to repair damaged tissue structures within the heart. Experiments show that bioprinted cardiac tissue possess cardiomyocytes orientated in an organized fashion and contracting capabilities, both consist with native heart tissue characteristics.[57,58]

Fabrication of Biomimetic Cardiac Tissues

Inducing the maturation of cardiomyocytes remains a problem in the field of cardiac tissue engineering and cardiac regeneration. Specifically, addressing this issue within the complexity of the in vivo conditions of the human heart remains an important barrier in cardiac tissue engineering. A variety of

methods have been tested to grow biomimetic tissue in the laboratory that can adjust to the

complexities within the body. These methods include the Hydrogel method, decellularized bio scaffolds, microfabrication, and bioprinting as discussed in the previous section. Hydrogels are widely used for their ability to be molded into several geometries and their scalability. The cardiac tissue can be fabricated by joint gelatin of cells and a polymer or by having a casting mold to confine the cells in a particular organization, which can mimic cell organization found in native cardiac tissue by freeze-drying the hydrogel.[6]

Decellularized bio scaffolds aim to generate scaffolds with a native extracellular matrix ultrastructure and composition while removing all cells and genetic material present in the native tissue. The most immediate application of decellularized myocardial sheets is scaffold recellularization; however, the recellularization of such sheets with cardiomyocytes, which can be successfully achieved, does not translate to the goal of recellularizing human hearts, which requires a vast number of cardiomyocytes for which there is no current sustainable production method.[6]

The use of biomaterials with built-in electroconductive characteristics are used to mimic the electrical conductivity of the native heart tissue, and scaffolds infused with electroconductive elements create a conductive network across the constructed tissue, which imitate electrical conductivity seen in the heart.[59] Gelatin-based scaffolds have also gained attention in recent years with their great bio-affinity that encourages the regeneration of tissue, and how by strengthening such gelatin-based scaffolds, the natural tissue can be accurately mimicked. Gelatin-based scaffolds cansimplify the inclusion of cells and growth factors for optimal scaffold construction, and advanced production strategies for these scaffolds create highly configurable scaffold geometric shapes needed for specific medical necessities and to fabricate patient-specific implants.[60]

Advances in microfabrication have allowed for the detailed engineering of material features to resemble the in vivo conditions of an organ, particularly the heart. Simpler systems use microfabrication to pattern material substrates, to ease cardiomyocyte spreading and sarcomere axial alignment, and following experimentation, a substantial improvement in cardiomyocyte alignment was shown. Microfabrication methodologies can ultimately be harnessed to create complex tissues like the native myocardium, which is crucial for regenerative cardiac tissue to adjust to the in vivo construct and conditions of the native cardiac tissue.[6]

Limitations of Imaging Methods for the Heart

The rapid and complex changes that occur from heart morphology make imaging the heart during development a challenging task. Confocal microscopy, an advancement of fluorescence microscopy, offers higher spatial resolution and the ability to visualize subcellular details; however, this imaging technique is relatively slow and harsh on the sample, potentially causing phototoxicity and bleaching.[61,62] A major challenge in biomimetic cardiac tissue engineering is the scalability of the fabrication technique and the translation of these approaches to clinical

applications. The issues that remain around biomimetic cardiac tissue engineering include standardized production methods, optimizing functional and structural maturation, and ensuring long-term stability of synthetic cardiac tissue.[59] These challenges, ultimately, require significant attention to move forward with advancements in this field.

The advancements in 3D bioprinting have revolutionized potential avenues in cardiac treatment in the future, with the opportunity to fabricate microscale cardiac tissue for potential in vivo applications. However, limitations occur with the use of artificial prints, compared to the original hearts of large mammals, as these prints remain naïve in their generation of both the input and output mechanical strengths long-term. Furthermore, because of the complexity of heart movement, whether these 3D bioengineered bodies are able to achieve or even get close to perfect cardiac replication physically and chemically remains unknown.[56]

There are a vast number of imaging techniques to examine the cardiac system, and although there are specific advantages for each imaging technique that make these applicable in certain situations, each of them has their own limitations. These imaging techniques include radionuclide imaging, MRI, ultrasound, CT scan, optical imaging, and magnetic particle imaging (MPI).

Radionuclide imaging has the limitations of radioactive exposure, high cost of operation, poor spatial resolution, and a short imaging time. MRIs are also expensive and require heavy involvement to operate. Ultrasounds have low tissue contrast, and they are dependent on an operator. CT scans have ionizing radiation which is extremely dangerous, and they offer limited soft tissue discrimination. Optical imaging offers poor penetration depth, poor spatial resolution, and it is only used in preclinical trials. Lastly, MPIs are unable to discriminate live cells from dead cells.

CONCLUSION

CVDs have had a fatal impact on the human population. Thus, studying heart and cardiac tissue repair has become a forefront issue to combat the effects of CVDs. The effects of CVDs result in damage of cardiac tissue and the death of cardiac cells. The heart soon experiences degraded function, triggering other dysfunctions within the body that ultimately result in death. Therefore, exploring human regenerative capacity and the potential application of heart and cardiac regeneration has evolved as a new therapeutic to engineering functional cardiac tissue following CVDs. The potential of cardiac regeneration is very promising for cardiac repair, and the progress made in the field through tissue engineering has shown the beneficial outcomes that can be achieved through cardiac regeneration.

The focus on the production of cardiac cells following CVDs is crucial in restoring appropriate cardiac function. Cells form the basis of living tissue, making the induction of cells important for regenerative purposes, especially cardiomyocytes which once introduced into the body are important for cardiac function. Through various applications of cell culture, specifically 2D and 3D cultures, these cardiac cells can be effectively understood and examined. Growing these cells in an artificial environment is extremely important in the context of cardiac

regeneration, as more cardiac cells can be produced and introduced into the human body as a supplement to compensate for the loss of cells following cell death. Cellular reprogramming also plays a crucial role in cardiac regeneration, by allowing cells to be changed to another cell type which could assist the process of cardiac regeneration by differentiating greater populated cells into cardiomyocytes which are less abundant in the body.

The field of cardiac regeneration actively growing, with a variety of approaches being explored to help promote and achieve regeneration of the heart. Cells sources have also been of extreme importance in engineering cardiac tissue to contribute towards regeneration of the heart. Cells sources for cardiac tissue engineering refer to the variety of cell types that can be used in cardiac tissue engineering constructs, including cardiomyocytes, induced pluripotent stem cells or isolated rodent hearts, cardia vascular cells and fibroblasts, various progenitor and stem cells and spheroids. Discovering and utilizing various cells sources to acquire cardiomyocyte cells is crucial to engineer cardiac tissue, as these cells appear to be the most prevalent cardiac cell population in the heart, and the need to acquire these cells is extremely important for regenerative applications to succeed.[6]

Various existing models have helped propel the concept of cardiac regeneration to become a forefront technique for therapeutics in the future to treat damaged cardiac tissue. The use of a variety of imaging techniques has been crucial in allowing internal areas of the cardiac system and the heart, as well as problems with a particular person's heart, to be examined and understood externally. This is crucial in cardiac regeneration, in particular, as particular constructs of cardiac tissue could then be induced after understanding a person's current heart state and the areas that are damaged. The utilization of imaging techniques also showcases the efficacy of the regenerative tissue and its ability to work within the body, which is useful when evaluating the use of the construct and understanding how it contribute to regeneration through a non-invasive procedure. However, the use of imaging techniques, and the development of models relating to cardiac regeneration have their inherent limitations, serving as a setback for the progress of cardiac regeneration. The need to address these issues is crucial in developing therapeutics and constructs that can advance cardiac regeneration and better suit their applications for human use. Possible directions for future myocardial regeneration include the consolidation of transplantation of cells with "true" regenerative potential, tissue engineering with various scaffolds and cell types, a stimulation of resident cell sources by cytokines or growth factors, and a direct reprogramming of scar tissue by delivery of various transcription factors or miRNAs63.

Cardiac regeneration is shaping to be a promising field in the future to treat damaged cardiac tissue following CVDs. The applications of cardiac regeneration are endless, and the hope generated by the possibility of cardiac regeneration is likely to be carried into the future. The process of cardiac regeneration could ultimately

revolutionize cardiac tissue repair and treatment, and the promise of cardiac regeneration could be the step needed to save countless lives.

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Author Contributions

Krishay Patel provided full contribution to the research and writing of the manuscript. The manuscript was drafted through the Collegiate Mentorship Program with the help of Dr Rosalyn Abbott (rabbott@andrew.cmu.edu)

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