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Applications and Advancements in Stem Cells for Cardiac Tissue Engineering

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ABSTRACT

Heart disease has continued to be the leading cause of death in the world (Mc Namara et al., 2019). Despite the growing need for a therapeutic, regenerative medicine approach, there has been a lack of advancements resulting in possible treatment options for these patients. Researchers have developed various approaches to combat the adversities of heart disease, ranging from small-scale cardiac patches to large-scale whole organ regeneration approaches. Although these findings are still preliminary, they provide potential therapeutic approaches. The main ingredient towards this promising direction are stem cell-derived cardiomyocytes, in which induced pluripotent stem cells are reprogrammed through signaling pathways to mimic characteristics of mature cardiac cells. With these self-renewing cardiac cells, researchers have been able to formulate hydrogel patches that mimic the environment of the cardiac tissue to eventually mend the injury and pump synchronously. For deeper penetration and favored nutrient flow, a process known as FRESH 2.0 has created a ventricular scaffold printing method that aids in the oxygen, nutrients, and signaling flow for bioprinted organ parts (Lee et al., 2019). In developing these tissues, SWIFT has progressed the needed cellular density to produce a cardiomyocyte rich artificial organ (Skylar-Scott et al., 2019). These successes still face drawbacks with perfusable vascularization, cell density on a whole organ scale, consistent maturation of cardiomyocytes, and clinical applications. Nonetheless, researchers have made significant advancements for regenerative medicine regarding the cardiac tissue, and will continue to expand their look towards a successful replication of the whole organ.

Introduction

Heart disease has been the leading cause of death in the United States since 1950, affecting 1 in every 5 Americans (Tsao et al., 2023). There has been a growing prevalence of heart disease in recent years, contributing to an average of about 697,000 deaths in the US per year (Tsao et al., 2023). The pressing concerns of heart disease involve unhealthy cardiac tissue associated with heart attacks, congestive heart failure, congenital heart disorder, and more. Cardiac tissue, or myocardium, is a specialized tissue that forms the heart, contracting and releasing synchronously to pump blood throughout the body. The muscle at the center of the circulatory system, the heart, is the necessary organ for pumping blood, along with circulating nutrients and oxygen through pumping ventricles (Rehman & Rehman, 2023). Healthy cardiac tissue is present with cardiomyocytes beating in an orchestrated, paced manner. Unhealthy tissue is irreversibly damaged, and unable to contract efficiently, or in more severe cases, not able to contract at all. To address this obstacle, current treatments involve prescription drugs or clinical treatments that, although help relieve discomfort and aid in survival after tissue damage, are only temporary solutions. The only permanent solution is a heart transplant, however organs are scarce, meaning that most people will not have access to a donor heart (Cameli et al., 2022). Additionally, there is a risk of immune rejection and it is challenging to predict whether or not the recipient's immune cells will accept the transplanted heart (Cameli et al., 2022). These shortcomings bring to light the future of regenerative medicine. A promising approach in the field utilizes stem cells as for an approach to cardiovascular regenerative medicine. The potential of stem cells can apply to cardiac tissue engineering with treatments ranging from bioengineered

patches to larger scale bioprinting for whole organ engineering (Kwon et al., 2018; Lee et al., 2019; Mei & Cheng, 2020).

Stem cells have unbound potential. These cells are found in the body with two main properties: the ability to self replicate, and the ability to differentiate into a specialized cell type. The ability to self replicate allows for an endless cell supply, granting their therapeutic potential. Stem cells are able to differentiate into other cell types, taking on the phenotype and function of that specialized cell type. The differentiation process occurs through the activation of specific cell signaling pathways (Lian et al., 2012). Research in this field has enabled protocols for the differentiation of stem cells into specific cell fates, including cardiomyocytes. These differentiated cardiomyocytes are similar to those found in the in vivo cardiac tissue, which could enable them to serve as a unit in a functioning heart. Each level of damage to the cardiac tissue increases in complexity from surface to internal scarring, leaving challenges and progress that are continuously researched in this field.

Current Advancements

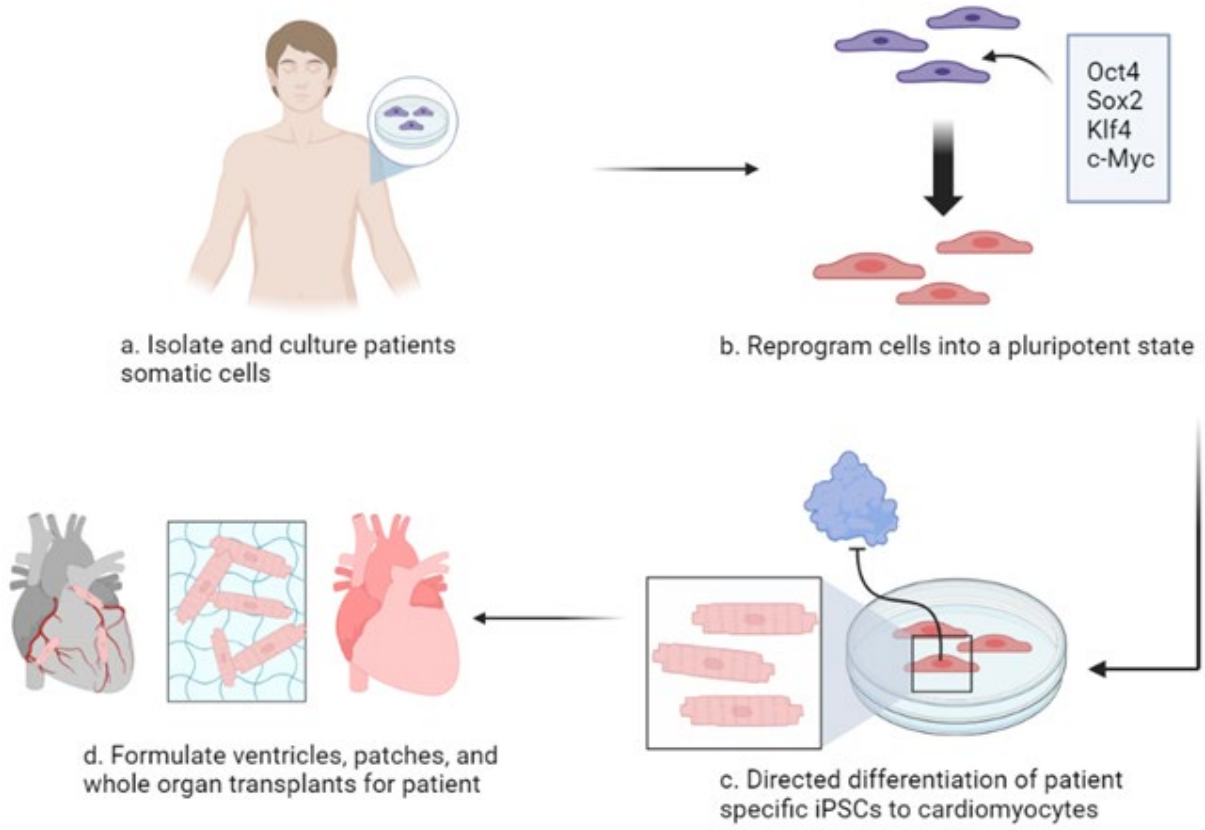

Figure 1. Therapeutic applications of patient specific stem cell-derived cardiomyocytes for heart disease. Patient derived somatic cells (a) are reprogrammed via Yamanaka factors into a pluripotent state (b). These iPSCs can then be differentiated into cardiomyocytes (c) for applications in the development of cardiac patches, ventricles, and whole organ replacements (d).

The most common cardiovascular disease onset is heart attacks , which leave patients with scarred, non-functional heart tissue. Stem cell based cardiac patches are of growing interest to restore function in scarred tissue following heart attacks. These patches are produced from biocompatible materials, commonly hydrogels, and

later seeded with combinations of growth factors and stem cell differentiated cardiomyocytes, to generate replicable and efficiently beating cardiomyocyte patches (Lian et al., 2012; Mei & Cheng, 2020). For patients with more severe heart defects, such as congenital heart disease, there is a need for whole ventricle replacement. Ventricle bioprinting has come to the forefront as a potential therapy for these patients. This can occur through the printing of a biocompatible scaffolding material, typically collagen, that can be layered with stem cell derived cardiomyocytes to eventually replace the necrotic area with flowing nutrients necessary for the survival of cardiac tissue (Kwon et al., 2018; Lee et al., 2019). Lastly, bioprinting methods have also been developed for patients requiring whole heart replacements. In this review, I will discuss how stem cell derived cardiomyocytes have the potential to generate therapeutic cardiac patches, ventricles, and eventually whole hearts.

Stem cells were first discovered and applied to regenerative medicine in 1963 through a self-renewing population found in the bone marrow. Until recently, embryonic stem cells and somatic stem cells were the primary stem cells that scientists used. With a breakthrough discovery in 2006, researchers found that certain specialized somatic cells could be genetically reprogrammed to assume a stem cell-like state (Figure 1a). This new cell type is known as induced pluripotent stem cells (iPSCs) (Poliwoda et al., 2022).

Advancements in the stem cell based cardiac regenerative medicine field can also be attributed to the discovery of induced pluripotent stem cells (iPSCs). iPSCs are somatic cells that have been reprogrammed back into an embryonic-like, pluripotent, state through the introduction of transcription factors required for pluripotency, known as Yamanaka factors (Figure 1b) (Liu et al., 2008). Akin to embryonic stem cells, iPSCs have shown the ability to differentiate into cell fates within the three germ layers. It has been shown that iPSCs can also be differentiated into desired cell types, including cardiomyocytes (Figure 1c) (Lian et al., 2012). Proving as an alternative, iPSCs-which are derived from adult cells by in vitro induction of pluripotency-are also non invasive and reduce immune rejection through their autologous transplants . Through the direct translation of a person's somatic cells, iPSCs are patient specific and embedded with the genetic information of the individual. The individual derived iPSCs allow for patient drug screening, drug testing, disease modeling, and regenerative medicine approaches (Figure 1d) unique to the individuals genetic and epigenetic makeup. As iPSCs genetically match somatic cells with their ability to differentiate through similar features, they have become popular for regeneration of patient specific somatic cells (Kwon et al., 2018).

One such protocol for the differentiation of iPSCs into cardiomyocytes was developed by Lian et al. in 2012 (Lian 2012) . This process began by seeding single cell iPSCs into a dish, allowing them to reach a certain level of confluency, and then introducing the proper media types and cell signaling molecules that direct the stem cells towards a cardiomyocyte fate. The differentiation process begins by switching media types from one that promotes pluripotency to one that directs cardiomyocyte differentiation. In this, activation of the Wnt signaling pathway is achieved through glycogen synthase kinase 3 (GSK3) inhibition by the small signaling molecule, CHIR99021. After 24 hours, Doxycycline was added to the medium to promote beta catenin knockdown required for purification of the induced cardiomyocyte population. This media condition is maintained for a subsequent 96 hours until contraction of the cardiomyocytes is observed, at which point media is finally switched to B27 with insulin to promote cardiomyocyte in vitro maturation (Lian et al., 2012).

Bioengineered Methods

Today, there is a prevalence of around three million people affected by myocardial infarction, otherwise known as heart attacks worldwide (Mechanic et al., 2023). It is unfortunately the leading cause of death in both men and women in the United States and worldwide. This phenomenon is caused due to loss of oxygen supply to the tissue of the heart, causing a block of vital nutrients which results in local tissue death. The dead tissue is permanently damaged because cardiac tissue is unable to self-renew. In compromise, the body replaces the dead tissue with non-functional fibrous tissue. This leaves the patient with impaired cardiac performance, susceptible

to forces that may damage more organs within the circulatory system (Harjola et al., 2017). Although pharmaceutical advancements have been made to increase survival rates for patients suffering from myocardial infarctions, there is a lack of long-term treatment options for survivors. One field of interest is through the generation of stem cell loaded cardiac patches (Figure 2a-b). These patches aim to restore heartbeat function through an incorporation with the host heart (Lian et al., 2012; Mei & Cheng, 2020).

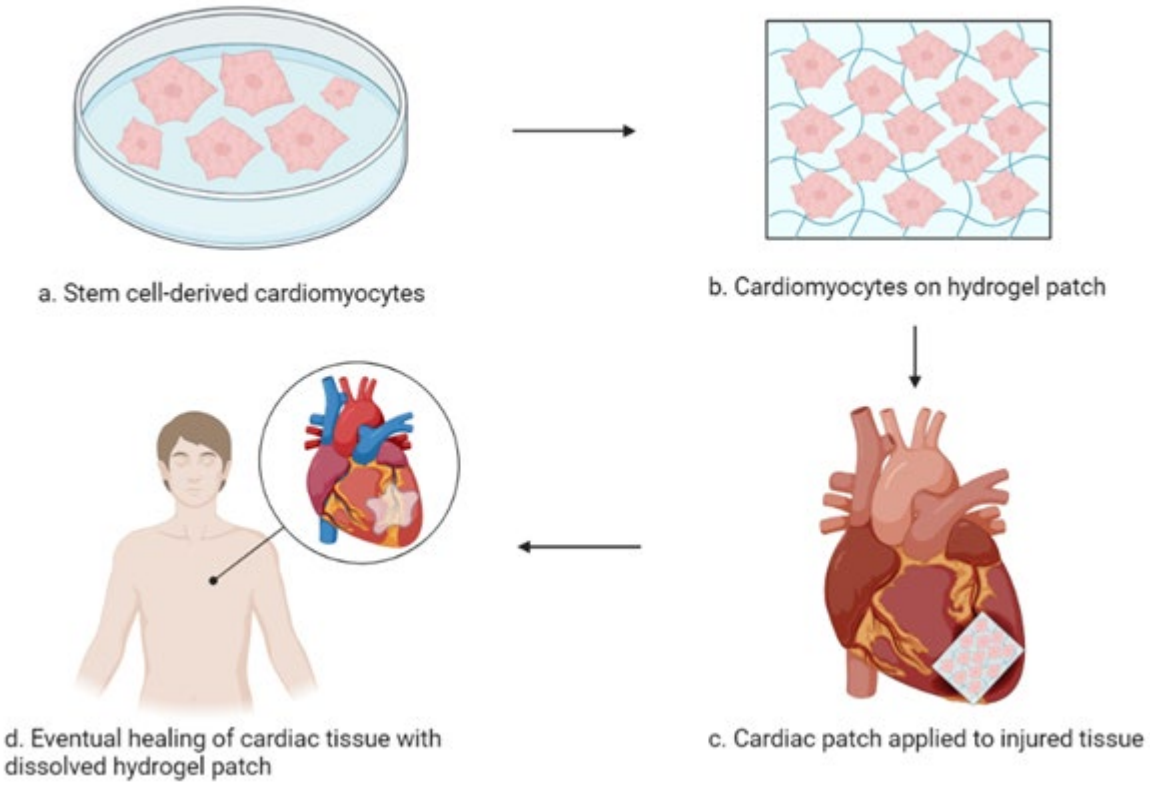

Figure 2. Cardiomyocytes seeded on bioengineered hydrogels can restore cardiac function lost in myocardial infarctions. Stem cell-derived cardiomyocytes can be seeded onto a hydrogel patch (a-b), mimicking the elasticity of the native heart tissue. These cardiac tissues can be surgically added to the heart (c) where the engineered cardiac patch will help restore native tissue function (d).

A cardiac patch consists of various biomaterials and scaffolding to create a hydrogel-like patch to then be placed on the area of damaged cardiac tissue (Figure 2c). There are three main components of cardiac patches: iPSC derived cardiomyocytes, biocompatible scaffolds and growth factors. Biomaterials used for the scaffold are carefully selected based on their ability to last in a cellular environment and mechanical support (Mei & Cheng, 2020; Nikolova & Chavali, 2019). Both synthetic and natural materials are being explored for the fabrication of cardiac patches. Due to their strong mechanical properties, synthetic materials such as polymer vinyl alcohol (PVA) are being clinically tried for their excellent potential in rebuilding damaged cardiac tissue. Natural materials such as collagen, fibrin, alginate, hyaluronic acid, gelatin, and a decellularized extracellular matrix (ECM) are different from synthetic materials in their manner to successfully replicate an original cellular microenvironment (Brovold et al., 2018; Nikolova & Chavali, 2019). Along with this, natural scaffolding materials have great biocompatibility compared to synthetic scaffolding materials and offer more protection against immune inflammation, allowing for their ideal therapeutic functions. An alternative to both synthetic and natural scaffolding materials is a scaffold-less cardiac patch. This kind of patch aims to provide an

alternative to irregular cardiac arrhythmia and immune rejection, both traits that the synthetic and natural scaffold materials fall victim to. This alternative uses cultured cell sheets that have more efficient cell communication along with a more realistic cellular microenvironment. The cell sources that are eventually loaded onto the scaffolds range through cardiac stem cells, mesenchymal stem cells, and pluripotent stem cells. In the case of repairing cardiac tissue, generated cardiomyocytes from iPSCs using protocols are grown on a scaffold, beginning to beat synchronously when matured. To aid in their ability to beat in orchestrated unison with the host cardiomyocytes, electric currents can be run through the cell plates to shock them into beating together. The cardiac patches can then be sutured onto damaged tissue to fully restore function that was lost during myocardial infarction (Figure 2d) (Mei & Cheng, 2020).

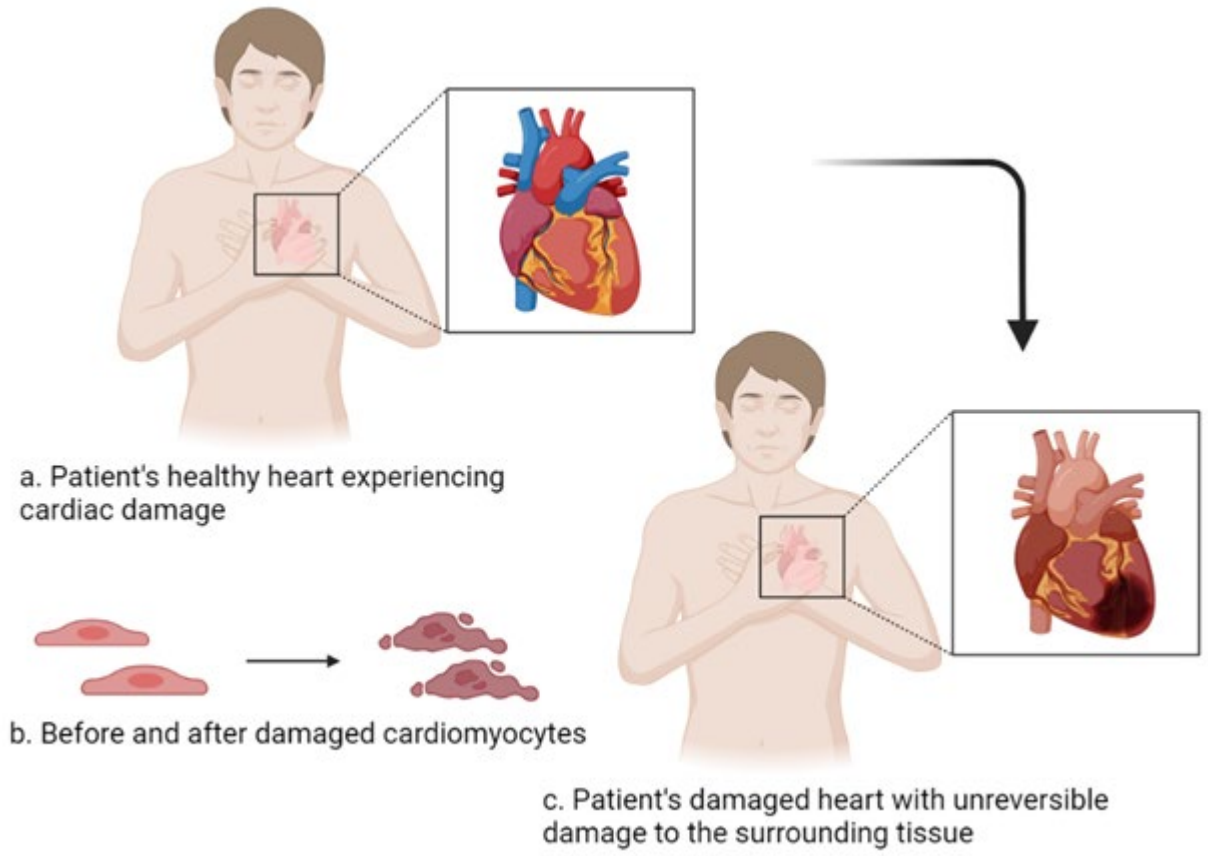

Figure 3. Effects of cardiac damage on tissue. Cardiac tissue becomes damaged following injury (a). This is a result of individual cardiomyocyte apoptosis within the tissue (b). The resulting damaged region becomes fibrotic and non-functional, resulting in irreversible damage (c).

Larger scale heart defects and abnormalities exist (Figure 3a-b), affecting whole ventricles as well as the whole heart. Although cardiac patches aid in tissue regeneration, their restriction to surface applications of the cardiac tissue prevent them from penetrating more severe defects. For this, different approaches have been developed including cardiac bioprinting. In the United States, congenital heart disease (CHD) is a genetically inherited disease that affects nearly 1% or 40,000 babies born each year (Gilboa et al., 2016). The defects of CHD can range from mild to severe, sometimes requiring whole ventricle replacement (Figure 3c). This in mind, there are many obstacles for creating a collagen scaffold that mimics the bio-environment and efficiency of a normal functioning organ. Work done by 3D printing collagen using freeform reversible embedding of

suspended hydrogels (termed FRESH) has successfully reproduced a patient-specific anatomical ventricle structure of their heart . “Fresh works by extruding bio-inks within a thermoreversible support bath composed of a gelatin microparticle slurry that provides support during printing and is subsequently melted away at 37°C.” This method is capable of bioprinting components of the patient's heart at different scales depending on their defect severity. Collagen, a vital component of our extracellular matrix (ECM), has been the ideal material for biofabrication for its mechanical strength, structural tissue organization, and adhesive qualities that allow for cell signaling. Although an ideal biomaterial, collagen, when used to bioprint components of an organ, is formed as a hydrogel. This native form of collagen is printed through temperature sensitivity, a factor difficult to control. In addition, hydrogels are not structurally sound, sagging when printed past a few layers (Lee et al., 2019). Instead of temperature, FRESH uses rapidly changing pH for collagen self-assembly within a buffered support material, allowing for collagen bioink that has not been chemically modified. This enhances mechanical properties of the finished print through a richer collagen supply that makes for a complex structural and functional tissue. Along with creating a stronger alternative bioink, FRESH’s support bath involves gelatin microparticles to create a porous 3D build, promoting microvascularization and cell infiltration (Lee et al., 2019). In relation to vascularization, fibronectin and the proangiogenic molecule recombinant vascular endothelial growth factor (VEGF) were used in the collagen bioink, resulting in a confirmed growth of an extensive vascular system. The 3D printed collagen scaffolds made through FRESH can then be seeded with the cardiomyocyte derived stem cells to develop ventricles, achieving a functional ventricle within the heart (Lee et al., 2019). FRESH has developed a proven concept with the ability to mimic the structure, mechanics, and biologic features of tissue and has built a foundation that opens more possibilities for whole 3D bioprinted functional organs . Even with the extensive improvements of FRESH’s cardiac bioengineered techniques, difficulties arise with amassing billions of cells for a density rich organ has given light to new research methods that mimic the cellular dense microenvironment furthermore (Lee et al., 2019).

Heart failure patients and those requiring heart transplants need to find alternative ways to generate whole organs. Organ transplants are scarce and often face an immune rejection response from the host receiver. In addition, the fabrication of a whole organ proves extremely difficult, requiring billions of functionally organized cells that are supplied with nutrients through an extensive vascular system. Work relying on sacrificial writing into functional tissue (SWIFT) has addressed the lack of cellular density and microstructural complexity. This method uses a mix of cardiac organ building blocks (OBB) within an extracellular matrix (ECM) composed of collagen and Matrigel to create an ideal ECM for creating perfusable vascular channels in tissues with the required cellular density for organ engineering. This solution, which is cooled, can then be casted with gelatin bioink that is eventually filled within a tissue matrix that develops into complicated and viable vascular channels. SWIFT can embed vascular channels into living matrices composed of an array of OBBs. To exemplify this, SWIFT performed three experimentals, but in specific to cardiac regeneration, they created a functional and perfusable tissue with human iPSC-derived cardiac OBBs using a suspension culture from EBs to generate highly efficient cardiomyocytes using the protocol described previously in this paper (Skylar-Scott et al., 2019). Conclusively, the compacted cardiac matrix had an estimated cell density of 240 million cells/ml and a cardiomyocyte density of 180 million cells/ml. In adding a branching channel within the matrix, the cardiac tissue progressively increased in synchronized beating along with spontaneous contraction. This work was then translated into creating cardiac spheroids which serve as organ building blocks for tissue development mimicking the cellular density and complicated perfusable channels exhibited by organs in vivo. This work done by SWIFT has allowed for personalized organ development with the possible scalability of in vivo organs’ cellular density and complicated micro vascularization (Skylar-Scott et al., 2019).

Limitations

Cardiomyocyte differentiation protocols have been shown to be ~95% efficient, however generating large volumes of cardiomyocytes remains difficult. For this, bioreactors have been developed that aid in generating large volumes of viable cardiac cells. Furthermore, methods such as FRESH and SWIFT have addressed the difficult task of creating perfusable vascular channels. There is also a need to account for the lacking maturity of generated cardiomyocytes which in comparison to adult cardiac tissue, have a ~1:20% contractile strain ratio . Work has been done to increase maturity including electrical stimulation, and different chemical signaling molecules, which have been shown to increase cell maturity, and in turn, further aid in contraction of the cardiomyocytes. Lastly, as work continues in this field, researchers are finding new methods of developing cardiac patches and whole organ-scale models for regenerative medicine purposes.

Conclusion and Future Directions

The prevalence of heart disease is as concerning as ever, affecting every 1 in 5 Americans, and contributing as the leading cause of death worldwide. Cardiac tissue engineering has brought different bio engineered techniques to prevent and aid in regeneration of the cardiac tissue. To begin any process, there are generated cardiomyocytes from iPSCs which use the Wnt signaling pathway through small signaling molecules in the matrix, yielding cardiomyocytes with high efficiency. These cardiomyocyte derived iPSCs can synchronously beat with spontaneous contractions, allowing for regeneration of the adult cardiac tissue that has been irreversibly damaged. The clinical applications currently being explored include cardiac patches and cardiac bioprinting. Concerning superficial damage from phenomena such as myocardial infarction, cardiomyocytes can be loaded onto a hydrogel-like patch to be sutured onto the damaged area, aiding in revival of contracting tissue. With larger scale damages or abnormalities, bioprinting whole organs or organ parts have been researched through techniques such as FRESH and SWIFT, mimicking a microenvironment that promotes vascularization along with supporting the difficult scale of cell density. Currently, researchers are consistently exploring ways to increase the complicated functions of perfusable vascular channels along with the efficiency of maturing cardiomyocytes. Moreover, the clinical applications for these methods are being explored, aiming for consumer availability to improve many heart disease affected patients.

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