Tampere University

Malak Abdel Hafez

MATERIALS USED IN ENGINEERED HEART TISSUES

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Supervisor: Mari Pekkanen-Mattila
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Abstract

Malak AbdelHafez: Materials Used in Engineered Heart Tissue
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Cardiovascular-related diseases are the leading cause of death globally. The treatment methods used in treating cardiovascular diseases like heart transplantation have limitations like donor shortages and risk of rejection. This demand for new treatment is high. A possible treatment option is using cardiomyocytes derived from human induced pluripotent stem cells (hiPSCs-CM) in engineered heart tissue or EHTs to repair the heart.

This study aims to review and explore the effectiveness of different materials used in the EHTs by comparing the results yielded by articles and research in that field.

The findings demonstrated that incorporating more than one cell type and material in the EHTs improved cardiac function, infarct size, and remuscularization. Incorporating mechanical and electrical stimulation greatly impacted the EHTs, resulting in better alignment of the cells, more efficient electrical signaling, and a stronger, more coordinated contraction of the tissue.

While EHTs hold promise for treating cardiovascular diseases, several challenges need to be addressed. These include the maturation phenotype of hiPSC-CMs and the safety of hiPSC-derived tissues. As well as improving the scalability and reproducibility of EHT production and ensuring the long-term viability and integration of transplanted tissues within the patient's heart. Additionally, understanding the immune response to hiPSC-derived tissues and mitigating the risk of arrhythmias are crucial areas for future investigation. Overall, while significant progress has been made, the transition from experimental models to widespread clinical use necessitates continued innovation and collaboration across multiple disciplines. Further research is still needed to develop safe and effective treatments that can provide long-term solutions for cardiovascular disease. The development of new biomaterials and the improvement of cell technologies can significantly affect the development of EHT technology and its clinical use in the future.

Keywords: Engineered heart tissue, Human Induced pluripotent stem cells, cardiovascular diseases, heart tissue, bioengineering

TIIVISTELMÄ

Malak AbdelHafez: Muokatussa Sydänkudoksessa käytetyt Materiaalit
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Sydän- ja verisuonitaudit ovat johtava kuolinsyy maailmanlaajuisesti. Sydän- ja verisuonitautien hoitomenetelmillä, kuten sydämensiirrolla, on rajoituksia, kuten luovuttajapula ja hylkimisriskit. Uusien hoitomenetelmien tarve on suuri. Yksi mahdollinen hoitovaihtoehto on käyttää ihmisen pluripotenteista kantasoluista peräisin olevia kardiomyosyyttejä (Human induced pluripotent stem cells derived cardiomyocytes (hiPSCs-CM)) muokatuissa sydänkudoksissa (Engineered Heart tissue) tai EHT:itä sydämen korjaamiseen.

Tämän tutkimuksen tavoitteena on tarkastella ja tutkia eri materiaalien tehokkuutta EHT:ssa vertailemalla alan artikkelien ja tutkimusten tuloksia. Tulokset osoittivat, että useamman kuin yhden solutyypin ja materiaalin yhdistäminen EHT:issä paransi sydämen toimintaa, infarktialueen kokoa ja uudelleenlihastumista. Mekaanisen ja sähköisen stimulaation sisällyttäminen vaikutti yleisesti EHT:ihin, mikä johti solujen parempaan linjaus, tehokkaampaan sähköiseen signaaliin ja vahvempaan, koordinoidumpaan kudoksen supistumiseen.

Vaikka EHT:t lupaavat sydän- ja verisuonitautien hoidossa, useita haasteita on käsiteltävä. Näitä ovat hiPSC-CM:iden kypsymisfenotyyppi ja hiPSC-peräisten kudosten turvallisuus. Se myös parantaa EHT-tuotannon skaalautuvuutta ja toistettavuutta sekä varmistaa implantoidun kudoksen pitkän aikavälin elinkelpoisuuden ja integroitumisen potilaan sydämeen. Lisäksi immuunivasteen ymmärtäminen hiPSC-peräiselle kudokselle ja rytmihäiriöriskin vähentäminen ovat tärkeitä osa-alueita tulevalle tutkimukselle. Kaiken kaikkiaan merkittävästä edistymisestä huolimatta siirtyminen kokeellisista malleista laajaan kliiniseen käyttöön vaatii jatkuvaa innovaatiota ja yhteistyötä useiden tieteenalojen välillä. Tarvitaan edelleen lisätutkimusta, jotta voidaan kehittää turvallisia ja tehokkaita hoitomuotoja, jotka pystyvät tarjoamaan pitkäkestoisia ratkaisuja sydän- ja verisuonitautien hoitoon. Uusien biomateriaalien kehittäminen ja solutekniikoiden parantaminen voivat merkittävästi vaikuttaa EHT-teknologian kehittymiseen ja sen kliiniseen käyttöön tulevaisuudessa.

Avainsanat: Muokattu sydänkudos, ihmisen aiheuttamat pluripotentit kantasolut, sydän- ja verisuonisairaudet, sydänkudos, biotekniikka

PREFACE

This thesis project aims to compare different materials used in engineered heart tissue constructs and provides an overview of the field of engineered heart tissue.

I would like to thank my supervisor Mari Pekkanen-Mattila for being so patient and guiding me though the process. I would also like to thank my friends and family for their continued support.

Tampere, 20 August 2024

Malak AbdelHafez

CONTENTS

ABBREVIATIONS

EHTs	Engineered heart tissue
hiPSCs	Human induced pluripotent stem cells
hiPSC-CMs	Human induced pluripotent stem cells derived cardiomyocytes
iPSCs	Induced pluripotent stem cells
hCMPs	Human cardiomyocyte patches
hiPSC-COs	Human induced pluripotent stem cells derived cardiomyocyte organoids
hESC-CMs	Human embryonic stem cell derived cardiomyocytes
PGA	Polyglycolic acid
PLCL	Poly(l-lactic-co-ε-caprolactone)
PLGA	Poly(D,L-lactic-co-glycolic acid)
CM	Cardiomyocytes
PC	Pericytes
MC	Mural cells
EC	Endothelial cells
LV	Left ventricle
ECM	Extracellular matrix

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1. INTRODUCTION

Cardiovascular diseases (CVDs) are the leading cause of death worldwide, accounting for approximately 17.9 million deaths annually (World Health Organization). These diseases include a variety of conditions, including coronary heart disease and cerebrovascular disease, which significantly impair the function of the heart and its associated vasculature. Among the most severe manifestations of CVDs is myocardial infarction (heart attack), where the sudden blockage of coronary arteries results in the death of cardiac muscle tissue (Cleveland Clinic). The human heart, characterized by its limited regenerative capacity, struggles to repair such damage, often leading to permanent functional deficits (Li et al., 2022). Traditional treatment options such as medications, coronary artery bypass grafting, and heart transplants, while effective, are associated with significant risks and limitations, including donor shortages and immune rejection in transplant cases (Tonsho et al., 2014).

Recent advancements in heart treatments involve innovative approaches in regenerative medicine, bioengineering, and molecular therapy. Stem cell therapy aims to use stem cells to regenerate damaged heart tissue (Harvard health). Gene editing techniques like CRISPR-Cas9 are being explored to correct mutations linked to heart diseases (Motta et al., 2017). Exosome therapy uses vesicles to transfer beneficial molecules to damaged heart cells (Loyer et al., 2018). Another advancing and suggested method of addressing this issue is engineered heart tissues (EHTs). EHTs are bioengineered constructs designed to replicate the structure and function of natural heart tissue, with the aim of repairing or replacing damaged areas of the heart (Zhang et al., 2022). These tissues are composed of two main components: cardiomyocytes (CMs), which are the cells responsible for heart muscle contraction, and a supportive scaffold that provides the necessary structure for tissue development and recovery (Majid et al., 2020). By closely mimicking the environment in which native heart tissue operates, including the application of mechanical forces that promote cell maturation and function, EHTs offer a promising approach to cardiac repair. Beyond their potential in heart regeneration, EHTs also serve as valuable tools in cardiac disease modeling and drug testing, providing to mimic the arrangement and function of a natural heart tissue than traditional methods (Goldfracht et al., 2020).

This thesis explores the components, production processes, and clinical applications of EHTs, with a focus on the use of human induced pluripotent stem cells or hiPSCs as a source of cardiomyocytes. By examining the materials used in scaffold construction, the methodologies employed in cardiac tissue engineering, and the outcomes of EHT transplantation studies, The goal of this research is to offer an extensive overview of the potential of EHTs in treating cardiovascular diseases. Furthermore, it delves into the challenges associated with the maturation of hiPSC-derived cardiomyocytes (hiPSC-CMs) and the importance of creating a biomimetic environment that closely replicates the native extracellular matrix (ECM) of the heart. Through this exploration, the thesis seeks to contribute to the ongoing development of EHTs as a viable therapeutic option for patients suffering from severe cardiac conditions.

2. EHTS AND THEIR APPLICATIONS

The heart is a muscular organ with four chambers: the left ventricle (LV) and the

right ventricle (RV). The atria are known as the right and left atriums, respectively. Blood returns to the heart through the atria, with oxygen-poor blood from the body going into the right atrium through the superior and inferior vena cavae and oxygen-rich blood from the lungs going into the left atrium through the pulmonary veins. Blood is pumped from the heart by the ventricles: the pulmonary artery carries blood from the right ventricle to the lungs, and the aorta carries blood from the ventricle to the rest of the body. The myocardium, which is the middle muscle layer, the epicardium, which is the outside layer, and the endocardium, which is the inner layer, make up the heart wall. The myocardium contains cardiomyocytes, which are responsible for the contractile function of the heart. Endothelial cells (EC) line the blood vessels and the interior of the heart chambers, while fibroblasts contribute to the ECM and structural integrity (Cleveland Clinic). This process is shown in Figure 1 below. Additionally, the heart contains specialized cells such as pacemaker cells in the sinoatrial node that regulate heart rhythm (Burkhard et al., 2017), and vascular smooth muscle cells in the coronary arteries that control blood flow (Uchida, 2012).

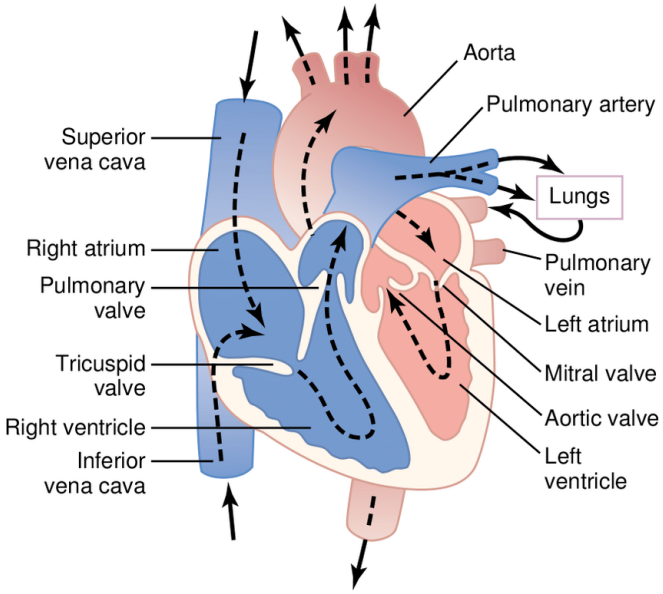

Figure 1: illustration depicting blood flow pathway through the heart Figure extracted from Guyton & Hall "Textbook of Medical Physiology" (Saunders, 2000).

Cardiovascular diseases are a group of disorders that impact the heart and its vessels, they include coronary heart diseases – a disease of the blood vessels supplying the heart muscle and cerebrovascular diseases – a disease of the blood vessels supplying the brain. These diseases can have huge impacts on the health of patients. For instance, coronary heart diseases cause myocardium infarctions, which is a sudden blockage in the arteries that cuts off oxygen to part of the heart's muscle causing it to die (Cleveland Clinic). Since the adult human heart lacks regeneration potential, any damage or defect to the heart is permanent and the heart does not have the ability to fix or heal itself (Li et al., 2022). Cardiovascular diseases considered the leading cause of death globally taking an estimated 17.9 million lives every year (World Health Organization). This issue gives rise for innovative treatment other than traditional methods like bypass surgery, pacemakers, and medications, which all come their own set of risks. Patients with a genetic heart defects or heart diseases like the ones mentioned above may require heart transplants (UPMC), which has many limitations, like donor shortages and risks of recipient rejections after transplantation (Tonsho et al.,

2014).

2.1 EHTs and Their Components

EHTs are bioengineered constructs that mimic the structure and function of natural heart tissue (Zhang et al., 2022).

They are developed by combining cardiomyocytes, which can be derived from stem cells, with supportive structures like scaffolds made of biomaterials. Biomaterials are natural or synthetic materials used to support, enhance or replace a biological function (Majid et al., 2020). Growth hormones can also be used in EHTs to promote proliferation, the growth of tissue cells (Burdick & Vunjak-Novakovic, 2009). EHTs should be designed to provide electrical and mechanical support to the heart. They also must be biocompatible to reduce the risk of immune rejection by the host, have appropriate mechanical properties to withstand the dynamic environment of the heart and support the formation of new blood vessels (Udriște et al., 2023). Figure 2 demonstrates the construction and arrangement of EHT one a recipient's heart.

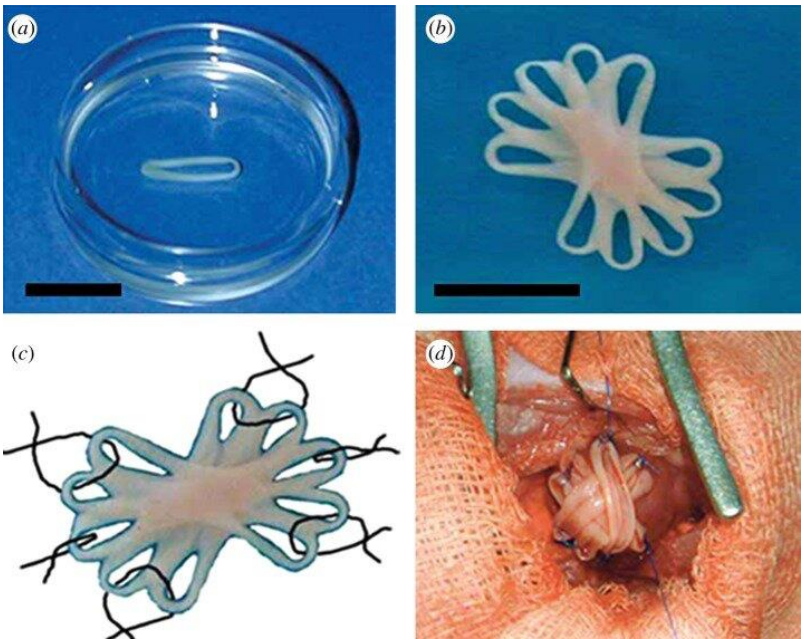

Figure 2: Developing EHTs. After stacking five single EHTs, the multi-loop EHTs (a) contracted synchronously, (b) were prepared for in vivo engraftment, and (c,d) were fixed on the recipient's heart using six single-knot sutures. (Zimmermann et al., 2006)

The end goal is to be used in applications like drug testing, disease modeling, and in the treatment of heart diseases. The creation of EHTs involves several key steps: differentiation of hiPSCs into cardiac or heart cells (which is the process in which the cells transition into cardiac cells), selection of suitable biomaterials, and the assembly of these components. (Mei et al., 2020)

The ECM in the heart is a network of proteins and sugars including collagen, elastin, fibronectin, and glycosaminoglycans, which collectively provide structure, function, and adaptability. The general architecture of the ECM is displayed in Figure 2. The ECM is crucial for maintaining cell growth, survival, spreading, proliferation, differentiation (Valiente-Alandi et al., 2017). In the context of EHTs and the selection of biomaterials for cardiac scaffolds, it is essential to replicate the ECM's composition and mechanical properties to ensure effective integration and function of the engineered

constructs. Biomaterials used in these scaffolds are often designed to include ECM-like proteins or peptides, such as collagen or fibronectin, which bind to proteins found on the surface of cells and mimic the natural ECM environment, thus enhancing cell attachment, proliferation, and tissue formation (Majid et al., 2020).

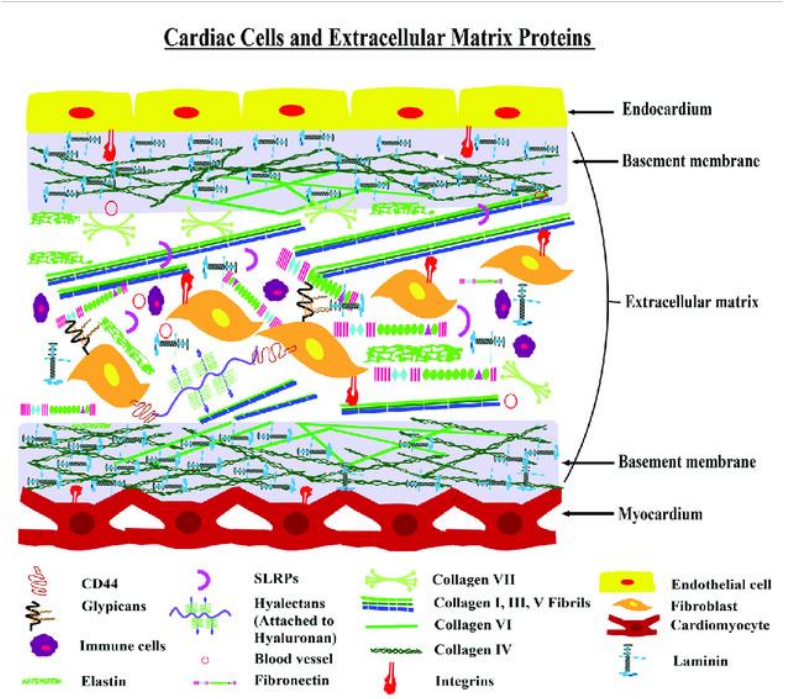

Figure 2: Fibroblasts and ECM proteins like collagen, elastin, and fibronectin are present in the heart's ECM. Additionally, several inflammatory cells are present in the interstitial matrix. (Sarohi et al 2022)

The biomaterials used in scaffolds must meet several key requirements: Biocompatibility, ensuring they are non-toxic and do not elicit an immune response; Biodegradability, allowing the material to degrade gradually as the cells form their own ECM, which eliminates the need for surgical removal; Mechanical properties that mimic those of native heart tissue to withstand the dynamic environment of the heart. Scaffolds should be sturdy enough to allow for surgical handling during surgery, and their mechanical qualities should match the anatomical site where they are to be implanted (Prasadh et al., 2018); Porosity, providing an interconnected structure for nutrient and oxygen diffusion. Cell migration and development within the scaffold depend on the interconnection of its pores. Small pores may restrict cell movement, and pore size may have an impact on adherence of cells (Murphy et al., 2010). (Jang et al., 2022)

Hydrogels are networks of three-dimensional polymers that can hold a lot of water without losing their structural integrity. These materials are composed of hydrophilic polymers that can swell and retain significant volumes of water, making them soft and flexible, like natural cardiac tissue. The polymers in hydrogels can be natural like gelatin, collagen, and alginate, synthetic like polyethylene glycol (PEG) and polyacrylamide, or a hybrid of both (Thang et al., 2023). Hydrogels are created through various polymerization processes, including free-radical polymerization, ionic gelation, and chemical cross-linking. These processes involve the formation of cross-links between polymer chains, creating a network that traps water molecules. The degree of cross-linking can be controlled to tune the physical properties of the hydrogel, such as its mechanical strength, porosity, and degradation rate (Slaughter et al., 2009).

They are often used as scaffolding materials in EHTs because of their biocompatibility, tunable mechanical properties, and capacity to replicate the heart's extracellular matrix. Bioactive substances like growth factors and peptides can be added to hydrogels to encourage cell adhesion, proliferation, and differentiation. (Burdick & Vunjak-Novakovic, 2009). These scaffolds provide a supportive environment for cardiac cells, facilitating the formation and organization of functional cardiac tissue. This biomimicry is crucial for the proper alignment and function of cardiomyocytes, leading to improved contractility and overall tissue performance (Zhang et al., 2013). Additionally, the hydrophilic polymers that made up hydrogels can be engineered to dissolve over time, allowing for gradual integration of the engineered tissue with the host tissue and promoting natural tissue regeneration (Rogers et al., 2014).

Human Pluripotent Stem Cells

In the area of regenerative medicine, pluripotent stem cells are a type of stem cells that have promising results. This is as a result of their ability for endless self-renewal and cell differentiation into every kind of human cell (Moradi et al 2019). The ability of the other two forms of stem cells, known as multipotent and unipotent, to differentiate into distinct tissues or cell types is limited, whereas pluripotent stem cells can give rise to any type of cell (Romito et al., 2016). Figure 4 depicts iPSC s before differentiation. These characteristics make pluripotent stem cells valuable tools for studying development and disease modeling.

One branch of pluripotent stem cells is hiPSCs. They were first discovered by Takahashi and Tamanaka in 2007 and are made by reprogramming adult somatic cells using a combination of transcription factors to a pluripotent state. Another branch of pluripotent stem cells is embryonic stem cells (ESCs), which are derived from the inner cell mass of the blastocyte-stage embryos ~~or~~ around 6 days after fertilization (Hassani et al., 2019). HiPSCs are ethically favorable since the process does not involve the destruction of embryos and are a patient-specific alternative to ESCs, though they may come with challenges related to genetic stability and consistency. The genetic instability of hiPSCs is linked to (i) pre-existing differences in the parental somatic cells, which can be revealed during the creation of iPSCs; (ii) mutations generated by reprogramming, and (iii) mutations which appear during the prolonged culture. While ESCs are potentially immunogenic, they also provide a highly reliable source of pluripotent stem cells since they do not undergo the stress of the reprogramming process. (Chehelgerdi et al., 2023)

The potential applications of pluripotent stem cells are vast. They offer opportunities for personalized medicine since the hiPSCs will be taken from the patient and as a result, can be differentiated into the desired cell type for transplantation, potentially over- coming immune rejection issues (Takahashi et al., 2007). Furthermore, pluripotent stem cells serve as invaluable tools for disease modeling, allowing researchers to study the mechanisms of various genetic disorders and develop novel therapeutic interventions by provide them with models to be tested on without the complications of using animals or human trials. (Andrysaik et al., 2021).

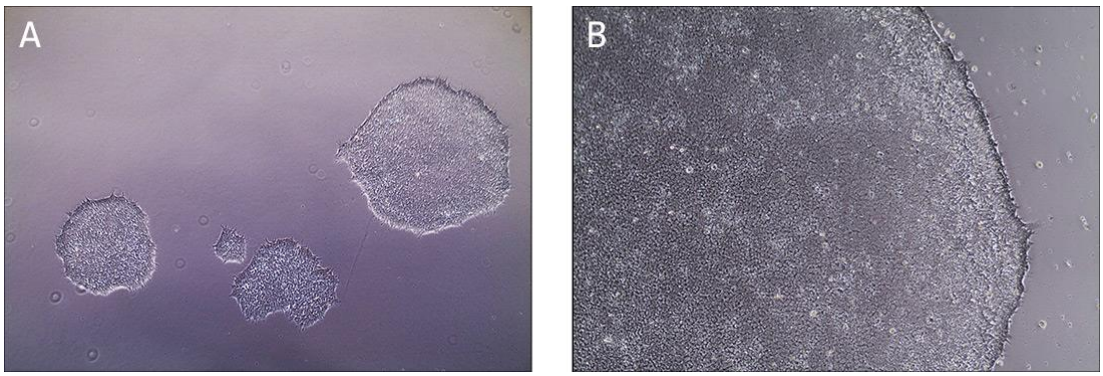

Figure 4: Undifferentiated iPSCs culture on dish. (ATCC)

The differentiation protocols of hiPSC-CMs

Differentiation of hiPSCs into functional cardiomyocytes is crucial for effective cardiac tissue creation. A protocol introduced by Lian et al. in 2012 efficiently directs hiPSCs to functional cardiomyocytes through temporal modulation of canonical Wnt signaling regulators in a growth factor-free and serum-free method, resulting in a high yield of cardiomyocytes of all subtypes however, mainly vantricular. The differentiation process typically lasts around 14-20 days as shown in figure 5. To achieve more accurate drug responses and improve therapeutic effects, it is important to use tissue-specific cells and promote their differentiation (Li et al., 2020). A recently developed protocol efficiently generates ventricular-like CMs with over 90% purity on a large scale using stirred tank bioreactor systems and continuous chemical WNT pathway control in early differentiation stages (Halloin et al., 2019). Significant advancements have been made in differentiating hiPSCs into atrial CMs. Retinoic acid has been used effectively to promote atrial cell differentiation through developmental signaling gradients (Li et al., 2020). Zhao et al. outlined a scalable tissue-cultivation platform with chamber-specific gene expression and drug responses that can electrophysiologically distinguish atrial and ventricular tissues. (Zhao et al., 2019). Various protocols involve co-expression of specific genes, activation of key signaling pathways, and modulation with specific factors to enhance pacemaker cell population (Li et al., 2020). This was done via developing cardiac pacemaker cells by combining specific genes from chickens and minks. By expressing certain genes, some heart precursor cells turned into pacemaker cells and adding activators during cell developments further increases the number of thesis pacemaker cells. (Li et al., 2020)

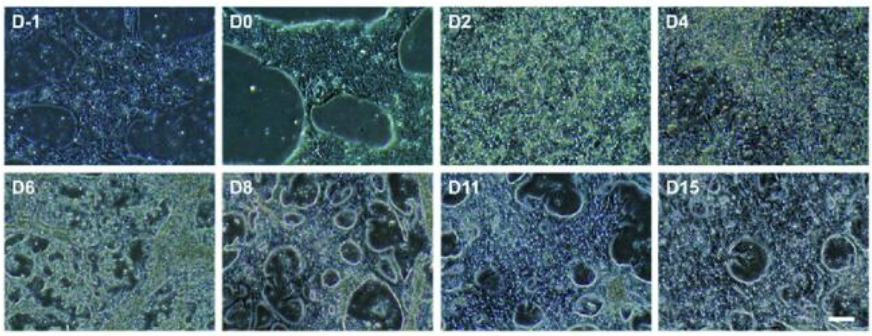

Figure 5: Heart differentiation in hiPSC-CMs during a 15-day period. In D-1: Cells appear as dense, compact colonies typical of undifferentiated stem cells. D2 and D4 show early differentiation begins, with cells starting to spread out. D6 and D8: Cells begin forming more organized, cardiac-like structures, with some areas potentially showing early beating. D15 shows cells exhibiting mature cardiomyocyte characteristics, including

organized, striated fibers and synchronized beating activity. (Reinal et al., 2023)

The Importance and Impact of Scaffolds in EHTs

One major issue is that hiPSC-CMs often exhibit an immature phenotype where they typically resemble fetal or neonatal cardiomyocytes in terms of structure, function (Knight et al., 2021). Conduction velocity is the speed at which an electrical impulse travels through the heart. Immature hiPSC-CMs exhibit slower conduction velocity and reduced muscle fiber organization with underdeveloped contractile properties**.** The scaffold relies on the proper integration of electrical signals and the slower conduction velocities hinders this integration, leading to a reduction in replicating the coordinated beating of the heart. In addition, underdeveloped contractile properties in immature hiPSC-CMs affect the scaffold's structural integrity and durability. Mature cardiomyocytes can exert and withstand significant mechanical forces during contraction, but immature cells lack this capability and could experience degradation over time. (Yang et al., 2014)

Factor playing a major role in successful modeling of a functional EHT is to simulate the conditions of the adult human heart, which includes the functional harmony of different heart cell types, complexity of the ECM, and the specific microenvironment that regulate the maturation of cardiomyocytes (Santos et al., 2021).

Cardiac patches made of polyglycolic acid (PGA) and a poly(l-lactic-co-ε-capro-lactone) copolymer (PLCL) (Sugiura et al., 2016) and poly(D, L-lactic-co-glycolic acid) (PLGA) nanofibers (Li et al., 2017) were tested in animal models. They found that these biodegradable patches promoted the regenerative proliferation of cardiomyocytes in the host heart. They facilitated the maturation and organization of cardiomyocytes into functional tissue constructs, with the constructs closely mimicking native cardiac tissue. Gao et al. (2017) utilized multiphoton-excited 3D printing to create a native-like ECM scaffold, which was then seeded with cardiomyocytes, smooth muscle cells, and endothelial cells to form Human cardiomyocyte patches (hCMPs). Four weeks after the treatment, the hCMPs showed a significant improvement in heart function, reduced the size of damaged heart tissue (infarct), and increased the density of blood vessels and small arteries. Additionally, they promoted the growth of new cells. These results highlight the potential benefits of using 3D printing technology in combination with cell therapy to effectively repair and regenerate damaged heart tissue.

Maturation of hiPSC-CMs

CM maturation is essential for effective cardiac function, and several approaches are employed to achieve this in vitro. Prolonged culture times can induce some aspects of CM maturation, such as larger size and an improved ability to regulate the calcium concentrations in the cells, known as calcium handling. However, this approach is time-consuming and unable to induce T-tubules, which are tunnels in muscle cells that help with spreading electric signals (Kamakura et al., 2013). Biochemical signals released cells, including hormones and insulin play crucial roles in CM maturation (Laustsen et al., 2007). Extracellular matrices produced by non-CMs can mimic the in vivo environment and provides mechanical support for cardiomyocytes, facilitating cellular organization and maturation (Yoshida et al., 2018). Co-culture systems involving non-CMs such as endothelial cells are important for enhancing CM maturation (Yoshida et al., 2018). 3D culture systems, including EHTs, better replicate in vivo conditions compared to two-dimensional cultures, improving CM maturation through enhanced cell-cell interactions and mechanical loading (Ewart et al., 2017).

Tulloch et al.'s (2011) study examined how immature human myocardium using human embryonic stem cells, and hiPSC-CMs, respond to mechanical stress and vascularization in immature heart tissue. They discovered that applying mechanical stress in one direction greatly increases the number cardiomyocytes and improves the matrix fiber alignment which is the orientation of the fibers in the ECM. This alignment helps in forming the necessary proteins in the heart cells and organizing them into structured patterns, known as sarcomeric banding, which is crucial for proper heart muscle function. Cyclic stress conditioning further promoted cardiomyocyte enlargement and proliferation rates. Aiming to enhance cardiac tissue constructs' viability and functional maturation through cyclical stretching, Mihic et al. (2014) observed that stretched constructs had a higher proportion of cardiac troponin T-expressing cells, which have a more mature phenotype with longer cells, and this protein is important in muscle contraction. Additionally, they observed that the contractile elements were more developed, and that gap junction expression was elevated. Stretched structures contracted more frequently, as imaging revealed. This was done by seeding Human embryonic stem cell-derived cardiomyocytes (hESC-CMs) onto gelatin-based scaffolds and subjecting them to cyclical stretching.

2.2 EHT Production Process

The liquid collagen type I prepared from rat tails, the concentrated serum-containing culture medium, and Matrigel were combined with the cardiomyocytes derived from embryonic stem cells. Titrating with NaOH neutralized the pH. The reconstitution mixture was pipetted into casting molds and incubated to allow it to firm. There was culture medium including serum in each dish. After seven days in culture, EHTs were put into a modified stretch apparatus and attached on the working rods for an additional seven days of unidirectional stretch. Four glass columns could give a static stretch. The EHT beat steadily and unidirectionally in the direction of stretching after seven days of mechanical stretching. (Lü et al., 2010)

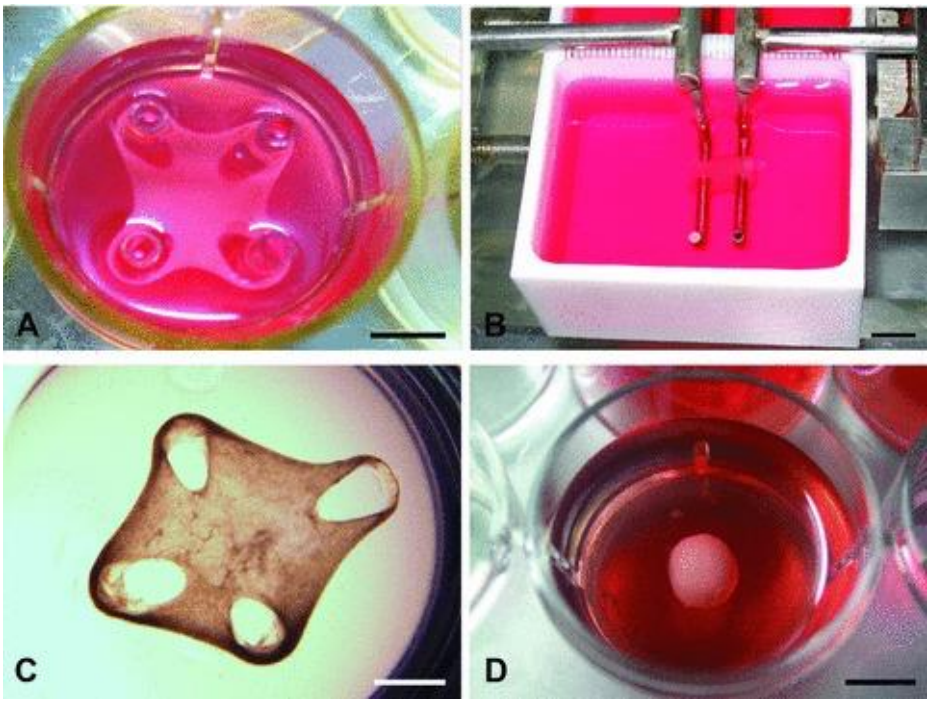

Figure 6: (A) After one day of culture, the EHTs gradually contracted and condensed. (B) EHTs stretched mechanically. (C) Microscopic image of an EHT that is contracting on its own (D) In seven days, the EHTs without static stretching will constrict into structures resembling contact lenses. (Lü et al., 2010)

2.3 EHT Testing on Animal models

EHTs from hiPSC-CMs can be made with a variety of materials. Wendel et al. created the patches from a mixture of cardiomyocytes and human pericytes (PCs) which are cells that wrap around the endothelial cells, embedded in a fibrin gel. At the same time, Shadrin et al utilized hydrogen modeling to achieve the resulting patches. After implantation into rat hearts, both studies showed improved cardiac function, successful integration, and proliferation. The new grafts also formed new blood vessels and robust electric coupling. Querdel et al. (2021) also developed cardiac tissue patches with a mesh structure and were transplanted into pigs and guinea pigs. This study showed the remuscularization was dose-dependent, with high-dose patches having an improved function of LV.

Significant remuscularization and a notable 30% improvement in cardiac function were found in a study using EHT strips from hiPSC-CMs and endothelial cells. These EHTs, transplanted onto large defects in guinea pig hearts, formed new blood vessels and established electrical coupling with host myocardial tissue. This demonstrates the potential to restore muscle and improve electrical and vascular integration (Weinberger et al., 2016). In a 2012 study, Miki et al. created a cardiac patch using mouse iPS cells and assessed cardiac function in a rat model of chronic myocardial infarction after the patch was implanted. According to the findings, the patch implantation greatly enhanced cardiac function and reduced cardiac remodeling, pointing to a possible heart failure treatment.

In contrast, Kensah et al (2012) expanded on this by using highly purified murine and hiPSCs-CMs to generate functional bioartificial cardiac tissue. They employed a collagen-based scaffold and identified three critical factors that cooperatively improve tissue formation and function: fibroblast addition, ascorbic acid supplementation, and increased static stretch.

The design requirements for EHTs resulting from hiPSCs can be carefully explored using computational modelling and microfabricated limitations. Thavandiran et al. (2013) have shown that a carefully chosen combination of a 3D matrix-based environment, uniaxial mechanical stress, and a mix of cardiomyocytes and fibroblasts can significantly enhance tissue performance and maturation by optimizing the design of aligned and functional 3D cardiac microtissues.

Developing a cell sheet-based technology by layering myocardial cell sheets derived from hiPSC-CMs without any scaffolding and transplanting them onto subcutaneous tissues of nude rats exhibited autonomous beating and a cardiac muscle-like structure with well-developed vascular systems (Komae et al., 2015). While Komae et al. used a cell sheet-based approach, Kawai et al. (2022) took another direction in constructing scaffold-free tubular EHTs using 3d structures derived from stem cells composed of multiple cardiac cells called hiPSC-CMs organoids (hiPSC-Cos) and bio-3D printing technology. There tubular EHTs, transplanted around mice's abdominal aorta and inferior vena cava, demonstrated superior in vivo maturation compared to hiPSC-

Cos, presenting an innovative method for creating highly mature and functional cardiac tissue.

Incorporating cardiomyocytes, endothelial cells, and cells located on walls of blood vessels called vascular mural cells (MC) derived from hiPSCs promoted functional maturation, myocardial replacement, and blood delivery to tissue highlighting the importance of recreating the complex cellular environment of the heart for effective cardiac tissue development (Masumoto et al., 2016). After inducing myocardial infarction in immune tolerant rat's hearts, the EHTs were implanted with different cell compositions to determine which combination of cells yields the best result. It was found that a combination of CM, EC, and MC resulted in better maturation and exhibits perfusion and myocardium replacement.

Meanwhile, Kawamura et al. (2012) explored the feasibility, safety, and therapeutic effectiveness of hiPSC-CM sheet transplantation for ischemic cardiomyopathy. They created hiPSCs-CM sheets using thermoresponsive dishes by infecting cells found in human skin with a retrovirus to establish human iPS cells and inducing cardiomyogenic differentiation via WNT signaling molecules. Transplantation of these sheets over myocardial infarcts in animal models showed improved cardiac function.

3.6 Analysis of Key Findings

This section delves into the comparative analysis and results of various studies that employed different materials and methodologies in cardiac tissue engineering, focusing on creating EHTs and their transplantation outcomes. By examining these studies' structural and functional results, we gain insights into the efficacy and potential of different approaches in cardiac repair.

3.6.1 Comparative Results

EHTs made with hiPSC-CMs supplemented with human brain PCs in a fibrin gel showed better LV ejection fraction (the percentage of blood pumped out of the LV and fractional shortening, which is the percentage of size reduction of the LV in systole (Clarius, 2022) compared to its control patch made with PCs only. The CM+PC group had denser CM on the surface and higher density of small blood vessels in an area of tissue. There was also a reduced region of scar tissue formed after heart muscle death due to lack of blood supply, in the CM+PC group and after 4 weeks, scar tissue was developed while the PC only group did not survive for 4 weeks in vivo. Patches made of hiPSC-CMs and hiPSC- derived endothelial cells using fibrinogen and thrombin were compared to cell-free fibrin grafts and engineered tissue made of endothelial cells only. Echocardiography assessment is a diagnostic tool that allows for the assessment of the structure and function of the heart. It showed that LV function improved in EHT group and remained unchanged in endothelial and cell-free group. There was also integration of the EHTs with the development of scar tissue, showing remuscularization in EHTs group with 12% of infarct area, while the endothelial and cell-free group showed no improvement in infarct size.

Using a multiphoton-excited 3D printer to create a native-like scaffold seeded with cardiomyocytes, smooth muscle cells, and endothelial cells in a 2:1:1 ratio along with photoactive gelatin methacrylate Gao et al found that there significantly higher LV ejection fraction and fractional shortening in the treated group with the 3d printed seeded scaffold than the unseeded scaffold. Additionally, the seeded group had reduced infarct size, greater infarcted region thickness, and increased small artery density. The number of cells undergoing a programmed death was significantly fewer in the border of the infarcted site in the treated group. The patches were positioned over the infarction site, leading to substantial cardiac function and structural integrity improvements compared to the control group.

Using a cardiac patch with a biodegradable scaffold composed of PGA and PLCL copolymer, reinforced with a woven fabric of polyglycolic acid and seeded with commercially obtained hiPSC-CMs, echocardiographic measurements indicated that the cell- seeded patches promoted regenerative proliferation of host cardiomyocytes, with a significant increase in positively stained markers for cardiomyocytes 16 weeks post-implantation showing substantial improvements in myocardial regeneration compared to the unseeded group. However, there was no statistical difference in the LV maximum and minimum diameters and LV ejection fraction at any time. Suggesting that the biodegradable patches created by Sugiura et al made no difference in the LV function after implantation.

Masumoto et al. (2016) created patches using a mixture of collagen type 1 and Matrigel, including hiPSC-CMs, EC, and vascular MC. All groups showed improved left

ventricular function and thick myocardium formation four weeks post-transplantation, with enhanced maturation and blood delivery to tissue in the transplanted tissues. The patches were implanted in myocardial infarction-induced rat hearts where the CM+EC+MC group showed the most significant cardiac function and tissue maturation improvements.

Comparatively, biodegradable scaffolds and 3D-printed native-like ECM scaffolds provided excellent environments for cell growth and integration, while fibrin-based constructs facilitated initial cell entrapment and ECM deposition but showed varied long-term viability. Studies incorporating multiple cell types, such as Gao et al. and Masumoto et al., demonstrated better functional and structural outcomes, underscoring the importance of a diverse cellular environment. Including endothelial and vascular cells appeared crucial for improved vascularization and myocardial regeneration. Echocardiographic assessments consistently showed improved left ventricular function and reduced infarct sizes in treated groups, with studies highlighting the benefits of electrical coupling and increased microvessel density.

3.6.2 Results of in vitro measurements

In vitro measurements of EHTs taken by Gao et al in 2017 showed that a native-like scaffold seeded with cardiomyocytes, smooth muscle cells, and endothelial cells has begun generating an increase in calcium concentrations in the cell called calcium transients. Seven days after the cells were seeded into the scaffold, there was a considerable rise in the expression of many genes necessary for contractile function, as well as in the maximum amount of calcium transients and contraction and relaxation speeds. Excellent functional electrophysiological communications were also seen between the cells, and the autofluorescence image demonstrated that the cells had aligned with the scaffold's channels. In vitro force measurements of EHTs made by Masumoto et al. showed that the best combination of cell types to yield preferential electric and mechanical characteristics is CM+EC+MC, suggesting that tissue maturation and function are altered by the usage of vascular cells. The application of MCs promoted improved alignment, a more developed CM structure, and the activation of several tissue maturation pathways.

3.6.3 Improving cardiac function and maturation

Several studies like Nunes et al in 2013 and Tulloch et al in 2011 have documented the contribution of electrical and mechanical stimulation to the improvement of cardiac tissue function and maturation. Nunes et al aimed to combined 3D cell culture with electrical stimulation to mature hiPSC-derived heart tissues. The engineered platform permitted the creation of 3D- aligned cardiac tissues with frequent striations. Electrical stimulation significantly im- proved organization in myofibril structure, conduction velocity, and electrophysiological and calcium-handling properties, showing an improved state of maturity of the re- modeling cardiomyocytes. Tulloch et al. (2011) employed tissue engineering techniques to study reactions to mechanical strain and vascularization. They discovered that myofibrillogenesis, sarcomeric banding, and cardiomyocyte and matrix fiber alignment are all frequently considerably enhanced by uniaxial mechanical stress conditioning. Further encouraging increases in cardiomyocyte hypertrophy and proliferation rates was cyclic stress conditioning. suggesting the condition in mechanical stress will significantly enhance the structure-function relationship of cardiac tissues.

Transplantation methods used suturing to apply the patches in vivo models like

rats, guinea pigs, and mice. Sugiura et al. (2016) also used fibrin glue to attach the patches along with the sutures. The success of these methods depended on the materials and cell types used, with multipotent cell combinations and supportive scaffolds showing the most promise.

study	Scaffold Material	Cells type(s)	Results	Transplantation
Wendel et al., (2015)	Fibrin	purified CMS and PCs	echocardiographic assessments showed higher left ventricular ejection fraction in CM+PC group and had a reduced infarct scar area.	Patches were applied by suturing them parallel to each other over epicardial surface.
Weinberger et al., (2016)	fibrinogen and thrombin	HiPSC-CMs and human EC	EHTs were integrated with scarring. There was remuscularization in EHTs group with 12% of infarct area. Echocardiography showed the LV function improved in EHT group.	The EHT was sutured into healthy myocardium adjacent to scar in the LV.
Gao et al., (2017)	gelatin methacrylate	3 different cell types were used in a 2:1:1 ratio of hiPSC-CM, hiPSC- SMC, and hip SC-EC.	Echocardiographic assessments showed that LV ejection fraction was significantly greater for animals in group 1 than group 2. The infarct size was also smaller in group 1.	Group 1 was treated with 2 human derived cardiac muscle patches and group 2 was treated with the scaffolds without the cells. These patches were positioned over the infarction site in the LV. There was also a control group where no treatment was applied.
Sugiura et al., (2016)	polyglycolic acid and poly (l-lactic-co-ε-caprolactone) copolymer	hiPSC-CMs	Initially the seeded patch didn't stain positive for a marker of CMs. 16 weeks post implantation, the area fraction showed the cell seeded promoted regenerative proliferation of host CMs.	Using adult male athymic nude rates were used. the cardic patch is sutured along the margin of the defect. Fibrin glue was also used along the edges of the patch.
Masumoto et al., (2016)	collagen type 1 and Matrigel	CM, EC and vascular MC	All groups with implanted hiPSCs derived engineered cardiac tissue shows improved LV function In addition, there was also improved maturation and prefusion	Induced Myo infarcted rat hearts had been divided into 3 groups. Group 1: CM+EC Group 2: CM+MC Group 3: CM+EC+MC and transplanted

Table 1. Comparison table outlining the scaffold materials, cell types, transplantation, and results of the studies. To be selected for comparison, studies must meet specific criteria, including being conducted in vivo and involving EHTs with a scaffold only. The studies compare the following parameters: LV function, assessed by measuring improvement through echocardiography after transplantation, and infarct size, evaluated by determining the area of infarct size to gauge the performance and remuscularization of the cardiac patch

3. CHALLENGES AND FUTURE DIRECTIONS

3.1 Challenges

While the use of pluripotent cells holds promise for generating engineered heart tissue, several challenges need to be addressed. One major challenge is the maturation of hiPSC-CMs. hiPSCs differentiated into cardiomyocytes often resemble immature fetal heart cells, rather than fully mature adult cardiomyocytes, limiting their functionality (Yang et al., 2014). The ability to produce larger amounts of uniform and high-quality cardiomyocytes is a challenge that needs to be addressed if engineered heart tissue were to be used in the real world (Miwa et al., 2020). While iPSC can be patient-specific, there are still concerns about the safety of PSC-derived tissues. Nguyen et al. highlighted the potential of tumorigenicity due to residual undifferentiated PSC. Ensuring that the engineered heart tissue does not trigger an immune response from the host is still a big challenge that must be addressed. This can be addressed by using the patient's own stem cells to reduce the risk of immune rejection and response. Using iPSCs in research and industry must come with legal and ethical considerations. These considerations include the donor's privacy and informed consent, the safety of the reprogramming strategy, and potential genetic modification. Lastly, the privacy and consent of the cell recipient should be emphasized. (Moradi et al., 2019). Addressing these challenges requires continued research and development to improve the differentiation, maturation, scalability, and safety of PSC-derived cardiomyocytes and engineered heart tissues.

3.2 Future Directions

Creating functional heart tissue constructs from pluripotent stem cells offers significant potential for treating cardiovascular diseases. A big challenge of hiPSC-CMs is maturation. One possible solution is through mimicking the heart's mature environment using biophysical and biochemical cues. Biophysical cues could be mechanical stretch and electrical stimulation, which promote cardiomyocytes' structural and functional maturation. Ruan et al. (2016) showed that applying cyclic mechanical stains and electrical pulses can increase engineered heart tissue's alignment, contractility, and electrical conductivity. The use of 3D bioprinting can help constrict precise and complex tissue structures that mimic the native heart tissue. This allows for spatial arrangements of multiple cell types and extracellular matrix components (Tripathi et al., 2022). Via gene-editing tools like CRISPR- Cas9, the properties of hiPSC-CMs can be enhanced by targeting certain genes associated with cardiomyocyte function and maturation (Han et al., 2023).

4. CONCLUSION

The studies reviewed highlight trends and breakthroughs in cardiac tissue engineering. Multicellular approaches, combining cell types like pericytes, endothelial cells, and fibroblasts with hiPSC-CMs, enhance vascularity, remuscularization, and tissue re- generation. Scaffolds made from biodegradable materials or via 3D printing support cardiomyocyte maturation and organization into functional tissues. Scaffold-free methods like cell sheet technology also improve tissue integration and maturation without scaffold-related issues. Materials used in scaffolds like collagen and gelatin highlight the potential and advantages of hydrogels in the EHT field.

Key challenges remain, including scalability for clinical use, long-term tissue viability, immune rejection, and the need for standardized protocols and rigorous preclinical testing. Despite these challenges, the advances in cardiac tissue engineering offer new hope for treating cardiovascular conditions and diseases. Future research should focus on addressing these issues to translate engineered cardiac tissues into real-world applications.

5. REFERENCES

Andrysiak, K., Stępniewski, J., & Dulak, J. (2021). Human-induced pluripotent stem cell-derived cardiomyocytes, 3D cardiac structures, and heart-on-a-chip as tools for drug research. Pflugers Archiv : European journal of physiology, 473(7), 1061–1085. https://doi.org/10.1007/s00424-021-02536-z

Burdick, J. A., & Vunjak-Novakovic, G. (2009). Engineered Microenvironments for Controlled Stem Cell Differentiation. *Tissue Engineering Part A*, *15*(2), 205–219. https://doi.org/10.1089/ten.tea.2008.0131

Burkhard, S., van Eif, V., Garric, L., Christoffels, V., & Bakkers, J. (2017). On the Evolution of the Cardiac Pacemaker. *Journal of Cardiovascular Development and Disease*, *4*(2), 4. https://doi.org/10.3390/jcdd4020004

Chehelgerdi, M., Behdarvand Dehkordi, F., Chehelgerdi, M., Kabiri, H., Salehian- Dehkordi, H., Abdolvand, M., Salmanizadeh, S., Rashidi, M., Niazmand, A., Ah- madi, S., Feizbakhshan, S., Kabiri, S., Vatandoost, N., & Ranjbarnejad, T. (2023). Exploring the promising potential of induced pluripotent stem cells in cancer re- search and therapy. Molecular cancer, 22(1), 189. https://doi.org/10.1186/s12943-023-01873-0

Clariushd. (2022). *How to Measure Fractional Shortening | Clarius*. Clarius. https://clarius.com/classroom/how-to-measure-fractional-shortening/

Cleveland Clinic. (2022). *Heart Attack (Myocardial Infarction)*. Cleveland Clinic. https://my.clevelandclinic.org/health/diseases/16818-heart-attack-myocardial-infarction

Cleveland Clinic. (2024, January 26). *Heart: Anatomy and Function*. Cleveland Clinic; Cleveland Clinic. https://my.clevelandclinic.org/health/body/21704-heart

Ewart, L., Dehne, E. M., Fabre, K., Gibbs, S., Hickman, J., Hornberg, E., Ingelman-Sundberg, M., Jang, K. J., Jones, D. R., Lauschke, V. M., Marx, U., Mettetal, J. T., Pointon, A., Williams, D., Zimmermann, W. H., & Newham, P. (2018). Application of Microphysiological Systems to Enhance Safety Assessment in Drug Discovery. Annual review of pharmacology and toxicology, 58, 65–82. https://doi.org/10.1146/annurev-pharmtox-010617-052722

Gao, L., Kupfer, M. E., Jung, J. P., Yang, L., Zhang, P., Da Sie, Y., Tran, Q., Ajeti, V., Freeman, B. T., Fast, V. G., Campagnola, P. J., Ogle, B. M., & Zhang, J. (2017). Myocardial Tissue Engineering With Cells

Derived From Human-Induced Pluripo- tent Stem Cells and a Native-Like, High-Resolution, 3-Dimensionally Printed Scaf- fold. Circulation Research, 120(8), 1318–1325. https://doi.org/10.1161/circresaha.116.310277

Goldfracht, I., Protze, S., Shiti, A., Setter, N., Gruber, A., Shaheen, N., Nartiss, Y., Keller, G., & Gepstein, L. (2020). Generating ring-shaped engineered heart tissues from ventricular and atrial human pluripotent stem cell-derived cardiomyocytes. Nature Communications, 11(1). https://doi.org/10.1038/s41467-019-13868-x

Hall, J. E., & Guyton, A. C. (2000). *Guyton and Hall textbook of medical physiology* (12. ed.). Saunders Elsevier.

Halloin, C., Schwanke, K., Wiebke Löbel, Franke, A., Szepes, M., Santoshi Biswanath, Wunderlich, S., Merkert, S., Weber, N., Osten, F., Jeanne, Polten, F., Kai Christoph Wollert, Kraft, T., Fischer, M., Martin, U., Gruh, I., Kempf, H., & Zweigerdt, R. (2019). *Continuous WNT Control Enables Advanced hPSC Cardiac Processing and Prognostic Surface Marker Identification in Chemically Defined Suspension Culture*. *13*(2), 366–379. https://doi.org/10.1016/j.stemcr.2019.06.004

Han, J. L., & Entcheva, E. (2023). Gene Modulation with CRISPR-based Tools in Human iPSC-Cardiomyocytes. Stem cell reviews and reports, 19(4), 886–905. https://doi.org/10.1007/s12015-023-10506-4

Hassani, S. N., Moradi, S., Taleahmad, S., Braun, T., & Baharvand, H. (2019). Transition of inner cell mass to embryonic stem cells: mechanisms, facts, and hypotheses. *Cellular and molecular life sciences : CMLS*, *76*(5), 873–892. https://doi.org/10.1007/s00018-018-2965-y

Heart Transplant Candidacy: Criteria & Requirements | UPMC. (2024). UPMC | Life Changing Medicine. https://www.upmc.com/services/transplant/heart/candidates#:~:text=Heart%20Conditions%20that%20Can%20Lead%20to%20Transplant%20Surgery&text=Arrhythmia%3A%20An%20irregular%20heartbeat

Jang, JW., Min, KE., Kim, C. et al. Review: Scaffold Characteristics, Fabrication Methods, and Biomaterials for the Bone Tissue Engineering. Int. J. Precis. Eng. Manuf. 24, 511–529 (2023). https://doi.org/10.1007/s12541-022-00755-7

Kawai, Y., Tohyama, S., Arai, K., Tamura, T., Soma, Y., Fukuda, K., Shimizu, H., Nakayama, K., & Kobayashi, E. (2022). Scaffold-Free Tubular Engineered Heart Tissue from Human Induced Pluripotent Stem Cells Using Bio-3D Printing Technology in vivo. Frontiers in Cardiovascular Medicine, 8. https://doi.org/10.3389/fcvm.2021.806215

Kawamura, M., Miyagawa, S., Miki, K., Saito, A., Fukushima, S., Higuchi, T., Kawamura, T., Kuratani, T., Daimon, T., Shimizu, T., Okano, T., & Sawa, Y. (2012). Feasibility, Safety, and Therapeutic Efficacy of Human Induced Pluripotent Stem Cell-Derived Cardiomyocyte Sheets in a Porcine Ischemic Cardiomyopathy Model. Circulation, 126(11_suppl_1). https://doi.org/10.1161/circulationaha.111.084343

Kensah, G., Lara, A. R., Dahlmann, J., Zweigerdt, R., Schwanke, K., Hegermann, J., Skvorc, D., Gawol, A., Azizian, A., Wagner, S., Maier, L. S., Krause, A., Dräger, G., Ochs, M., Haverich, A., Gruh, I., & Martin, U. (2012). Murine and human pluripotent stem cell-derived cardiac bodies form contractile myocardial tissue in vitro. European Heart Journal, 34(15), 1134–1146. https://doi.org/10.1093/eurheartj/ehs349

Knight, W. E., Cao, Y., Lin, Y. H., Chi, C., Bai, B., Sparagna, G. C., Zhao, Y., Du, Y., Londono, P., Reisz, J. A., Brown, B. C., Taylor, M. R. G., Ambardekar, A. V., Cleve land, J. C., Jr, McKinsey, T. A., Jeong, M. Y., Walker, L. A., Woulfe, K. C., D'Alessandro, A., Chatfield, K. C., … Song, K. (2021). Maturation of Pluripotent Stem Cell-Derived Cardiomyocytes Enables Modeling of Human Hypertrophic Cardiomyopathy. Stem cell reports, 16(3), 519–533. https://doi.org/10.1016/j.stemcr.2021.01.018

Komae, H., Sekine, H., Dobashi, I., Matsuura, K., Ono, M., Okano, T., & Shimizu, T. (2015). Three-dimensional functional human myocardial tissues fabricated from induced pluripotent stem cells. Journal of Tissue Engineering and Regenerative Medicine, 11(3), 926–935. https://doi.org/10.1002/term.1995

Laustsen, P. G., Russell, S. J., Cui, L., Entingh-Pearsall, A., Holzenberger, M., Liao, R., & Kahn, C. R. (2007). Essential role of insulin and insulin-like growth factor 1 receptor signaling in cardiac development and function. Molecular and cellular biology, 27(5), 1649–1664. https://doi.org/10.1128/MCB.01110-06

Li, J., Hua, Y., Miyagawa, S., Zhang, J., Li, L., Liu, L., & Sawa, Y. (2020). hiPSC-Derived Cardiac Tissue for Disease Modeling and Drug Discovery. *International Journal of Molecular Sciences*, *21*(23), 8893. https://doi.org/10.3390/ijms21238893

Li, J., Liu, L., Zhang, J., Qu, X., Kawamura, T., Miyagawa, S., & Sawa, Y. (2022). Engineered Tissue for Cardiac Regeneration: Current Status and Future Perspectives. *Bioengineering*, *9*(11), 605. https://doi.org/10.3390/bioengineering9110605

Li, J., Minami, I., Shiozaki, M., Yu, L., Yajima, S., Miyagawa, S., Shiba, Y., Morone, N., Fukushima, S., Yoshioka, M., Li, S., Qiao, J., Li, X., Wang, L., Kotera, H., Nakatsuji, N., Sawa, Y., Chen, Y., & Liu, L. (2017). Human Pluripotent Stem Cell-Derived Cardiac Tissue-like Constructs for Repairing the Infarcted Myocardium. Stem Cell Reports, 9(5), 1546–1559. https://doi.org/10.1016/j.stemcr.2017.09.007

Lian, X., Zhang, J., Azarin, S. M., Zhu, K., Hazeltine, L. B., Bao, X., Hsiao, C., Kamp, T. J., & Palecek, S. P. (2012). Directed cardiomyocyte differentiation from human plu- ripotent stem cells by modulating Wnt/β-catenin signaling under fully defined con- ditions. Nature Protocols, 8(1), 162–175. https://doi.org/10.1038/nprot.2012.150

Loyer, X., Zlatanova, I., Devue, C., Yin, M., Howangyin, K.-Y., Klaihmon, P., Guerin, C. L., Kheloufi, M., Vilar, J., Zannis, K., Fleischmann, B. K., Hwang, D. W., Park, J., Lee, H., Menasché, P., Silvestre, J.-S., & Boulanger, C. M. (2018). Intra-Cardiac Release of Extracellular Vesicles Shapes Inflammation Following Myocardial Infarction. *Circulation Research*, *123*(1), 100–106. https://doi.org/10.1161/circresaha.117.311326

Lü, S., Li, Y., Gao, S., Liu, S., Wang, H., He, W., Zhou, J., Liu, Z., Zhang, Y., Lin, Q., Duan, C., Yang, X. J., & Wang, C. (2010). Engineered heart tissue graft derived from somatic cell nuclear transferred embryonic stem cells improve myocardial performance in infarcted rat heart. *Journal of Cellular and Molecular Medicine*, *14*(12), 2771–2779. https://doi.org/10.1111/j.1582-4934.2010.01112.x

Majid, Q. A., Fricker, A. T. R., Gregory, D. A., Davidenko, N., Hernandez Cruz, O., Jabbour, R. J., Owen, T. J., Basnett, P., Lukasiewicz, B., Stevens, M., Best, S., Cameron, R., Sinha, S., Harding, S. E., & Roy, I. (2020). Natural Biomaterials for Cardiac Tissue Engineering: A Highly Biocompatible Solution. *Frontiers in Cardiovascular Medicine*, *7*. https://doi.org/10.3389/fcvm.2020.554597

Masumoto, H., Nakane, T., Tinney, J. P., Yuan, F., Ye, F., Kowalski, W. J., Minakata, K., Sakata, R., Yamashita, J. K., & Keller, B. B. (2016). The myocardial regenerative potential of three-dimensional engineered cardiac tissues composed of multiple human iPS cell-derived cardiovascular cell lineages. Scientific Reports, 6(1). https://doi.org/10.1038/srep29933

Mei, X., & Cheng, K. (2020). Recent Development in Therapeutic Cardiac Patches. Frontiers in cardiovascular medicine, 7, 610364. https://doi.org/10.3389/fcvm.2020.610364

Mihic, A., Li, J., Miyagi, Y., Gagliardi, M., Li, S. H., Zu, J., Weisel, R. D., Keller, G., & Li, R. K. (2014). The effect of cyclic stretch on maturation and 3D tissue formation of human embryonic stem cell-derived cardiomyocytes. Biomaterials, 35(9), 2798–2808. https://doi.org/10.1016/j.biomaterials.2013.12.052

Miki, K., Uenaka, H., Saito, A., Miyagawa, S., Sakaguchi, T., Higuchi, T., Shimizu, T., Okano, T., Yamanaka, S., & Sawa, Y. (2012). Bioengineered myocardium derived from induced pluripotent stem cells improves cardiac function and attenuates cardiac remodeling following chronic myocardial infarction in rats. Stem cells translational medicine, 1(5), 430–437. https://doi.org/10.5966/sctm.2011-0038

Miwa, T., Idiris, A., & Kumagai, H. (2020). A novel cardiac differentiation method of a large number and uniformly-sized spheroids using microfabricated culture vessels. Regenerative therapy, 15, 18–26. https://doi.org/10.1016/j.reth.2020.04.008

Moradi, S., Mahdizadeh, H., Šarić, T., Kim, J., Harati, J., Shahsavarani, H., Greber, B., & Moore, J. B., 4th (2019). Research and therapy with induced pluripotent stem cells (iPSCs): social, legal, and ethical considerations. Stem cell research & therapy, 10(1), 341. https://doi.org/10.1186/s13287-019-1455-y

Motta, B. M., Pramstaller, P. P., Hicks, A. A., & Rossini, A. (2017). The Impact of CRISPR/Cas9 Technology on Cardiac Research: From Disease Modelling to Therapeutic Approaches. *Stem Cells International*, *2017*, 1–13. https://doi.org/10.1155/2017/8960236

Murphy, C. M., & O'Brien, F. J. (2010). Understanding the effect of mean pore size on cell activity in collagen-glycosaminoglycan scaffolds. Cell adhesion & migration, 4(3), 377–381. https://doi.org/10.4161/cam.4.3.11747

Nguyen, T. D., Chooi, W. H., Jeon, H., Chen, J., Tan, J., Roxby, D. N., Lee, C. Y., Ng, S. Y., Chew, S. Y., & Han, J. (2024). Label-Free and High-Throughput Removal of Residual Undifferentiated Cells From iPSC-Derived Spinal Cord Progenitor Cells. Stem cells translational medicine, 13(4), 387–398. https://doi.org/10.1093/stcltm/szae002

Nunes, S. S., Miklas, J. W., Liu, J., Aschar-Sobbi, R., Xiao, Y., Zhang, B., Jiang, J., Massé, S., Gagliardi, M., Hsieh, A., Thavandiran, N., Laflamme, M. A., Nanthakumar, K., Gross, G. J., Backx, P. H., Keller, G., & Radisic, M. (2013). Biowire: a platform for maturation of human pluripotent stem cell–derived cardiomyocytes. Nature Methods, 10(8), 781–787. https://doi.org/10.1038/nmeth.2524

Prasadh, S., & Wong, R. C. W. (2018). Unraveling the mechanical strength of biomaterials used as a bone scaffold in oral and maxillofacial defects. Oral Science International, 15(2), 48–55. https://doi.org/10.1016/s1348-8643(18)30005-3

Querdel, E., Reinsch, M., Castro, L., Köse, D., Bähr, A., Reich, S., Geertz, B., Ulmer, B., Schulze, M., Lemoine, M. D., Krause, T., Lemme, M., Sani, J., Shibamiya, A., Stüdemann, T., Köhne, M., Von Bibra, C., Hornaschewitz, N., Pecha, S., Weinberger, F. (2021). Human Engineered Heart Tissue Patches Remuscularize the Injured Heart in a Dose-Dependent Manner. Circulation, 143(20), 1991–2006. https://doi.org/10.1161/circulationaha.120.047904

Reinal, Ignacio & Ontoria-Oviedo, Imelda & Selva, Marta & Casini, Marilù & Peiro, Esteban & Fambuena, Carlos & Climent, Andreu M. & Balaguer, Julia & Cañete, Adela & Mora, Jaume & Raya, Angel & Sepulveda, Pilar. (2023). Modeling Cardiotoxicity in Pediatric Oncology Patients Using Patient-Specific iPSC-Derived

Cardiomyocytes Reveals Downregulation of Cardioprotective microRNAs. 12. 1378. https://doi.org/10.3390/antiox12071378

Repairing the heart with stem cells. (2013, March 1). Harvard Health. https://www.health.harvard.edu/heart-health/repairing-the-heart-with-stem-cells

Romito, A., & Cobellis, G. (2016). Pluripotent Stem Cells: Current Understanding and Future Directions. Stem Cells International, 2016(9451492), 1–20. https://doi.org/10.1155/2016/9451492

Ruan, J. L., Tulloch, N. L., Razumova, M. V., Saiget, M., Muskheli, V., Pabon, L., Reinecke, H., Regnier, M., & Murry, C. E. (2016). Mechanical Stress Conditioning and Electrical Stimulation Promote Contractility and Force Maturation of Induced Pluripotent Stem Cell-Derived Human Cardiac Tissue. *Circulation*, *134*(20), 1557–1567. https://doi.org/10.1161/CIRCULATIONAHA.114.014998

Santos, A. R. M. P., Jang, Y., Son, I., Kim, J., & Park, Y. (2021). Recapitulating Cardiac Structure and Function In Vitro from Simple to Complex Engineering. Micromachines, 12(4), 386. https://doi.org/10.3390/mi12040386

Sarohi, V., Chakraborty, S., & Basak, T. (2022). Exploring the cardiac ECM during fibrosis: A new era with next-gen proteomics. *Frontiers in molecular biosciences*, *9*, 1030226. https://doi.org/10.3389/fmolb.2022.1030226

Scafa Udriște, A., Niculescu, A. G., Iliuță, L., Bajeu, T., Georgescu, A., Grumezescu, A. M., & Bădilă, E. (2023). Progress in Biomaterials for Cardiac Tissue Engineering and Regeneration. Polymers, 15(5), 1177. https://doi.org/10.3390/polym15051177

Shadrin, I. Y., Allen, B. W., Qian, Y., Jackman, C. P., Carlson, A. L., Juhas, M. E., & Bursac, N. (2017). Cardiopatch platform enables maturation and scale-up of human pluripotent stem cell-derived engineered heart tissues. Nature Communications, 8(1). https://doi.org/10.1038/s41467-017-01946-x

Slaughter, B. V., Khurshid, S. S., Fisher, O. Z., Khademhosseini, A., & Peppas, N. A. (2009). Hydrogels in Regenerative Medicine. *Advanced Materials*, *21*(32-33), 3307–3329. https://doi.org/10.1002/adma.200802106

Stem cell Culture Guide. (n.d.). ATCC. https://www.atcc.org/resources/culture-guides/stem-cell-culture-guide

Sugiura, T., Hibino, N., Breuer, C. K., & Shinoka, T. (2016). Tissue-engineered cardiac patch seeded with human induced pluripotent stem cell derived cardiomyocytes promoted the regeneration of host cardiomyocytes in a rat model. Journal of Cardiothoracic Surgery, 11(1). https://doi.org/10.1186/s13019-016-0559-z

Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda,

K., & Yamanaka, S. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*, *131*(5), 861–872. https://doi.org/10.1016/j.cell.2007.11.019

Thang, N. H., Chien, T. B., & Cuong, D. X. (2023). Polymer-Based Hydrogels Applied in Drug Delivery: An Overview. Gels (Basel, Switzerland), 9(7), 523. https://doi.org/10.3390/gels9070523

Thavandiran, N., Dubois, N., Mikryukov, A., Massé, S., Beca, B., Simmons, C. A., Deshpande, V. S., McGarry, J. P., Chen, C. S., Nanthakumar, K., Keller, G. M., Radisic, M., & Zandstra, P. W. (2013). Design and formulation of functional pluripotent stem cell-derived cardiac microtissues. Proceedings of the National Academy of Sciences of the United States of America, 110(49). https://doi.org/10.1073/pnas.1311120110

Tonsho, M., Michel, S., Ahmed, Z., Alessandrini, A., & Madsen, J. C. (2014). Heart trans- plantation: challenges facing the field. Cold Spring Harbor perspectives in medicine, 4(5), a015636. https://doi.org/10.1101/cshperspect.a015636

Tripathi, S., Mandal, S. S., Bauri, S., & Maiti, P. (2022). 3D bioprinting and its innovative approach for biomedical applications. MedComm, 4(1), e194. https://doi.org/10.1002/mco2.194

Tulloch, N. L., Muskheli, V., Razumova, M. V., Korte, F. S., Regnier, M., Hauch, K. D., Pabon, L., Reinecke, H., & Murry, C. E. (2011). Growth of Engineered Human Myocardium With Mechanical Loading and Vascular Coculture. Circulation Research, 109(1), 47–59. https://doi.org/10.1161/circresaha.110.237206

Uchida, S. (2012b). Further application: adult stem cells and tissue regenerations. In *Elsevier eBooks* (pp. 137–169). https://doi.org/10.1533/9781908818126.137

Valiente-Alandi, I., Schafer, A. E., & Blaxall, B. C. (2016). Extracellular matrix-mediated cellular communication in the heart. *Journal of Molecular and Cellular Cardiology*, *91*, 228–237. https://doi.org/10.1016/j.yjmcc.2016.01.011

Weinberger, F., Breckwoldt, K., Pecha, S., Kelly, A., Geertz, B., Starbatty, J., Yorgan, T., Cheng, K. H., Lessmann, K., Stolen, T., Scherrer-Crosbie, M., Smith, G., Reichenspurner, H., Hansen, A., & Eschenhagen, T. (2016). Cardiac repair in guinea pigs with human engineered heart tissue from induced pluripotent stem cells. Science translational medicine, 8(363), 363ra148. https://doi.org/10.1126/scitranslmed.aaf8781

Wendel, J. S., Ye, L., Tao, R., Zhang, J., Zhang, J., Kamp, T. J., & Tranquillo, (2015). Functional Effects of a Tissue-Engineered Cardiac Patch From Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes in a Rat Infarct Model. Stem Cells Translational Medicine, 4(11), 1324–1332. https://doi.org/10.5966/sctm.2015-

0044

World Health Organization. (2024). Cardiovascular Diseases. World Health Organization. https://www.who.int/health-topics/cardiovascular-diseases#tab=tab_1

Yang, X., Pabon, L., & Murry, C. E. (2014). Engineering adolescence: maturation of human pluripotent stem cell-derived cardiomyocytes. Circulation research, 114(3), 511–523. https://doi.org/10.1161/CIRCRESAHA.114.300558

Yoshida, S., Miyagawa, S., Fukushima, S., Kawamura, T., Kashiyama, N., Ohashi, F., Toyofuku, T., Toda, K., & Sawa, Y. (2018). Maturation of Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes by Soluble Factors from Human Mesenchymal Stem Cells. Molecular therapy : the journal of the American Society of Gene Therapy, 26(11), 2681–2695. https://doi.org/10.1016/j.ymthe.2018.08.012

Zhang, D., Shadrin, I. Y., Lam, J., Xian, H., Snodgrass, H. R., & Bursac, N. (2013). Tissue-engineered cardiac patch for advanced functional maturation of human ESC-derived cardiomyocytes. *Biomaterials*, *34*(23), 5813–5820. https://doi.org/10.1016/j.biomaterials.2013.04.026

Zhang, Y., Mu, W., Zhang, Y., He, X., Wang, Y., Ma, H., Zhu, T., Li, A., Hou, Q., Yang, W., Ding, Y., Ramakrishna, S., & Li, H. (2022). Recent advances in cardiac patches: Materials, preparations, and properties. ACS Biomaterials Science & Engineering, 8(9), 3659–3675. https://doi.org/10.1021/acsbiomaterials.2c00348

Zhao, Y., Rafatian, N., Feric, N. T., Cox, B. J., Aschar-Sobbi, R., Wang, E. Y., Aggarwal, P., Zhang, B., Conant, G., Ronaldson-Bouchard, K., Pahnke, A., Protze, S., Lee, J. H., Huyer, L. D., Jekic, D., Wickeler, A., Naguib, H. E., Keller, G. M., Vunjak-Novakovic, G., . . . Radisic, M. (2019). A platform for generation of Chamber-Specific cardiac tissues and disease modeling. *Cell*, *176*(4), 913-927.e18. https://doi.org/10.1016/j.cell.2018.11.042

Zimmermann, WH., Melnychenko, I., Wasmeier, G. *et al.* Engineered heart tissue grafts improve systolic and diastolic function in infarcted rat hearts. *Nat Med* **12**, 452–458 (2006). https://doi.org/10.1038/nm1394