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2D and 3D *in-Vitro* models for mimicking cardiac physiology

Gerges Abdelsayed [a], Danish Ali [a], Andrew Malone [a], Jemil Saidi [a], Manoj Myneni [b], Keshava Rajagopal [b], Faisal H. Cheema [b,c,*], Aamir Hameed [a,d,*]

[a] *Tissue Engineering Research Group (TERG), Department of Anatomy and Regenerative Medicine, Royal College of Surgeons in Ireland, RCSI University of Medicine and Health Sciences, Dublin, Ireland*
[b] *Tilman J. Fertitta Family College of Medicine, University of Houston, Houston, TX, United States*
[c] *HCA Healthcare Research Institute, Nashville, TN, United States*
[d] *Trinity Centre for Biomedical Engineering (TCBE), Trinity College Dublin (TCD), Dublin, Ireland*

A B S T R A C T

Cardiovascular diseases are the leading cause of morbidity and mortality and a huge economic burden on the healthcare system globally. Both pharmacological and device based treatment options have emerged over the years, however, it is still a 'holy grail' to effectively treat some cardiovascular conditions, for example, heart failure. Any treatment option whether it is drug therapy or a device therapy, has to go through a rigorous regulatory approval process. This requires robust pre-clinical research and clinical trial results. In order to proceed to the clinical trials, pre-clinical research is very important and may take methodologies which are at the interface of biology and engineering, for example, *in-vitro, ex-vivo* and *in-vivo* models. This paper focusses on the 2D and 3D *in-vitro* models to mimic the pathophysiology of a specific cardiovascular disease and their advantages and limitations.

1. Introduction

Cardiovascular diseases (CVD's) are responsible for an estimated 17.9 million deaths each year, accounting for 31% of all deaths worldwide and making CVD the number one killer globally (Cardiovascular diseases (CVDs) [Internet] 2021). Since three quarters of these deaths occur in low- and middle-income countries, there has been a large demand for the development of new, cost-effective managements (Cardiovascular diseases (CVDs) [Internet] 2021). Before a new drug or device is introduced to the market, it must first demonstrate safety and efficacy in extensive testing processes (Myers and Moore, 1987). The new drug and device approval process in the United States takes an average of 12 and 7 years, respectively (Van Norman, 2016). This process consists of preclinical and clinical trials (The phases of preclinical and clinical trials [Internet], 2021). Clinical trials are classified into four phases, each completed on human subjects to study safety, and effectiveness of the novel therapy (The phases of preclinical and clinical trials [Internet], 2021).

However, since the accurate evaluation of novel cardiovascular drugs and devices carries an unacceptably high risk in humans, the therapy must first be proven safe in preclinical trials (Mathur et al., 2016, Development, and Approval Process | Drugs | FDA [Internet] 2021). These trials utilize *ex-vivo, in-vivo*, and *in-vitro* methods to demonstrate preliminary efficacy, toxicity, pharmacokinetic, and safety information (The phases of preclinical and clinical trials [Internet], 2021).

The use of *ex-vivo* beating heart models is limited by their rapidly deteriorating electrophysiological and hemodynamic functions and are costly (Kappler et al., 2019). *In-vivo* models utilize mammalian hearts isolated from rats, rabbits, Guinea pigs, canines, and swine (Park et al., 2020, Hill et al., 2005). Using gene knockout experimental animal models, various extracellular matrix (ECM) proteins may be studied using these methods (Hall and Ogle, 2018). However, the complexity of these models had made it difficult to isolate the effects of individual proteins on specific cell processes or pathological states (Hall and Ogle, 2018). Additionally, these models fail to fully recapitulate the human pathophysiology, are costly, and may require a great deal of time to develop target disease models, which is especially true for complex

Abbreviations: CVDs, Cardiovascular diseases; 2D, two-dimensional; 3D, three-dimensional; ECM, extracellular matrix; HiPSCs, Human induced pluripotent stem cells; LQTS, long QT syndrome; DT-MRI, diffusion tensor magnetic resonance imaging; HFpEF, Heart failure with preserved ejection fraction; hPSC, human pluripotent stem cell.

* Corresponding authors at: Aamir Hameed, Department of Anatomy and Regenerative Medicine, Royal College of Surgeons in Ireland - RCSI University of Medicine and Health Sciences, Dublin, Ireland, 123 St. Stephen's Green, Dublin, Ireland, Faisal H. Cheema, Tilman J. Fertitta Family College of Medicine, University of Houston, Houston, TX, HCA Healthcare Research Institute, Nashville, TN

E-mail addresses: Faisal.Cheema@HCAhealthcare.com (F.H. Cheema), aamirhameed@rcsi.ie (A. Hameed).

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cardiovascular disease conditions (In vitro vs. In vivo: Is One Better? | UHN Research [Internet] 2021, Savoji et al., 2019). *Ex-vivo* and *in-vivo* models require the use of animals, which has been the focus of growing ethical concerns (In vitro vs. In vivo: Is One Better? | UHN Research [Internet] 2021). Thus, the evaluation of cardiovascular drugs or devices may be done *in-vitro* under experimental conditions closely mimicking *in-vivo* conditions, reducing the need for animal models (SWIER et al., 1989, Festing and Wilkinson, 2007).

In-vitro models allow for the study of the biophysical and biomolecular mechanisms in cardiac tissue, allowing for the advancement in understanding and development of new drugs, devices, and treatment strategies for various cardiac diseases (Mathur et al., 2016, Hall and Ogle, 2018, Duval et al., 2017). One of the earliest *in-vitro* cardiac models was created by P. Swier and their team in 1989 (SWIER et al., 1989). Swier et al. (1989) created a model that circulated 1.4 L of blood through a pneumatically driven 50 cc polyurethane right ventricle with tricuspid semilunar valves connected to a horseshoe-shaped blood reservoir and paved the way for modern day *in-vitro* device testing circulatory rigs . An ideal *in-vitro* cardiac model should precisely recapitulate the physiological or pathological conditions of the human heart, including the tissue structure, extracellular matrix (ECM) network orientation, and circulation (Mathur et al., 2016). Although two-dimensional (2D) *in-vitro* models have predominated since their inception in the 1900's, three-dimensional (3D) systems have been emerging, thanks to recent technological advancements, such as 3D bioprinting (Hall and Ogle, 2018, Duval et al., 2017, Advantages and Disadvantages [Internet], 2021).

2. Objectives

Using existing research, this paper aims to provide an overview of 2D and 3D *in-vitro* cardiac models and how they are produced, and discuss the advantages and limitations to each.

3. Methods

A review of the international published literature was completed in March 2022 of studies on 2D and 3D *in-vitro* cardiac models. Using Medical Subject Heading (MeSH) terms, the database Medline OVID was used to discover appropriate studies. Using the Boolean terms AND/OR, the following MeSH terms were used: 2D OR 3D AND *In-Vitro* AND Cardiac, results were limited to English language (See Appendices for search results). This search yielded 57 results. All abstracts were screened. Full texts of selected abstracts were then reviewed in detail. Additional articles were gathered using the reference lists of included studies and through independent searches. Data on 2D and 3D *in-vitro* cardiac devices was extracted and compared.

4. Results

4.1. 2D In-vitro Models

2D *in-vitro* models consist of cells that are cultured on flat dishes then placed onto coated surfaces, where they are able to adhere and spread using microfabrication-based patterning techniques (Mathur et al., 2016, Advantages and Disadvantages [Internet] 2021). Microfabrication is the development of miniscule structures on a micrometre or smaller scale (What is Microfabrication? - Monroe Engineering [Internet] 2021). The most commonly used microfabrication-based patterning technique is photolithography, which allows for the creation of a desired pattern on the surface of a light-sensitive substrate through exposure of specific regions to ultraviolet (Betancourt and Brannon-Peppas, 2006). The development of such techniques has allowed researchers to manipulate aspects of the cellular microenvironment, such as colony and cardiac myocyte topography, geometry, and cell morphology and function while maintaining nutrient delivery and chemical cues to the culture (Mathur et al., 2016, Annabi et al., 2013, Motlagh et al., 2003). These techniques have been extensively used to maximize the 2D models resemblance to its *in-vivo* counterpart and to model various diseases, such as conduction disorders (Savoji et al., 2019).

The cardiac action potential is shaped by ion channels, allowing for effective cardiac contraction and rhythm (Nattel et al., 2007). Dysfunction of these ion channels accounts for the underlying pathogenesis of many cardiac diseases (Savoji et al., 2019, Nattel et al., 2007). Many high throughput screening methods have been developed to study ion channel activity, including ligand binding assay, flux-based assay, fluorescence-based assay, and automated electrophysiological assays (Yu et al., 2016). Human induced pluripotent stem cells (HiPSCs) isolated from patients with various inherited arrhythmias can used to model these disorders, such as long QT syndrome (LQTS) (Savoji et al., 2019, Itzhaki et al., 2011). In 2011, Itzhaki et al developed an *in-vitro* model of congenital LQTS using HiPSCs isolated from a patient with type 2 LQTS (Itzhaki et al., 2011). This model was studied using whole-cell patch-clamp and microelectrode arrays, which allow for the measurement of impulse propagation (Itzhaki et al., 2011, Microelectrode Array - an overview | ScienceDirect Topics [Internet] 2021). This study revealed a significant prolongation of the action potential duration in type 2 LQTS cells due to a reduction of the cardiac potassium current I_{Kr} when compared to healthy control myocytes (Itzhaki et al., 2011). Further research by Lahti *et al* has revealed that LQTS cells are also more sensitive than controls to potentially arrhythmogenic drugs, including sotalol (Lahti et al., 2012). 2D cardiac *in-vitro* models such as this have served an important role in studying the pathophysiological mechanisms and drug sensitivities of LQTS and many other arrhythmic disorders, including LEOPARD syndrome and Timothy syndrome (Savoji et al., 2019, Lahti et al., 2012). On account of these characteristics, pharmaceutical companies have been utilizing 2D *in-vitro* cardiac models to study functional properties and cardiotoxicity in pre-clinical stages of drug development for decades (Savoji et al., 2019).

Although these 2D models are useful in investigating cardiac myocytes at the cellular level, they cannot fully recapitulate the complex biological and mechanical properties of the human heart due to a lack of the architectural and functional properties of 3D human organs (Mathur et al., 2016). This has driven the demand for the development of 3D cardiac *in-vitro* models, which may be able to provide a more representative micro-environment (Savoji et al., 2019).

4.2. 3D In-vitro Models

3D cardiac *in-vitro* models are better able to recreate the morphology of the human heart, maximizing their similarity with *in-vivo* physiological and pathological conditions (He et al., 2020). They are more suitable in simulating the cell-cell and cell-ECM interactions, physiological cues, and overall microenvironment of the heart (Savoji et al., 2019). There are three main classifications of 3D cardiac *in-vitro* models, including mini-tissue, heart-on-a-chip, and heart constructs (He et al., 2020).

Mini-tissue models allow for the creation of functional units with simple compositions, which can be used for high-throughput testing (He et al., 2020). These models may be formed using cell-laden hydrogel microfibers or bioprinting techniques (He et al., 2020). The use of cell-laden microfibers is one of the main approach's for creating engineered heart tissue (Savoji et al., 2019, He et al., 2020). This technique requires isolated heart cells (i.e. HiPSC's), a hydrogel scaffold formed from biopolymers (i.e. collagen or fibrin), and an aseptic chamber for culture of the hydrogel (a bioreactor) (Mathur et al., 2016, Savoji et al., 2019, Duval et al., 2017). Hydrogels allow for easily adjustable biochemical and mechanical properties, and they provide tissue-like water content (Duval et al., 2017). Mechanical load may also be applied to these models using flexible silicon posts without the need for extra stretching devices (Savoji et al., 2019). In 2017, Shadrin et al. (2017) developed a combined hydrogel scaffold and dynamic culture

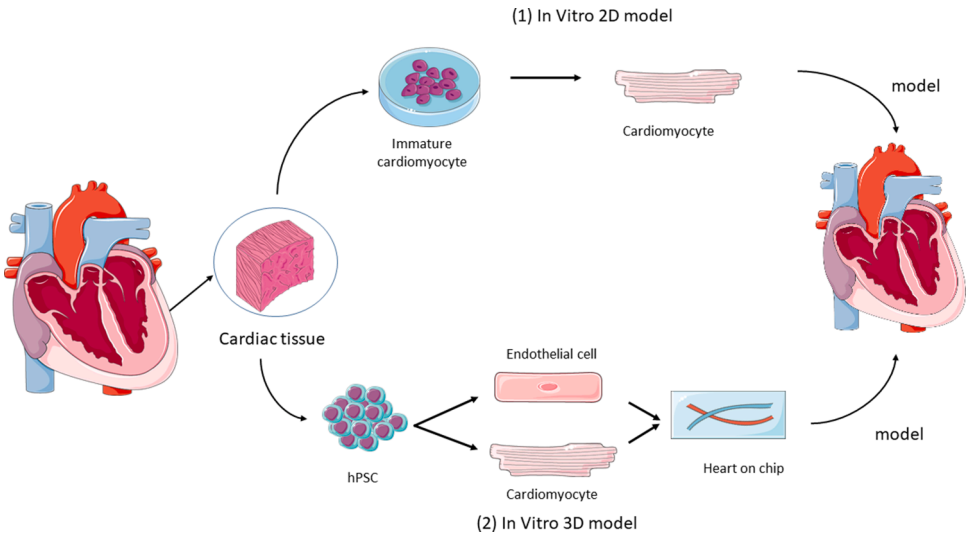

Figure 1. 2D and 3D *in-vitro* models

platform to generate large, functional heart tissue for human therapy called "Cardiopatch" . This platform was grown in the absence of exogenous stimulations and it demonstrated robust electromechanical coupling, constant I-bands and H-zones, and evidence of M-bands and T-tubules (Shadrin et al., 2017). Cardiopatches implanted on rat hearts robustly engrafted and maintained pre-implantation electrical function, with no increased incidence of arrhythmias (Shadrin et al., 2017). Alternatively, the main bioprinting method, inkjet bioprinting, uses thermal or acoustic forces to precisely distribute biomaterials onto an anticipated location, producing a 3D cardiac model (He et al., 2020).

Heart-on-a-chip is a 3D *in-vitro* model that aims to replicate the key functions of the heart (Team, 2021). These models can be used to mimic microenvironments and their influence on organ function, allowing for the study of human physiology and the production of artificial disease models (Team, 2021). *Heart-on-a-chip* models are created using microfluidics, which involves the use of miniature devices containing microchambers through which fluids flow or are confined (Team, 2021). These miniature devices are created using microfabrication techniques, such as microcontact 3D printing (Team, 2021). *Heart-on-a-chip* models may be classified as electrical or mechanical stimulation-based systems, depending on their main function (Kitsara et al., 2019). Electrical systems are useful for electrophysiology studies, while mechanical systems allow for the evaluation of contractility and structural assessment (Kitsara et al., 2019). Development of systems that can mimic specific disease, such as hypertrophic cardiomyopathy or myocardial ischemia, has already commenced (Kitsara et al., 2019). Novel chips that can be used to study the alignment of contractile apparatus and their gene expression profile have demonstrated utility in the quantification of stress, cellular architecture, and electrophysiology (Grosberg et al., 2011).

Cardiac constructs allow for recreation of accurate patient-specific hemodynamic conditions (Park et al., 2020). In 2020, Park et al. (2020) produced a bio-robotic hybrid heart consisting of an organic porcine endocardium with intact intracardiac structures attached to an active synthetic myocardium using a novel soft tissue silicone adhesive . The synthetic myocardium was programmed to replicate the complex biological motion of the heart using emerging techniques in soft robotics (Park et al., 2020). This allowed the heart to display physiological contractile motion with interventricular septum engagement (Park et al., 2020). Through the use of an electro-pneumatic control system, parameters such as heart rate, contractility, and stroke volume may be tuned to simulate various conditions, including exercise or disease states (Park et al., 2020). This model has shown promise in improving the longevity of the device when compared to *ex-vivo* and *in-vivo* hearts, and may be imaged using MRI, echocardiography, and computed tomography (Park et al., 2020). Using DT-MRI images of a patient, a patient-specific hybrid heart model may be produced and used to test intracardiac devices in a mock circulatory loop, optimizing the device's application to the specific patient (Park et al., 2020). Most recently, Malone (2022) have created a mock circulatory loop to simulate both healthy heart function and Heart Failure with preserved Ejection Fraction (HFpEF). This rig will be helpful to test the mechanical circulatory support devices for HFpEF.

5. Discussion

This paper has provided an overview of 2D and 3D cardiac *in-vitro* models. Fig. 1 shows an illustration of 2D and 3D *in-vitro* models.

These models offer great utility in studying the physiology and pathology of cardiac tissues, aiding in the development of effective pharmacotherapies. In the past, 2D models have been favoured. These models are generally easier to develop, inexpensive, and provide reliable information for studying cardiac myocytes at the cellular level. They are well established and have an abundance of published literature available. 2D models are standardized, allowing them to offer high-content screening of electrical and mechanical measurements (Mathur et al., 2016). However, 3D models are necessary to more accurately recreate the physiologically relevant 3-dimensional environment of the heart, allowing for more accurate reproduction of *in-vivo* conditions. Although evolving technologies such as improved hydrogel and bioreactor techniques have made 3D models easier to develop, the process is still difficult. Compared to their 2D counterparts, 3D models are not as well established. There is no standard approach for the development of 3D models in literature. Standardization is difficult since different cell types will react differently to particular 3D systems. More difficulties arise in finding viable cell types, scaffold materials, and designs that can accurately recreate the physiological cardiac tissue (Duval et al., 2017). Although this is the case, advancements in the field are constantly being made. For example, the use of pre-formed spherical microtissues loaded into microchambers and cultured under continuous perfusion inside organ-on-a-chip designs has allowed for the creation of simpler, more easily reproducible 3D cultures (Duval et al., 2017, Zuppinger, 2019, Kim et al., 2015). Another massive advancement is the bio-robotic hybrid heart mentioned previously. Table 1 gives the advantages and limitations of both 2D and 3D *in-vitro* models.

In the future, the ideal *in-vitro* model will accurately recapitulate the biological, mechanical, and electrical activity of cardiac tissue. Widespread acceptance of such a model will require effective and efficient engineering strategies to allow for automation and easy integration into

Table 1
Advantages and limitations of 2D and 3D *in-vitro* models

In vitro model 2 Dimensional (2D)	Advantages	Limitation	Reference
Primary Animal derived Cardiomyocyte (CM):Cardiac tissue is obtained from various animals which undergoes enzymatic digestion. The suspended cell, following digestion, are then used to generate 2D cardiac syncytia.	- Affordable - Easily establish protocol	- Ethical concerns - Mixed population of Non CM and CM	Van Gorp PRR et al 2020,Bingen B et al 2013
Human derived Pluripotent Stem Cell (PSC): Human embryonic stem cells, following aggregation and exposure to differentiation conditions form embryonic bodies containing partially differentiated cells, including PSC, and with use of induces further differentiate to non-CM, arterial and ventricular cells.	- Reduce animal laboratory use - Suitable for personalised disease modelling	- Costly - Time consuming	Van Gorp PRR et al 2020, Mummery CL et al 2012, Laksman Z et al 2017
3 Dimensional (3D)			
3D Scaffolds:3D printing has advanced the development of 3D scaffolds for *in vitro* use. Construction of full scaffold using AutoCAD software with the use of biocompatible material (hydrogel mixed with cells) has made it possible to print artificial organs or muscle.	- Biocompatibility - Biodegradable - High cell proliferation* - Good printability *(dependent on material)	- Varied mechanical strength - Biocompatibility*	Qasim M et al 2019, Mittal R et al 2019
Engineered Heart Tissue (EHT): EHT formation results from combining human pluripotent stem cells (hPSC) and Extra-Cellular Matrix (ECM) substrate forming a hydrogel.	- Application specific shape - Vasculature and stiffness can be modified for specific pathologies - Mechanical and electrical testing	- Large number of cells per tissue - shape is determined by scaffold mould	Zuppinger C. et al 2019, Ramirez-Calderon G et al 2022
Following few days of condensation they bioengineered hydrogel acquires its final scaffold properties.			

practice. These devices will consider an individual's variability in genes, environment, and lifestyle using patient-specific HiPSCs, allowing for more precise evaluation, prevention, and treatment of disease.

6. Conclusion

In conclusion, the field of 2D and 3D *in-vitro* cardiac devices is rapidly evolving. The ideal approach to using these tests utilizes a combination of currently implemented strategies, including both 2D and 3D *in-vitro* models. Such an approach offers great potential in helping researchers uncover novel discoveries that may accelerate the development of effective therapies to prevent and/or treat cardiac disorders.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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