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CARDIOPULSE

Global spotlights

The link between regeneration and extracellular matrix in the heart—can three-dimensional *in vitro* models uncover it?

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Myocardial regeneration therapy—lost in clinical translation?

The question of why the human heart only has a very limited innate capacity to regenerate after injuries have prompted numerous scientists to investigate regenerative therapies for the heart. Despite extensive studies, the cardiovascular disease after myocardial infarction is still one of the main causes of morbidity and mortality worldwide. While some human tissues heal with a functional scar after injury or disease, that is, with replacement tissue that reiterates the functional properties of the injured tissue, myocardial scars do not support heart function (*Figure 1*). However, the fibrous scar in the heart is sufficient to maintain its structural integrity during exercise. This comes at a cost: contractility and, in some cases, myocardial excitation and conduction are usually impaired. Most of the research over the past 20 years has focused on cell-based and cell-derived therapies to either prevent excessive scarring after injury or to regenerate remodelled and scarred myocardial tissue. In preclinical trials with rat and mouse models of myocardial injury, great advances have been made in restoring myocardial function using cell-based or tissue-engineered products. While large animal models still showed favourable results, none of these products resulted in a clinically meaningful therapy that could withstand regulatory hurdles and achieve successful regeneration of myocardial tissue. In addition to the lack of suitable cardiac progenitor cells for heart repair, another issue is the regeneration of extracellular matrix (ECM), a complex and dynamic network of proteins that surrounds and interconnects cardiac cells, in the myocardium. Furthermore, Taylor and colleagues have shown in decellularized hearts that ECM can guide transplanted cells to form functionally beating myocardial tissue.[1] Additional efforts must be made to study the regeneration of proper ECM in the myocardial scar and to determine what formulation of cell and ECM composites are needed to form a functional myocardial tissue that integrates into existing tissue. However, a major limitation in the development and evaluation of such products and the study of the mechanisms of cardiac remodelling in humans is the lack of appropriate models.

Modelling the remodelling of the human heart

Some lower vertebrates such as the zebrafish have remodelling programs that ultimately repair heart injuries with functional myocardial tissue. However, this process is blocked in higher vertebrates such as humans regardless of age. At the cellular level, it is known that there are specific transcriptional and translational check points that prevent de novo cardiopoiesis.[2] However, the role of the ECM and its interaction with cardiac cells is not yet well understood. Although 99% of human genes have corresponding mouse orthologues, previous experience with animal models has shown that results can only be applied to humans to a limited extent. There are significant anatomical and physiological differences between the human heart and those of animals, such as tissue structure, heart beat rate, and electrophysiological properties, which can become more obvious and limiting in

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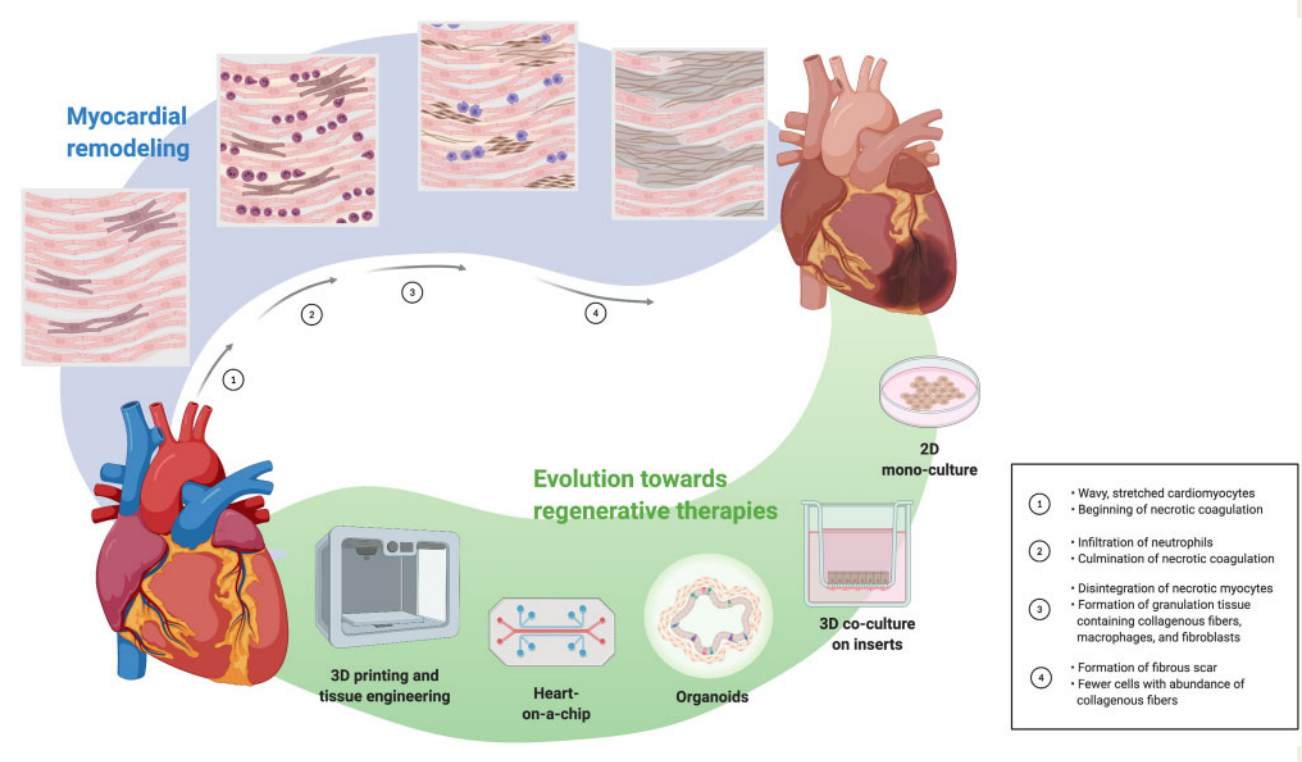

Figure 1 Myocardial remodelling and strategies for regeneration (created with BioRender.com).

Table 1 **Advantages and disadvantages of traditional 2D cell culture, 3D *in vitro* models, and rodent *in vivo* models**

Technique	Advantages	Disadvantages
2D cell culture (culture dish)	• Homogenous genetic population • Controlled environment • Well established methodology • High reproducibility • Cost-effective	• Static condition • No cellular heterogeneity • Lack of complex cell microenvironment • Lack of 3D tissue architecture and complexity • May not represent the *in vivo* phenotype
3D cell culture (organoid)	• *In vivo*-like complexity • *In vivo*-like architecture • Cell–cell and cell–extracellular matrix interactions • Sensitivity to drugs is similar to in vivo tissue	• More complex culture system • Static condition • Inefficient nutrient, waste, and gas exchange • Difficult to standardize
Microfluidic platform (organoid-on-a-chip)	• *In vivo*-like complexity • *In vivo*-like architecture • Fine control of the complex cell microenvironment • Ability to integrate various sensors and actuators • Good mass transport provided by fluid flow	• Requirement of special equipment (pumps, tubes) • Operational complexity • Difficult to be adapted for high-throughput screening
3D bioprinting	• Custom-made architecture • High reproducibility • High precision • High-throughput production • Vascularization of the engineered tissue	• Expensive equipment and materials • Difficult to be adapted to high-throughput screening
Animal model (rodent model)	• Readily available • Ease of handling and housing • Cost-effective • High reproduction rate	• Ethical concerns • No representation of the human physiology • Difficult to precisely model human diseases • Difficult to be adapted to high-throughput screening

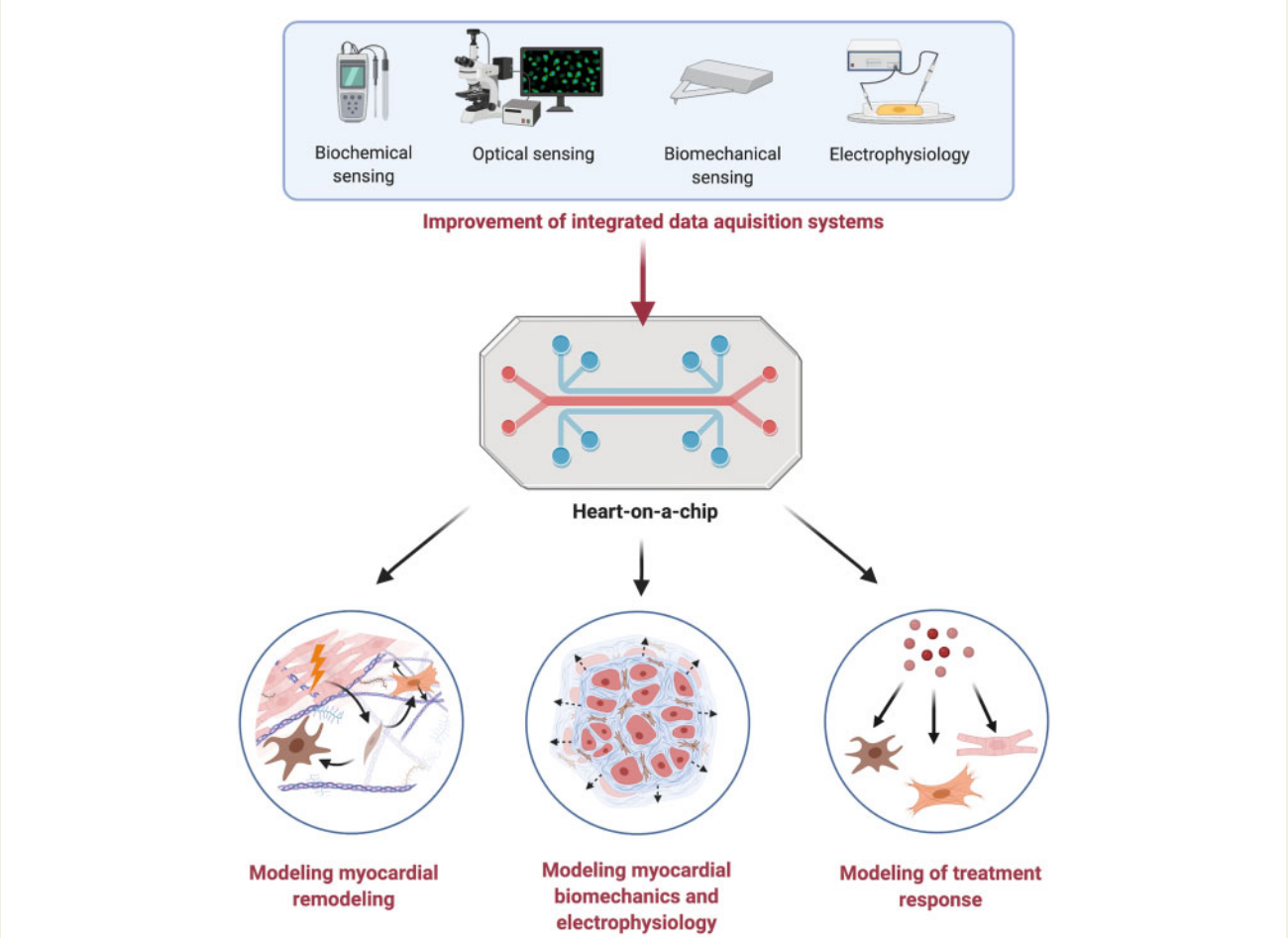

Figure 2 Improved integrated data acquisition systems are needed to develop next-generation heart-on-a-chip models for more comprehensive analysis of myocardial remodelling after heart injury, myocardial biomechanics, and response to treatment (created with BioRender.com).

pathological conditions. It is therefore important that more research be directed towards more complex tissue-engineered models that meet the following criteria:

i. Modelling cell-to-cell interactions and maintaining tissue homeostasis
ii. Acceptance of ECM modifications to study their role in tissue healing and homeostasis
iii. Mimicking of biomechanical and functional properties of myocardial tissue
iv. Ability to study the integration of functional myocardial tissue into existing tissue

Consequently, *in vitro* mimicking of the human heart with three-dimensional (3D) models would be beneficial. Recent developments in bioengineering organoids, organoid-on-a-chip platforms, and 3D bioprinting technologies could overcome some hurdles in modelling myocardial injury and cardiac remodelling and serve as a platform for developing strategies for tissue regeneration on a cellular and ECM level.[3] The possibility to cultivate cardiac cells in a 3D microenvironment that reflects the situation in the native organ *in vivo* could improve various biological studies that may not be performed directly in the human heart due to ethical issues and potential life-threatening complications. *Table 1* summarizes the advantages and disadvantages of traditional two-dimensional (2D) cell culture, *in vitro* 3D models, and *in vivo* animal models (rodent).

Organoids, organoids-on-a-chip, and 3D bioprinting—where do we stand?

Organoids are self-organizing 3D structures derived from stem cells that recapitulate essential aspects of tissue structure and function.[4] These miniaturized organs have the ability to mimic human tissue and organ architecture in terms of cell-to-cell and cell-to-matrix interactions, thereby exhibiting physiological organ functions and pathological conditions. Compared to animal models, organoids can be scaled down for high-throughput testing at a lower cost with less ethical concerns. In addition, organoid models can be utilized to study heart diseases using experimental manipulations that are problematic or even not feasible to be performed *in vivo* due to ethical or technical hurdles. One of the greatest challenges being encountered in designing cardiac organoids is the heterogeneity of the different cell populations composing the heart tissue and the association of various

diseases with specific cell types. On the technical level, organoid technology has challenges of uniformity and reproducibility in terms of size, shape, architecture, and cell composition. Another challenge is the lack of vascularization in these complex structures, which exposes organoids to necrosis and short lifetimes if not sub-cultured once they reach a certain size or when the organoid dimension exceeds the diffusion barrier. Manufacturing methods for cardiac organoids were first published in 2007 and have made a significant progress in recent years. For example, Keung *et al.*[5] created human ventricular-like cardiac organoid chambers from human embryonic stem cells, Voges *et al.*[6] established a human cardiac organoid injury model to mimic myocardial infarction, and Ronaldson-Bouchard *et al.*[7] made functional cardiac tissue from pluripotent stem cells. Future joint efforts from various scientific areas such as biology, pharmacology, toxicology, chemical engineering, and *in silico* modelling should ideally be carried out to achieve an even stronger impact. Overall, organoid technology can be considered one of the major technological breakthroughs of the past decade, as it possesses unique and powerful properties to revolutionize traditional *in vitro* research tools for modelling human heart development and diseases.

In addition, organoids-on-a-chip are engineered microfluidic systems that hold 3D tissues under precisely controlled conditions mimicking the complex structures and cellular interactions within and between different cell types and tissues *in vivo*. With microfluidic technology, tissue culture can be performed in a microenvironment with optimized temperature, pH, nutrient and oxygen supply, and waste removal. Sensors and actuators can be integrated into the micro-engineered devices to enable online and real-time monitoring and control of cellular processes.[8] By optimizing several key parameters, such as the cell-to-cell contact, cell-type composition, tissue architecture, and nutrient exchange, batch-to-batch fluctuations can be significantly minimized. Furthermore, improved integrated sensor technology holds the promise of more comprehensive data acquisition (*Figure 2*). Although imitating the cardiovascular system is still challenging due to its high dynamic nature such as blood flow, mechanical stretching, and electrical stimulation, heart-on-a-chip platforms have been successfully generated in previous years.[9] This development offers unprecedented opportunities to establish *in vitro* models of human heart tissue that enable us to study the fundamental mechanisms of diseases, early drug discovery, safety assessment and risk management of lead compounds, and then eventually the reduction and replacement of animal experiments.

Recently, 3D bioprinting has emerged as a promising technology that combines both cells and biomaterials as printing materials to create geometrically defined functional living tissue constructs in three dimensions.[10] Data for 3D bioprinted models can be obtained from 3D scanners, computed tomography, magnetic resonance imaging, and ultrasound systems using appropriate software. Based on a high-precision layer-by-layer building process, 3D bioprinting can be used to create a complex tissue architecture with a spatio-temporal distribution of cells and bioactive factors to accurately imitate native physiological niches and complex tissue microenvironments. Bioprinting also enables the vascularization of the engineered tissue constructs, which offers additional versatility in the manufacture of vascularized organoids. Although the bioprinting technology is still in its early stages, it has already been used to create cardiovascular tissues. We believe that 3D bioprinting is a feasible approach to create a physiologically relevant and robust cardiac model by reproducing *in vivo* tissue composition and complexity.

Let's go 3D!

The development of 3D cardiac tissue models that mimic heart structure and physiology *in vivo* is revolutionizing our understanding of disease mechanisms (*Figure 1*). Although many tools and reagents are commercially available for creating and analysing 3D *in vitro* models, the technology is still rarely used in academic research laboratories or in industry. One of the possible reasons for this challenge is that the standard 2D culture has been well established, and therefore it is more convenient to compare results from the previous studies. In addition, 2D culture is highly accepted in the scientific community, is probably more cost-effective, and more standardized. However, 3D culture models are becoming increasingly popular for modelling human diseases and testing therapeutics *in vitro*, especially in terms of how large pharmaceutical companies perceive this technology for the long run. Furthermore, in contrast to 2D models, 3D models could prove to be more conducive to investigate cell-to-ECM interactions. The ultimate goal is to achieve *in vivo*-like settings that reflect the complex structure of ECM and various cell types in myocardial tissue as well as the physiological function of the heart. These models could also make it possible to refine strategies to improve on ECM production and formulations in order to maintain cell transplants. Despite recent advances in 3D culture models, the full potential has not yet been completely exploited, but hopefully will further increase with more refined technical innovations. In terms of data analysis of using 3D cell culture technologies, improved sensors, image analysis techniques, advanced microscopy and imaging, machine learning and artificial intelligence capturing cellular features in high throughput, and data acquisition methods have already lowered the hurdle to use 3D *in vitro* models as biological tools. In the future, the combination of organoid platforms, microfluidics, and 3D bioprinting is foreseen to provide a highly anticipated technology for developing next-generation *in vitro* heart models. Continuous efforts are still required to ensure the accuracy and reproducibility of these models and to improve techniques required for the automation purposes.

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A new evidence on pulmonary circulation discovery: A text of Ibn Luqa (860–912 AD)

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The history of pulmonary circulation discovery has been long-time debated. For centuries, William Harvey (1578–1657) was reported as the first scholar who described pulmonary circulation mechanism in his book entitled *De Motu Cordis* (On the Motion of the Heart; 1628)[1]; while, before him, the credit of this great anatomical discovery was given to Michael Servetus (*c.*1511–1553). Servetus was a Spanish philosopher, theologian, and polymath hypothesizing pulmonary circulation in his book *Christianismi Restitutio* (Restoration of Christianity; 1553).[2] After him, such a theory was further developed by the Italian anatomist Renaldus Columbus (1516–1559), and finally elaborated through a mechanistic approach by Harvey.[1]

Only after 1920s, it was revealed that Servetus had an access to books authored by Ibn al-Nafis (1213–1288), the Syrian physician who presented the lesser circulation by rejecting the previous theory of Avicenna (980–1032).[3] Specifically, Avicenna speculated the transferring of blood between left and right ventricles via small pores in the septum between the ventricles. A theory derived from that of Galen of Pergamon (c.129–c.200), the famous Greek physician.[4]

Very recent studies show that the modern theory circulated among some Persian scientists even before Ibn al-Nafis, indeed in his writings Akhawayni (died 983) introduced both theories: pulmonary circulation and pores in the septum.[5] Even before, pulmonary circulation was mentioned in *Vazidegihā-i-Zādisparam,* a Sassanid Pahlavic text, implying that such a theory was known (and presumably originated) in the Sassanid era (224–637) of ancient Persia.[6] However, further studies are needed to find the missing links up to the present.

We herein aim to present another historical evidence of pulmonary circulation in a text that belongs to pre-Ibn-al-Nafis era. This text, attributed to Ibn Luqa (860–912), can be a significant reference for tracking the discovery of pulmonary circulation in the history of cardiovascular system.

Qusta Ibn Luqa was born in Baalbek (modern-day in Lebanon) in 860 AD. Later, he emigrated to Baghdad, the capital of the Abbasid Caliphate, and one of the centres of science in the era of the Islamic Golden Age (9–13th century).[7] Ibn Luqa was a respected physician, scientist, and translator. He translated non-Arabic texts into Arabic under the supervision of Hunain Ibn Ishaq, a notable Nestorian Christian translator and physician in the House of Wisdom (*Beit al-Hekmeh,* an early academy) in Baghdad (*Figure 1*).[8] It seems that he had also close relations with Iranians;[9] and probably had access to ancient Persian sources. Finally, Ibn Luqa left Baghdad to Armenia upon an invitation from the Armenian king and died there in 912. As a prolific author and translator, he wrote about 60 among books and manuscripts.[10]

Figure 1 A painting of a class in '*Beit al-Hekmeh*' of Baghdad; depiction by Yahya ibn Vaseti in the *Maqama of Hariri* (13th century), preserved in Bibliothèque nationale de France.

In his Arabic books entitled *On the Difference of Spirit and Soul* (*Figure 2*), Ibn Luqa discusses *ruh* (spirit) and *nafs* (soul) claiming that they belong to medicine and philosophy, respectively. He expounds

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