Modeling the heart with Novoheart's MyHeart™ platform

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Reliable and predictive human-specific *in vitro* heart models can revolutionize drug discovery and development. With the advent of pluripotent stem cell technologies, human cardiomyocytes can now be readily produced in large quantities. Using tissue engineering techniques, they can be further assembled into cardiac tissues of specific 2D and 3D configurations, to create models that behave and function like the native human heart. Novoheart (BC, Canada) uniquely offers the MyHeart™ Platform of bioengineered human heart constructs, designed to provide researchers with effective models of either healthy or diseased human hearts. As *in vitro*, human-based assays become more widely accepted, the next decade could witness a shift away from animal testing towards more accurate and scalable human assays like the MyHeart™ Platform.

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Traditional discovery and development of novel drugs and therapeutics continue to be an unacceptably inefficient and expensive process [1]. A major reason has been due to the lack of appropriate human models to simulate the normal and pathological physiology of our native human heart, let alone the genetic diversity across different ethnic groups. Currently, in the preclinical discovery and development stages of a new drug, animal models and simple cellular models are used to predict safety and efficacy. Yet, it is well known that animal physiology is highly species-specific and, consequently, laboratory animals poorly predict human responses to drugs, leading to false negatives and false positives that compromise overall successes. For example, the mouse heart rate is 500 bpm, compared with 60–80 bpm for humans. As such, the US FDA (MD, USA) has been advocating the use of nonrodent models to study the proarrhythmic potential of new drugs. Other large animal models, such as dogs and nonhuman primates, likewise have their own nonhuman-like attributes. The use of *in vitro* assays based on human cardiomyocytes derived from pluripotent stem cells has the potential to revolutionize drug development by providing virtually unlimited access to human heart cells capable of long-term viability in culture. However, standard *in vitro* human cellular models do not consider the more complex environment of the heart as an organ comprising tissues. As an industry standard extensively utilized by pharmaceutical companies to evaluate cardiotoxicity, the 'hERG assay' involves nonhuman/noncardiac Chinese hamster ovary, shortened to CHO, cells and the human embryonic kidney-derived HEK293 cells that have been modified to overexpress the 'rapid' delayed rectifying potassium channel protein as but one of the dozens of critical ion channels and pumps present in native human cardiomyocytes. The FDA recognizes the severe deficits of this approach and is actively seeking alternatives in partnership with industrial and academic bodies as part of the Comprehensive *in vitro* Pro-arrhythmia Assay initiative. There is thus a major, long-standing gap between animal preclinical models and human patients, and we believe Novoheart's (BC, Canada) cardiac tissue engineering technologies can be utilized to fill this gap. In particular, our MyHeart™ Platform offers models of healthy human heart tissue for screening cardiotoxicity, as well as *in vitro* models of human heart diseases, giving drug developers a powerful tool for identifying novel targets and testing efficacy in the drug discovery process.

Novoheart's foundational technologies are the direct outcome of almost two decades of ongoing collaborations, leveraging cross-disciplinary expertise among our founding scientists in stem cell biology, tissue engineering, cardiac electrophysiology, material science, machine learning, as well as hardware and software development. Novoheart developed its comprehensive platform of human pluripotent stem cell-derived, bioengineered human heart constructs, collectively known as the MyHeart™ Platform, which includes:

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Human ventricular cardiomyocytes

Derived from human embryonic stem cells or induced pluripotent stem cells, using a proprietary small molecule-based differentiation process, our human ventricular cardiomyocytes (hvCMs) are highly enriched for the ventricular-specific phenotype typical of the major pumping ventricular chambers of the heart, which are the chambers of primary concern for cardiotoxicity and therapeutic intervention. These hvCMs have been extensively characterized using molecular, electrophysiologic and -omics based techniques, and serve as the fundamental building blocks for constructing the various 2D- and 3D-MyHeart™ Platform constructs as described below [2–10].

Human ventricular cardiac anisotropic sheet

Disturbances in cardiac electrical conduction are a major cause of cardiotoxicity. However, life-threatening electrophysiological phenomena such as arrhythmias are multicellular phenomena that cannot be accurately predicted using conventional single-cell measurements. The human ventricular cardiac anisotropic sheet (hvCAS) assay uses an aligned monolayer of hvCMs enabled by our proprietary microgrooved substrate technology to reproduce the electrical anisotropy observed in the native heart. The assay allows investigators to literally visualize the occurrence of adverse electrical conduction patterns that could be life-threatening in patients [11–15]. As an example, in the Cardiac Arrhythmia Suppression Trial conducted between 1986 and 1998, several drugs classified as ‘anti-arrhythmics’ based on preclinical screening in animals were found to unexpectedly and ironically increase mortality by causing lethal arrhythmias after testing in over 1700 patients. Using hvCAS, the proarrhythmic properties of flecainide, a drug that failed in the Cardiac Arrhythmia Suppression Trial study, could be visualized for the first time *in vitro* [11].

Human ventricular cardiac tissue strip

Similar to electrical conduction, muscle contraction is a multicellular process that cannot be accurately predicted using single-cell measurements such as calcium transients or substrate impedance. Therefore, human ventricular cardiac tissue strip (hvCTS) has been developed to recapitulate the contractile properties of native human heart muscle. Designed to mimic a thin ventricular muscle strip (trabecula), each hvCTS is about 10-mm long and 0.5-mm in diameter with 1 million hvCMs embedded within a 3D collagen-based extracellular biomaterial. Grown in a custom bioreactor device, real-time, noninvasive measurements of the contractile force from the hvCTS can be readily obtained [16]. The hvCTS assay has been rigorously tested in blinded studies and validated by comparison to native human heart muscle preparations [15–22].

Human ventricular cardiac organoid chamber

Ultimately, the heart is a pump that serves the life-sustaining role of circulating blood throughout the body. Cardiologists are most interested in measures that quantify pump performance, such as ejection fraction, cardiac output and developed pressure. Novoheart has pioneered the first functional human mini-ventricle, or ‘heart-in-a-jar,’ specifically engineered to recapitulate essential aspects of a pumping human left ventricle [23]. Each human ventricular cardiac organoid chamber (hvCOC) is a hollow spherical shape about a centimeter in diameter, with a wall thickness of about 10 cardiomyocytes thick, and comprises about 10 million hvCMs in a 3D collagen-based biomaterial. Real-time pressure and volume measurements allow unprecedented assessment of pressure–volume relationships that define cardiac contractility in clinically familiar terms. The hvCOC is also compatible with optical mapping to combine the best of the hvCAS and hvCTS assays into a single platform with the highest cardiac biofidelity available to date. In addition, the heart-like environment of the hvCOC accelerates the maturation of resident hvCMs to yield molecular signatures and functional responses more similar to the adult human heart [18,23].

The above tissue-engineered cardiac constructs, as extensively published, are being used for modeling various heart diseases so as to improve our understanding of the underlying pathophysiology, and to discover new biomarkers and druggable targets.

One reason for Novoheart’s focus on heart diseases is that heart failure is a global pandemic; there were approximately 64.3 million cases reported worldwide in 2017, with a rising trend in prevalence [24]. Every year, the global economic burden of heart failure is estimated at over US\$100 billion [25]. Accounting for approximately 50% of heart failure cases, in particular, heart failure with preserved ejection fraction (HFpEF) is a major and increasing public health problem worldwide, with a poor understanding of its pathological mechanisms and diverse etiology. Due to these complexities, no model to date has proven to effectively mimic the clinical presentation of HFpEF, including a number of animal models [26]. Therefore, drug developers lack an effective tool for testing

drug efficacy *in vitro* and, as a result, clinical outcomes for HFpEF have not improved over the last decades as no effective therapies are currently available.

With a shared interest in bringing new therapeutic solutions to patients with heart failure, Novoheart and AstraZeneca (Cambridge, UK; Gothenburg, Sweden) have teamed up to develop the world's first human-specific *in vitro*, functional model of HFpEF, using Novoheart's expertise in engineering human mini-hearts, like the hvCOC. The goal is to establish a new *in vitro* model that reproduces key phenotypic characteristics of HFpEF. Unlike the currently used animal models, engineered hvCOCs can be created with specific cellular and matrix compositions, as well as patient-specific human-induced pluripotent stem cells, that allow control over their biological constituents and biophysical properties to mimic those observed in HFpEF patient hearts. This partnership aims to provide a unique assay for understanding the mechanisms of HFpEF, identification of new therapeutic targets and assessment of novel therapeutics for treating HFpEF patients. This hopes to bridge the aforementioned gap, in order to help accelerate the drug discovery process by providing human-specific preclinical data [27].

Prior to modeling HFpEF, Novoheart already has an established record of peer-reviewed studies on cardiac disease modeling, including the recently published collaboration with Pfizer on the first human-specific *in vitro* models of Friedreich's Ataxia (FRDA), developed with our MyHeart™ Platform assays [15]. A rare neuromuscular degenerative disease that affects over one in 50,000 people worldwide, FRDA is caused by a defective *FXN* gene, which often leads to lethal heart complications [28]. This new disease model was created using genetically modified, as well as FRDA patient-derived, cells successfully capturing both electrical and mechanical defects of the heart observed in FRDA patients using hvCAS and hvCTS assays, respectively. Again, Novoheart's FRDA models overcome limitations in traditional animal models and offer an innovative and powerful human-based platform to develop new therapies for FRDA's cardiac symptoms, for which no effective treatments are currently available.

It has been generally accepted that human-based *in vitro* assays present attractive alternatives to traditional animal models. Nevertheless, challenges remain with disease modeling in human-based, stem cell-derived assays. For example, simple 2D cell cultures cannot recapitulate most clinical symptoms, limiting their ability to properly demonstrate drug efficacy through the abrogation of symptoms. Simple *in vitro* assays derived from stem cells are also less mature compared with their equivalents in adults, again limiting their predictive capabilities. Another advantage of our 'human heart-in-a-jar' is that the 3D heart-like structure provides a promaturation milieu, yielding gene expression patterns and functional responses that more closely resemble the adult heart than 2D and simpler 3D models [23].

Moreover, the challenges of disease modeling increase with disease complexity, and diseases with complicated or poorly understood etiology are more difficult to re-create. When the pathology involves multiple organs, *in vitro* models of any one organ in isolation may be insufficient to model such diseases.

Nevertheless, it is encouraging that advanced strategies are being developed for overcoming such challenges. For example, tissue engineering methodologies can be utilized to alter biophysical properties to mimic pathological conditions even without a full understanding of the genetic or epigenetic underpinnings. Other environmental stressors (such as hypoxia) may be used to simulate disease conditions, while genetic manipulations (RNA interference, gene editing, etc.) offer additional tools to elicit pathological phenotypes. As *in vitro* models of different organs become better developed, their integration could provide systems-level models with enhanced capabilities for representing human physiology and pathophysiology.

As these *in vitro*, human-based assays become widely accepted by industry and regulators, we foresee within the next decade a shift away from the animal testing toward more accurate and scalable human assays, which will substantially enhance the efficiency of the drug discovery and development process and help deliver safer and more effective drugs to patients in need, in shorter time and at lower cost.

Financial & competing interests disclosure

GKY Wong, KD Costa, B Fermini and RA Li hold equities in Novoheart which has a financial interest in the subject matter. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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