novoheart™

Novoheart is a global biotechnology company that is at the forefront of this new era of precision medicine. With its innovative MyHeart™ Platform, comprising a suite of cardiac research tools that can be customized, Novoheart promises to revolutionize the pharmaceutical research and discovery process, taking centre stage as precision medicine transforms the face of health care.

About Novoheart

Novoheart is a publicly listed, global stem cell biotechnology company with R&D laboratories and offices in the United States, Canada, and Hong Kong.

Novoheart's mission and vision are to revolutionize drug discovery and the development of heart therapeutics with various proprietary bioengineered human heart constructs, and to further develop them into transplantable grafts for cell-based regenerative heart therapies with superior safety and efficacy.

Novoheart offers a one-stop solution for your cardiac research by pioneering an array of next-generation human cardiac tissue models.

Modeling of the human heart *in vitro* is technically challenging: the heart is an organ of great complexity, with diverse cell types organized into a specialized 3-D architecture for optimal function as a physiological pump. Novoheart is committed to developing the best bioengineered human heart tissues for studying cardiac functions. Our suite of proprietary technologies mimics the heart's unique properties, including its pumping mechanism, for applications in drug discovery, toxicology and efficacy screening, disease modeling, and cardiac research. Novoheart also offers consulting services for all studies using human stem cell-derived cardiac models: we offer our customers a wide range of expertise in areas including cardiac mechanics and electrophysiology, stem cell biology, tissue engineering, hardware/software engineering, machine learning, and more.

MyHeart™ Platform

for Cardiac Research

The world's only one-stop human cardiac research platform.

Find out more about the MyHeart™ Platform at **novoheart.com**.

Evaluating the state of cardiac health requires extensive assessment of a diverse set of functional outputs ranging from electrophysiology to contractile function. Novoheart's MyHeart™ Platform is the perfect tool, with its versatility and all-round capabilities for comprehensive analysis as well as answering specific questions about cardiac function. Four distinct assays, each designed for assessing specific functional outputs, are available and can be combined to provide valuable insights that researchers need for their studies. For added flexibility, the entire MyHeart™ Platform can also be customized using stem cell lines provided by the client.

- **Human-derived platform offers clinical relevance**
- **Readily adaptable and customizable to iPSC disease models (e.g. familial cardiomyopathy[1-3], BRAF-mediated hypertrophic cardiomyopathy[4], long QT syndrome[5], dilated cardiomyopathy, congenital heart diseases, etc.)**
- **Supported by in-house hardware and software to maximize sensitivity, accuracy, and throughput**

[1]Karakikes *et al.* (2015) *Nat Commun* 6: 6955, [2]Stillitano *et al.* (2016) *Eur Heart J* 37: 3282-3284, [3]Ceholski *et al.* (2018) *J Mol Cell Cardiol* 119: 147-154 , [4]Cashman *et al.* (2016) *PLoS One* 11(1): E0146697, [5]Stillitano *et al.* (2017) *Elife* 6: E19406.

	HUMAN VENTRICULAR CARDIOMYOCYTES (hvCMs) *for HCS*	**HUMAN VENTRICULAR CARDIAC ANISOTROPIC SHEET (hvCAS)** *for arrhythmogenicity*	**HUMAN VENTRICULAR CARDIAC TISSUE STRIP (hvCTS)** *for contractility*	**HUMAN VENTRICULAR CARDIAC ORGANOID CHAMBER (hvCOC) ("human heart-in-a-jar")** *exclusive tool for PV loop, EF, CO, etc.*
Electrophysiology				
Action potential	○	○	○	○
Ionic current composition	○			
Calcium transient	○	○	○	○
Multicellular conduction		●	○	○
• Anisotropic electrical conduction		●		○
Volume-conducted ECG				●
Contractility				
Cell shortening	○			
Real-time force measurement			●	
Pressure-volume (PV) loop				●
Ejection fraction				●
Cardiac output				●
Others				
• Single cell assays (e.g., metabolic activity, viability, etc.)	○	○	○	○

● Best in class

hvCMs

for *In Vitro* Cellular Studies

- >90% pure population of ventricular cardiomyocytes
- Available for clinically relevant cardiac disease phenotypes and genetic backgrounds, and can be produced from Novoheart's stock human pluripotent stem cell lines or customer-supplied lines to support specific experimental needs
- Highly reproducible across a wide range of applications including metabolic assays, electrophysiological measurements, omics studies, and generating engineered cardiac tissue constructs

Novoheart's human ventricular cardiomyocytes (hvCMs) are terminally differentiated cardiomyocytes derived from human pluripotent stem cells (hPSCs) using Novoheart's proprietary differentiation method[1,2]. These cells have been extensively characterized for their electrophysiology[3,4], calcium homeostasis[5-7], transcriptome[8,9], microRNAome[10,11], and proteome[12,13]. They exhibit ventricular-like action potentials and express ventricular-specific myosin light chain MLC2v. With virtually homogeneous ventricular properties, these cells provide the consistency necessary to achieve reliable and reproducible results in downstream applications.

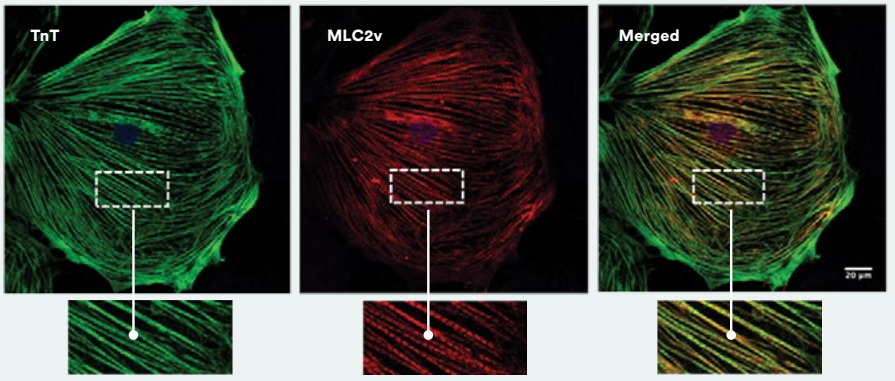
References:

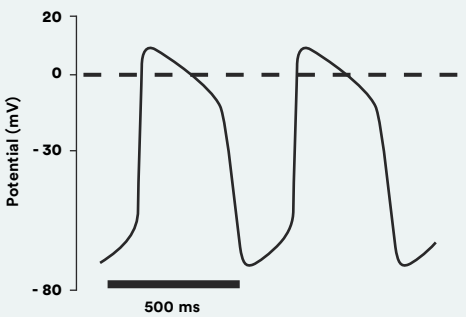
[1]Weng *et al.* (2014) A simple, cost-effective but highly efficient system for deriving ventricular cardiomyocytes from human pluripotent stem cells. *Stem Cells Dev* 23: 1704-1716.

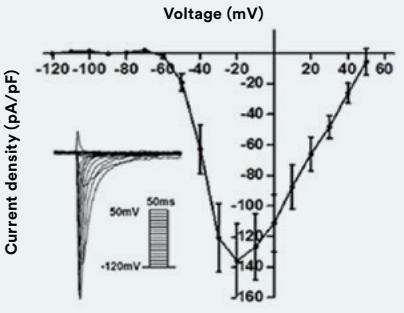
[2]Karakikes *et al.* (2014) Small molecule-mediated directed differentiation of human embryonic stem cells toward ventricular cardiomyocytes. *Stem Cells Transl Med* 3(1): 18-31.

[3]Fu *et al.* (2010) *Stem Cells Dev* 19(6): 773-782, [4]Lieu *et al.* (2013) *Circ Arrhythm Electrophysiol* 6(1): 191-201, [5]Liu *et al.* (2007) *Stem Cells* 25(12): 3038-3044, [6]Lieu *et al.* (2009) *Stem Cells Dev* 18(10): 1493-1500, [7]Li *et al.* (2014) *Heart Rhythm* 11(1): 133-140, [8]Poon *et al.* (2013) *PLoS One* 8(10): E77784, [9]Chow *et al.* (2013) *Stem Cells Dev* 22(19): 2678-2690, [10]Wilson *et al.* (2010) *Circ Cardiovasc Genet* 3(5): 426-435, [11]Moore *et al.* (2008) *Biochem Biophys Res Commun* 372(4): 553-558, [12]Poon *et al.* (2015) *Circ Cardiovasc Genet* 8(3): 427-436, [13]Fu *et al.* (2011) *PLoS One* 6(11): E27417.

Human pluripotent stem cell (hPSC)-derived cardiomyocyte immunostained for cardiomyocyte-specific marker troponin T (TnT) and ventricular-specific marker myosin light chain MLC2v.







Majority of the cardiomyocytes produced with our proprietary differentiation protocol exhibit a ventricular (v) action potential (AP) phenotype, as shown by patch clamp AP measurement (left). I_{Na}, a major ionic current contributing to the cardiac ventricular AP, is illustrated as an example; other currents such as I_{K1}, I_{Kr}, and I_{Ks} were also fully characterized (right).

hvCAS

for Assessing Arrhythmias

Effective modeling of arrhythmias *in vitro* is challenging: by definition they are multicellular events that can only be recorded by monitoring conduction patterns in electrically coupled cardiomyocytes. Conventional assays can only use surrogate markers for arrhythmia: the hERG assay, for example, uses inhibition of a single potassium channel exogenously expressed in non-cardiac cell lines as an indicator for proarrhythmic risk. Although these remain the standard tests for arrhythmogenicity, global drug regulators recognize their pitfalls and are actively seeking better alternatives, notably in the Comprehensive *in vitro* Proarrhythmia Assay (CiPA) initiative. Human pluripotent stem cell-derived cardiomyocytes are inherently prone to arrhythmic events when cultured as randomly oriented monolayers. Novoheart's human ventricular Cardiac Anisotropic Sheet (hvCAS) assay utilizes specially designed microgrooved substrates that physically guide hvCMs to align in a similar manner to that in the native human ventricle[1-4]. The aligned cells show anisotropic electrical conduction which has been validated to reduce the baseline arrhythmogenicity compared to monolayers without a cardiomimetic preferential conduction axis[2].

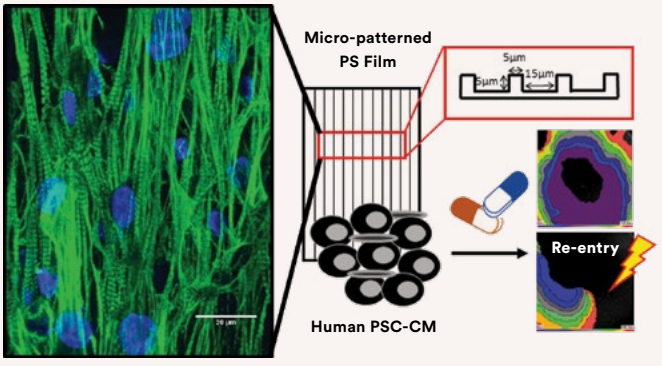
- First validated multicellular optical approach for quantifying cardiac arrhythmia risk, with measurements of cell-to-cell properties and phenomena that are unavailable in single cell studies
- Proprietary substrate optimized for aligning cellular preparations which can mimic the electrical coupling and conduction pattern of the human heart
- Provides stable electrophysiological baseline for comparison with drug treatment or pathological effects
- Optical electrophysiological measurements with voltage-sensitive probes provide much higher spatial resolution than multi-electrode array (MEA) recordings
- Arrhythmias can be directly visualized and detected as spiral wave propagation of electrical signal
- Clinically arrhythmogenic drugs such as flecainide, procainamide, and cisapride have been shown to induce spiral wave formation in hvCAS

References:

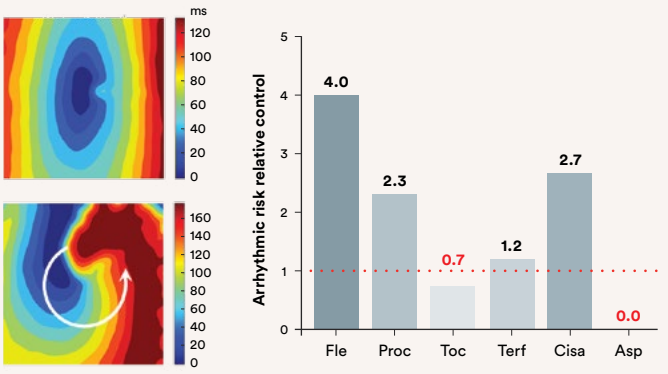
[1]**Shum *et al.* (2017) A micropatterned human pluripotent stem cell-based ventricular cardiac anisotropic sheet for visualizing drug-induced arrhythmogenicity. *Adv Mater* 29: 1602448.**

[2]**Wang *et al.* (2013) Effect of engineered anisotropy on the susceptibility of human pluripotent stem cell-derived ventricular cardiomyocytes to arrhythmias. *Biomaterials* 34: 8878-8886.**

[3]Luna *et al.* (2011) *Tissue Eng Part C Methods* 17(5): 579-588, [4]Chen *et al.* (2011) *Adv Mater* 23(48): 5785-5791.



A diagram illustrating an application of hvCAS composed of human pluripotent stem cell-derived cardiomyocytes cultured on micro-patterned polystyrene (PS) substrate for screening drug-induced re-entry arrhythmias. On the left, immunostaining of cardiac troponin T (green, with blue DAPI staining of nuclei) reveals anisotropic alignment of hvCMs on the substrate.



An isochronal map of an hvCAS under baseline condition exhibiting an elliptical pattern that indicates anisotropic conduction (top left) and of an hvCAS undergoing re-entry arrhythmia (bottom left). Arrhythmic risk assessment by hvCAS for tested drugs (right). All drugs known to be associated with Torsades de Pointes, a specific type of ventricular arrhythmia, showed an elevated risk in hvCAS assessment. Fle: flecainide, Proc: procainamide, Toc: tocainide, Terf: terfenadine, Cisa: cisapride, Asp: aspirin.

hvCTS

for Assessing Human Ventricular Muscle Contractility

Contractile performance is an essential function of the human heart, yet conventional 2-D cardiomyocyte cultures are inadequate for assessing contractility as they cannot perform physiological contractions on rigid plasticware. Tissue engineering offers a superior contractile assay in the form of Novoheart's human ventricular Cardiac Tissue Strip (hvCTS), which is structurally and functionally similar to native trabecular muscle. This assay consists of aligned hvCMs in a 3-D hydrogel mixture that is constructed using Novoheart's custom-designed bioreactor with integrated force-sensing posts at the ends[1-10]. This model has been validated as a sensitive and reliable predictor of clinical effects of drugs or pathologies on cardiac contractility[1-5].

- Contractile muscle strip designed for direct and non-invasive assessment of contractility through measurement of active and passive force
- Sensitive and reliable predictor of clinical effects of drugs and pathologies on cardiac contractility, validated in blinded drug screening study and disease models
- Custom-designed bioreactor not only enables tissue fabrication, but also allows non-invasive and repeated assessments for longitudinal studies over days or weeks
- Can be electrically paced for controlled beat rate

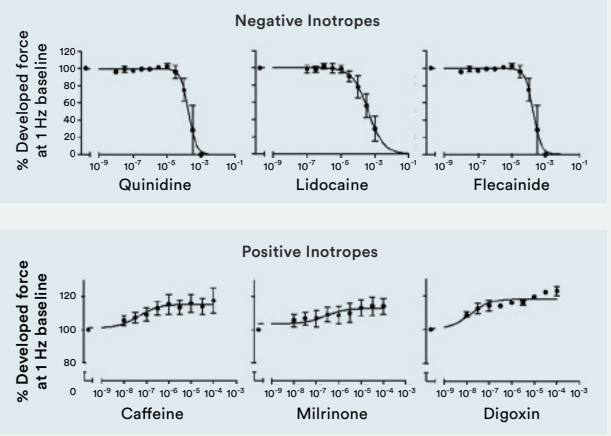
References:

[1]Turnbull *et al.* (2014) Advancing functional engineered cardiac tissues toward a preclinical model of human myocardium. *FASEB J.* 28: 644–654.

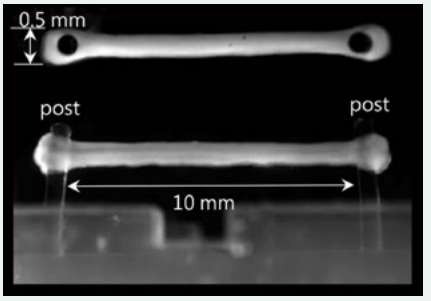
[2]Lee *et al.* (2017) Machine learning of human pluripotent stem cell-derived engineered cardiac tissue contractility for automated drug classification. *Stem Cell Reports* 9(5): 1560-1572.

[3]Stillitano *et al.* (2016) *Eur Heart J.* 37: 3282-3284, [4]Ceholski *et al.* (2018) *J Mol Cardiol* 119: 147-154, [5]Cashman *et al.* (2016) *PLoS One* 19;11(1): E0146697, [6]Cashman *et al.* (2016) *J Vis Exp* 109: E53448, [7]Mayourian *et al.* (2017) *Circ Res* 121(4): 411-423, [8]Ceholski *et al.* (2017) *Stem Cell Res* 23: 77-86, [9]Mayourian *et al.* (2018) *Circ Res* 122(1): 167-183, [10]Mayourian *et al.* (2018) *Circ Res* 122(7): 933-944.

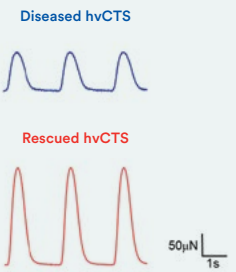
Blinded drug tests on hvCTS predicted and classified known negative, positive, and null inotropes.

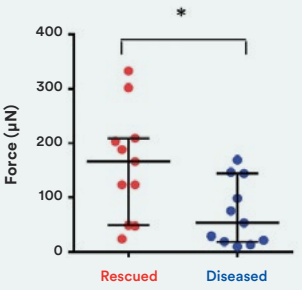


Top and side views of hvCTS anchored by flexible posts in the custom-designed bioreactor.

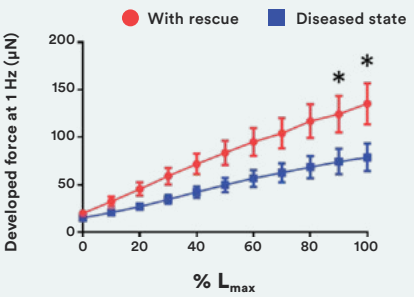


hvCTS disease model in custom bioreactor showing compromised contractile function which was rescued by overexpression of the wild type gene.





Advanced isometric force measurement system is capable of detecting more subtle effects on hvCTS function, as demonstrated here in a disease model with or without rescue.



“Heart-in-a-jar”

for the most sophisticated model of cardiac function

Novoheart’s fluid-ejecting 3-D human ventricular Cardiac Organoid Chamber (hvCOC), a.k.a. “human heart-in-a-jar” is the only technology available to date that enables the clinically informative assessment of human cardiac pump performance which no other human engineered heart tissues on the market are capable of. Combined with complementary custom-designed hardware and software, the best-in-class “human heart-in-a-jar” allows drug screening and disease modeling with unprecedented biofidelity.

Reference:

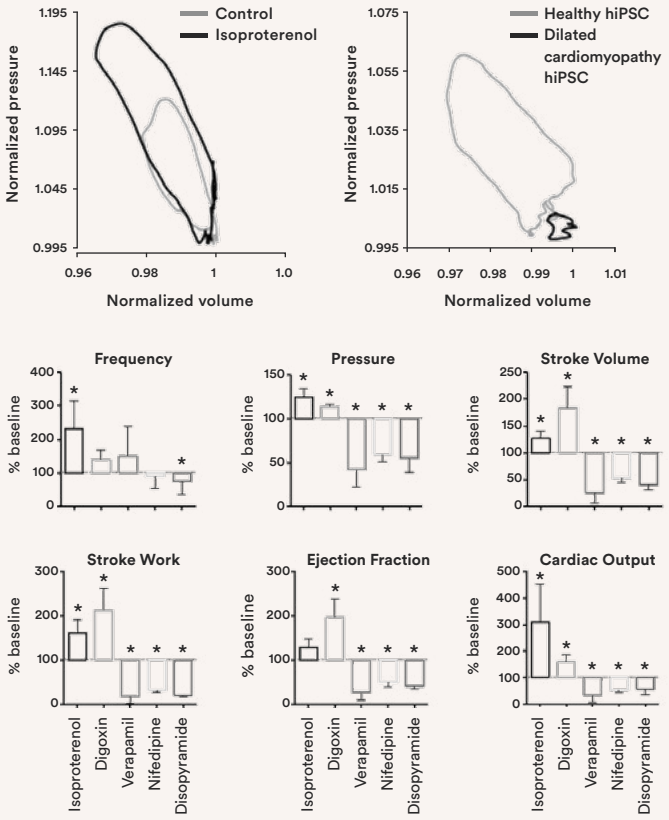
Li R. *et al.* (2018) Bioengineering an electromechanically functional miniature ventricular heart chamber from human pluripotent stem cells. *Biomaterials* 163: 116-127.

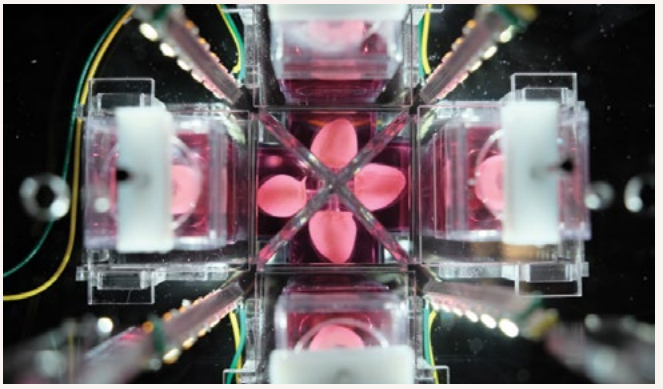
- Custom-designed bioreactor for 3-D chamber fabrication followed by mechanical and electrophysiological measurements
- Fluid-pumping human ventricular chamber provides unique measurements of:
  - o Pressure
  - o Stroke volume
  - o Stroke work
  - o Ejection fraction
  - o Cardiac output

- 3-D environment enhances maturation of ventricular cardiomyocytes, providing a superior model for adult ventricular function
- Ideal for testing pharmacological responses and disease modeling
- Supports controlled electrical pacing by field or point stimulation

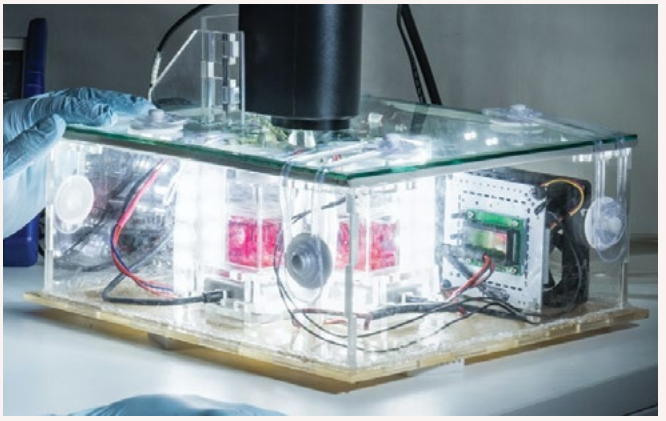
- Version 2.0 now enhanced with hardware and software engineering and integrated with Novoheart’s proprietary machine learning platform to deliver higher efficiency, throughput, accuracy, and sensitivity compared to the first generation
- Enhanced sensitivity to positive inotropes

The “human heart-in-a-jar” model uniquely offers 3D cardiomimetic fluid pumping with physiological pressure/volume relationships, for applications including drug screening and disease modeling.





Our bioreactor design allows precise temperature and environmental control, and simultaneous, 24-7 monitoring of multiple “human heart-in-a-jar” constructs, to capture intermittent or infrequent events.



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