A tissue-engineered scale model of the heart ventricle

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Laboratory studies of the heart use cell and tissue cultures to dissect heart function yet rely on animal models to measure pressure and volume dynamics. Here, we report tissue-engineered scale models of the human left ventricle, made of nanofibrous scaffolds that promote native-like anisotropic myocardial tissue genesis and chamber-level contractile function. Incorporating neonatal rat ventricular myocytes or cardiomyocytes derived from human induced pluripotent stem cells, the tissue-engineered ventricles have a diastolic chamber volume of ~500 µl (comparable to that of the native rat ventricle and approximately 1/250 the size of the human ventricle), and ejection fractions and contractile work 50–250 times smaller and 10⁴–10⁸ times smaller than the corresponding values for rodent and human ventricles, respectively. We also measured tissue coverage and alignment, calcium-transient propagation and pressure-volume loops in the presence or absence of test compounds. Moreover, we describe an instrumented bioreactor with ventricular-assist capabilities, and provide a proof-of-concept disease model of structural arrhythmia. The model ventricles can be evaluated with the same assays used in animal models and in clinical settings.

Laboratory models of the heart are used to gain a mechanistic understanding of heart function in health and disease, and to test the safety and efficacy of potential therapeutics. The heart is studied at multiple scales in vitro, from cellular assays to excised or engineered tissues and ‘organ-on-chip’ microphysiological systems that recapitulate integrated aspects of specific pathological conditions. Functional readouts obtained using engineered heart tissues include contractile forces and electrophysiological measurements, but direct comparison with natural ventricle pressure and volume dynamics requires three-dimensional (3D) contractile cardiac chambers. To obtain these measurements, animal models and isolated heart preparations are evaluated using preclinical and cardiac chambers. To obtain these measurements, animal models and isolated heart preparations are evaluated using preclinical and clinical measurement modalities such as catheterization, echocardiography, and magnetic resonance imaging. Data obtained using these methods allow direct comparison with patient data, but differences in genetics, physiology and disease aetiology limit the utility of animal models for developing therapeutic interventions. These and other limitations associated with animal models, for example, high maintenance costs and low experimental throughput, motivate the development of alternative in vitro cardiology assays based on human cells.

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have emerged as a promising tool for in vitro cardiology, with the potential to eliminate interspecies and interpersonal variations through patient-specific derivation. These cells are assembled into functional engineered heart tissues, including muscular thin films or 3D muscle strips, to model contractile pathophysiologies, promote hiPSC-CM maturation, and produce drug responses that are increasingly comparable to human patients. However, the lack of in vitro models based on hiPSC-CMs that accurately reproduce the architecture and functional output of the heart chambers is a significant limitation because tissue performance cannot be directly compared with animal or human heart performance, which are evaluated by measuring changes in chamber pressure and volume. Engineered cardiac organoid chambers comprising cardiomyocytes embedded in isotropic hydrogels show significant promise for obtaining intra-ventricular pressure measurements, but the lack of a scaffold to guide cell assembly hampers the formation of organized tissues that recapitulate the laminar architecture of the native myocardium. Instead, we decided to use 3D scaffolds to provide anisotropic cues that guide the same laminar tissue formation we obtained on patterned monolayers and extend these design rules to tissues composed of multiple cell layers. Based on reports describing anisotropic cardiomyocyte assembly in fibrous scaffolds, we reasoned that by taking design inspiration from the human myocardial tissue architecture and recreating it using a nanofibre production system, we could build 3D tissue-engineered ventricle scaffolds. Nanofibres provide biochemical and nanotopographical structural cues with sufficient fidelity to guide cell adhesion, orientation, shape and assembly. We therefore hypothesized that ventricle-shaped nanofibrous scaffolds would promote cardiomyocyte assembly into functional 3D tissue-engineered ventricle chambers.

To test whether nanofibrous scaffolds are suitable for ventricle chamber tissue engineering, we first determined the minimal essential features of human ventricle structure that can be reliably recapitulated using nanofibre production systems. We fabricated ellipsoidal thin-walled chambers composed of a nanofibrous synthetic–natural scaffold that by taking design inspiration from the human myocardial tissue architecture and recreating it using a nanofibre production system, we could build 3D tissue-engineered ventricle scaffolds. Nanofibres provide biochemical and nanotopographical structural cues with sufficient fidelity to guide cell adhesion, orientation, shape and assembly. We therefore hypothesized that ventricle-shaped nanofibrous scaffolds would promote cardiomyocyte assembly into functional 3D tissue-engineered ventricle chambers.

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polymers—protein blend and seeded them with neonatal rat ventricular myocytes (NRVMs) or hiPSC-CMs. Synchronous chamber-level contraction was observed for both cell types after three to five days of culture in the scaffolds. The resulting tissue-engineered ventricle chambers were sutured to tubing or bioreactor components through which catheter sensors were introduced and stable contraction of both NRVM and hiPSC-CM ventricles permitted time-dependent pressure and volume measurements. We then provide a proof-of-concept structural arrhythmia disease model by measuring spontaneous calcium activity of an engineered rat ventricle before and after inflicting geometrically controlled injuries. Our findings confirm that tissue-engineered model ventricle contraction can be monitored by catheter sensors, and suggest that model ventricles are suitable for cardiac arrhythmia studies. These results have implications for preclinical cardiology and regenerative medicine research where human organ models are sought to improve translation of therapeutic strategies.

Model assumptions for tissue-engineered ventricle chambers
To begin, we developed a model cardiac chamber based on tabulated structural and functional properties of the human left ventricle. In the native heart, overlapping myocardial fibres are arranged into distinct laminae of four to six myocytes thick and separated from adjacent laminae by an extracellular collagen network. We reasoned that we could recapitulate the structure of model myocardial laminae within an ellipsoidal chamber geometry. Our overall strategy to produce a scale model of the human left ventricle chamber for pressure–volume catheterization is depicted schematically in Fig. 1a. We made the following assumptions from the outset: (1) we would build a scale model of the human left ventricle chamber with a diastolic chamber volume of ~500 μl (~2 × rat, ~1/250 human); (2) the ventricle wall would not be vascularized and its thickness limited to ~0.1 mm (~1/10 rat, ~1/100 human) to maintain high cell viability within the diffusion-limited environment; (3) the ventricle scaffold material would be a well-known protein–polymer mixture to ensure production reproducibility using existing nanofibre systems; and (4) cardiomyocyte alignment would be circumferential throughout the scaffold, representing a thin-walled approximation of the helical alignment within the native ventricular wall.

Ventricle scaffold
Healthy ventricular musculature (myocardium) arises from precisely coordinated multiscale integration of physical forces transmitted between cells and the extracellular matrix (ECM), as well as between neighbouring cells. The myocardial ECM is fibrillar and anisotropic, and provides nanotopographical cues that guide cardiomyocyte alignment and assembly, ultimately forming a helicoid structure in tissues composed of immature cells where cell geometry, calcium activity, and direction of force transmission lead to the wall motion of amplitude, frequency, and speed. The kinetic energy associated with the wall motion of amplitude, frequency, and speed scales as \( \rho R A \omega^2 \), while the potential energy of deforming the shell is dominated by long wavelength bending deformations and scales as \( E_h (A / R^2) B^2 \), where \( E_h \) is the bending stiffness, and the curvature scales as \( A / R \). Balancing the two yields \( E_h (A / R^2) B^2 \sim \rho R A \omega^2 \), so that \( \omega \sim \sqrt{E_h / (\rho R)} \). Using parameter values from our scaffolds (\( R = 5 \text{ mm}, h = 0.1 \text{ mm}, 50 \text{kPa} < E < 500 \text{kPa} \)) gives bending resonant frequencies of 0.67 Hz < \( f_b < 2 \text{ Hz} \), where \( f_b = \omega / (2\pi) \), which match the contraction rates of cardiomyocytes. This suggests a natural design principle to engineer artificial ventricles as a function of the natural frequencies of cardiomyocyte contraction.

Tissue-engineered ventricle chamber
To build our model ventricle chambers, we seeded ventricle-shaped scaffolds with either NRVMs or hiPSC-CMs. Synchronous, coordinated ventricle contraction developed spontaneously after three to five days of culture (Supplementary Videos 3 and 4) and persisted for the duration of experimental procedures (Supplementary Video 5), conducted fourteen days after seeding. Calcium imaging, which has been used as a surrogate for the action potential, revealed continuous excitation propagation on the ventricle surfaces, confirming electrical continuity throughout the ventricle constructs (Fig. 1d and Supplementary Videos 6 and 7). Mean calcium wavefront propagation velocity from apex to base was 9.33 cm s\(^{-1}\) or 5.2 cm s\(^{-1}\) for NRVM or hiPSC-CM ventricles, respectively. These values are comparable to those observed in tissues composed of immature cells where cell geometry, calcium handling, gap junction expression and spatial distribution, as well as other factors limit the conduction velocity when compared with mature tissues. Immunostaining confirmed fibre-directed anisotropic cell alignment and infiltration within the chamber wall (Fig. 2, Supplementary Figs 3 and 4, and Supplementary Video 8), enabling us to quantify cell alignment using a metric known as the orientational order parameter (OOP) that ranges from zero (random organization) to one (perfect alignment). Both NRVM and hiPSC-CM ventricles showed high alignment based on OOP analysis of F-actin stains (Supplementary Fig. C), with OOP values of 0.84 ± 0.02 (NRVM) and 0.85 ± 0.02 (hiPSC-CM).

Intraventricular pressure and volume measurements
To determine whether our engineered ventricles exhibited in vivo-like chamber contraction, we measured time-dependent intraventricular pressure and volume by catheterization (Fig. 3 and Supplementary Fig. 5), an established method for heart chamber performance evaluation. We sutured the engineered ventricles around tubing and inserted a pressure–volume (PV) catheter into the chamber midway.
from base to apex (Supplementary Fig. 5a). We performed catheter calibrations (Supplementary Fig. 5) and acquired 2,000 data points per second (Supplementary Fig. 6). Exported data were processed for time- and frequency-domain analyses, which are commonly used to quantitatively assess cardiovascular function\(^5,^6\) (Supplementary Fig. 7). As an initial validation of our experimental system, we investigated ventricle contractile responses to gross alterations in the composition of the extracellular bath solution. Whereas regular beat frequencies were observed in calcium-containing M199 or Tyrode’s solution, exposure to calcium-free PBS led to broad beat-rate distributions (Supplementary Fig. 7b). Furthermore, we observed an expected reduction in beat rate, as judged by a shift in the frequency spectrum, as bath temperature was decreased from physiological 37 °C to less than ~30 °C (Supplementary Fig. 7c). These results demonstrate that tissue-engineered model ventricles can be functionally interrogated and myocardial performance assessed using catheter-based PV measurements (Fig. 3b). Measured differences in chamber pressure were ~50μmHg (rat or human) and the volume change was ~5μl (rat) or 1μl (human). Thus, ejection fractions were ~1% (rat) or ~0.2% (human) and stroke work was \( W_\text{S} \sim 0.25 \text{mmHg} \times \mu\text{l} (\text{rat}) \) or \( W_\text{S} \sim 0.05 \text{mmHg} \times \mu\text{l} (\text{human}) \).

A fundamental aspect of myocardial function is the response to adrenergic agonists, which has been studied extensively in both rodents and humans, making \( \beta \)-adrenergic response a good test of the ability of our engineered ventricles to recapitulate normal cardiac pharmacology\(^5,^6\). To assess the chronotropic response of our engineered ventricles to a \( \beta \)-adrenergic receptor agonist, we subjected them to concentrations of isoproterenol ranging from 0.1 nM to 0.1 mM. PV loops measured before and after isoproterenol exposure showed an isoproterenol-induced reduction in stroke work, concomitant with an increase in beat frequency (Fig. 3b,c). Continuous intraventricular pressure and volume measurements enabled frequency-domain analysis of the chronotropic response of either NRVM or hiPSC-CM ventricles (Supplementary Fig. 7d). The spontaneous beat rate of NRVM ventricles (~130 ± 15 bpm) was higher than hiPSC-CM ventricles (~85 ± 15 bpm), and both increased by ~40% following exposure to isoproterenol.
to $10^{-4}$ M isoproterenol. Ventricle beat rates showed a positive chronotropic response over the concentration range in both the NRVM and hiPSC-CM ventricles (Fig. 3c), as expected for healthy NRVM$^{59}$, hiPSC-CM$^{60}$, engineered hiPSC-CM cardiac tissues$^{16}$ and human patients$^{61}$.

**Bioreactor for modular assembly of ventricles and valves**

Laboratory models of biological tissues often benefit from culture in bioreactors or microfluidic technologies where fluid exchange can be automated and in situ measurements obtained. To support tissue-engineered ventricle culture, we built a self-contained instrumented heart bioreactor (HBR; Fig. 4, Supplementary Figs 8 and 9, and Supplementary Video 9). The HBR includes both intraventricular and extraventricular flow loops, separate chambers for optional valve inserts and additional access ports for catheters (Fig. 4a and Supplementary Fig. 8). Cyclic pressure applied to the extraventricular loop drives intraventricular fluid flow via assisted ventricle contraction, and unidirectional flow can be achieved using commercially available valves or with custom valve inserts in the HBR. We demonstrated assisted pumping in the absence of drugs by
controlling pressure delivered to the external (assist) channel using a pressure-driven programmable pump and cast-moulded silicone tricuspid valves to direct flow (Fig. 4a and Supplementary Fig. 9). In this configuration, matured ventricles can be exposed to externally controlled physiological or pathological pressure variations and PV loops acquired with or without valves (Fig. 4b), emphasizing the potential to study heart failure where differential effects of various organ substructures are measured. Lastly, to highlight the translational nature of this technology, we monitored ventricle contraction by ultrasound through removable elastomeric windows in the HBR (Fig. 4c, Supplementary Fig. 10, Supplementary Videos 10 and 11). Using small animal echocardiography equipment, we recorded both unassisted natural spontaneous ventricle contraction (Fig. 4c, left, and Supplementary Video 10) and HBR-assisted contraction (Fig. 4c, right, and Supplementary Video 11). Echocardiography performed in the absence of extraventricular tissues can improve image clarity compared with in vivo studies, underscoring the advantage of organ modularity in our HBR.

Fig. 3 | Intraventricular PV data obtained by tissue-engineered ventricle catheterization. a, An overview of the ventricle catheterization procedure. Catheters were fed through tubing on which the ventricle base was sutured. Ventricles were submerged within a 3.5 cm Petri dish bath, which was mounted on a temperature-controlled heating stage. Catheter readouts fed to signal amplification instruments provided real-time measurements of intraventricular pressure and volume. Y, single-sided amplitude spectrum of the fast Fourier transformation. b, Intraventricular pressure and volume measured by catheterization of NRVM- or Cor.4U hiPSC-CM-based ventricles. Exposure to isoproterenol (Iso) reduced stroke work of both rat and human ventricles. Here, pressure and volume were normalized by polynomial fit to remove measurement drift occurring over the course of multiple ISO doses.<sup>P</sup> < 0.05; P values were P = 0.04 (NRVM, N = 4 ventricles) and P = 0.038 (Cor.4U, N = 3 ventricles). c, Iso-proterenol concentration. d, Isoproterenol-dependent beat rates for rat (N = 8) and human hiPSC-CM (N = 4) engineered model ventricle chambers. Time-domain recordings of chamber volume were Fourier-transformed (FFT) to obtain beat rates. The spontaneous beat rate of NRVM ventricles (-130 ± 15 bpm) was higher than hiPSC-CM ventricles (-85 ± 15 bpm), and both increased by ~40% following exposure to 10<sup>−4</sup>M Iso. <sup>P</sup> < 0.05, <sup>**</sup>P < 0.001, compared with baseline (no ISO), one-way ANOVA with Tukey post-hoc test. Exact P values for Iso-dependent beat rates (Hz) were P = 0.0325 (NRVM, N = 8 ventricles) and P = 0.524 (Cor.4U, N = 4 ventricles). Exact P values for Iso-dependent beat rate (% increase) were P < 0.001 for baseline NRVM versus the highest Iso dose of 10<sup>−4</sup>M. Exact P values for NRVM at the smallest three doses (10<sup>−6</sup>M, 10<sup>−5</sup>M and 10<sup>−4</sup>M) versus the largest dose (10<sup>−4</sup>M Iso) were P = 0.003, P = 0.006 and P = 0.008, respectively. The exact P value for NRVM dosed with 10<sup>−4</sup>M Iso versus 10<sup>−4</sup>M Iso was P = 0.011. For Cor.4U, differences in beat rate were not statistically significant (P = 0.183). In all cases, measurements were performed on day 14. Data are presented as box plots with individual data points overlaid, where lower or upper edges of the box represent 25th or 75th percentiles, the middle bar is the median, dashed red bar is the mean, and whiskers are minimum and maximum values.
Structural arrhythmia disease model

In myocardial tissues, anatomical structures can introduce heterogeneities of impulse conduction that lead to ventricular tachycardia, including spiral waves anchored to anatomical defects\(^3\)\(^3\),\(^6\)\(^2\). Here, we show a proof-of-concept structural arrhythmia disease model by measuring spontaneous calcium activity of an engineered rat (NRVM) ventricle before and after inflicting geometrically controlled (1 mm diameter) injuries (Fig. 5). Plane wave propagation was observed before injury (Fig. 5a, top), whereas injured ventricles generated spiral waves that were pinned to the controlled anatomical defects.
anatomical defects (Fig. 5a, bottom panels, and Supplementary Video 12). Calcium fluorescence intensity measured near the rotor poles of a single-hole injury model showed consistent phase difference and a rotation rate of ~5 Hz (Fig. 5b, Supplementary Fig. 12, and Supplementary Video 13). The two-hole injury generated counterpropagating spiral waves that converged and propagated through the inter-hole region with each cycle. Black arrows in isochrone images indicate direction of calcium wave rotation and propagation. Scale bars, 3 mm. b Calcium fluorescence intensity measurements near the rotor poles of the single-hole injury showed consistent phase difference and a rotation rate of ~5 Hz. Experiments were performed at day 12 and spontaneous activity was recorded without external stimulation for all cases. Measurements shown in a were acquired using a 5 ms exposure window, whereas those shown in b were acquired using a 10 ms exposure window. In all cases, the temporal derivative of calcium fluorescence intensity is displayed as a heat map ranging from blue (minimum) to red (maximum), overlaid on a greyscale image of the ventricle surface. A and B (right) refer to points selected on opposite sides of the injury site. Graph in b shows time-dependent calcium intensity at points A and B, which are out of phase.

Discussion
Preclinical cardiology and regenerative medicine research will benefit from an expanding set of in vitro models where human heart structure and function are examined at multiple scales. Here, we took inspiration from the myocardial ECM and used nanofibers to extend to 3D tissue-engineered cardiac chambers the same engineering design principles that led to increasingly functional laminar cardiac tissues. For near-future design iterations that concentrate on myocardial engineering, we suggest several steps that may precede the addition of non-cardiomyocytes and ventricle vascularization. These ‘obstacles and opportunities’ facing bioartificial hearts include improvements to scaffolds, cardiomyocytes and culture protocols, briefly outlined below.

Scaffold engineering should account for the fact that the myocardium is a composite in which the dominant stiffness shifts from myocytes to the ECM with increasing strain, emphasizing the ECM’s role maintaining structural integrity. In our scaffold, gelatin was used to enhance cell and biomolecule adhesion during cell seeding, and the polymer backbone (PCL) provided structural features (microporosity, nanotopography, anisotropy) that resisted degradation during culture. These features provided sufficient structural integrity for ventricle culture and they guided cardiomyocyte assembly, but alternative nanofibrous materials used for cardiac tissue engineering may improve scaffold customization. For example, the tensile elastic modulus of our scaffold in the direction of fibre alignment (E ~ 500 kPa) was higher than healthy ventricular ECM (E ~ 350 kPa) and may have limited cardiomyocyte shortening during contraction. The balance between structural support during culture, scaffold degradation rate and ventricle mechanics should be refined. Alternative biodegradable polymers or bioprotein mixtures reflecting age-dependent composition of natural myocardial ECM may be used to improve scaffold elasticity, recapitulate specific disease or development phases, or promote scaffold replacement with cell-secreted ECM. Biomolecular nanofibres can be produced using precipitation systems for direct use as
scaffolds or dispersed in printable ‘inks’, where ink and print conditions influence printed nanofibre orientation. Our work suggests that including a nanofibrous component to printable inks would be advantageous for directing cardiomyocyte shape and assembly in printed scaffolds or tissues. Alternative chamber shapes, anatomical features and scaffold fibre alignments may be used to study their effects on cardiomyocyte assembly and electromechanical integration. In all cases, design rules for contractile 3D chambers should account for chamber contraction as a function of chamber size, shape and elastic modulus. Our calculations of resonant bending frequencies suggest a natural design principle to engineer cardiac chambers as a function of their intended beat rates.

Cardiomyocyte spontaneous beat rates in our model ventricles were similar to other in vitro assays based on NRVMs or hiPSC-CMs, showing moderate positive chronotropy (−40% beat rate). We expected to observe variance between ventricles, both in terms of beat rates and their response to isoproterenol, given the heterogeneous pace-making phenotypes within the cardiomyocyte population. Our observation of isoproterenol-induced stroke work reduction (Fig. 3b) is consistent with negative force–frequency relationships observed in tissues based on immature cardiomyocytes, including NRVMs and hiPSC-CMs. The moderate positive chronotropy and reduction of stroke work observed at high isoproterenol doses are characteristic of immature tissues exhibiting poorly developed calcium handling properties, suggesting that maturation protocols may be required to obtain positive force–frequency relationships, as observed in healthy adult human ventricular myocardium.

Critical to the advance of tissue-engineered ventricles will be the development of in situ stem cell proliferation, differentiation and maturation protocols. The cell density in our ventricles, estimated by counting immunostained (4,6-diamidino-2-phenylindole (DAPI)) NRVM ventricle surfaces and cross-sections (Supplementary Fig. 3), was ~90,000 cells mm⁻³ for a total cell number ~2.3 × 10⁶ in a 26 mm³ scaffold volume, which is approximately tenfold less than 21-day-old or 3-month-old rat hearts. The ejection fraction of our tissue-engineered rat ventricles was ~1% (1/50 ejection fraction of healthy rats), suggesting that discontinuities in cardiomyocyte coverage limited tissue-engineered ventricle contractile strength. Novel protocols that proliferate stem cells to confluence within the scaffolds may overcome the coverage limitation, provided that in situ differentiation and maturation can be efficiently achieved following the proliferation phase. Preliminary work in our laboratories suggests that we can achieve extended culture periods for NRVMs (Supplementary Fig. 12 and Supplementary Videos 14 and 15) and hiPSC-CMs (Supplementary Figs. 13 and 14) in our nanofibrous scaffolds. For example, proliferative hiPSC (line PGP1-iPSC) seeded in our scaffolds at a density of 10⁶ cells cm⁻² and cultured according to previously described protocols achieved confluence within 4 days of culture (Supplementary Fig. 13a, day 0). When cultured in differentiation media, the resulting hiPSC-CM showed spontaneous contraction and early stages of sarcomeric assembly within 8–12 days of culture (Supplementary Fig. 13a, day 12), and anisotropic sarcomeric assembly with early stages of z-disk alignment after 1 month in culture (Supplementary Fig. 13a, day 30). We also cultured a non-proliferative hiPSC-CM line (Cor.4U) in model ventricle scaffolds for 6 months, demonstrating that tissue anisotropy was preserved (Supplementary Fig. 14a) and stable PV loops could be recorded (Supplementary Fig. 14b). These preliminary results suggest that our scaffolds support functional myocardial tissue production using relatively small numbers of seeded hiPSC that can subsequently be cultured for extended time periods. In these cases, tissue maturation may benefit from bioreactor culture where tissues are exposed to physiological pressures and electromechanical stimulation. Similarly, pathophysiological studies using healthy and diseased human cells that may respond positively to ventricular assist should also be conducted. These future experiments, using systems like ours to evaluate hiPSC-CM tissue formation and electromechanical integration within biosynthetic scaffolds, will inform regenerative medicine practices that aim to restore myocardial function.

A direct comparison of engineered ventricle chamber contractile performance with those of ex vivo animal studies is challenging. For example, our thin-wall chambers powered by immature cardiomyocytes generated small differences in chamber pressure (~50 μmHg) and chamber volume (1–5 μl), equivalent to ejection fractions of ~0.2–1.0%, which are smaller than expected for healthy mammalian ventricles by factors of roughly 50–250. Similarly, the contractile work performed by our ventricles (Wₑ ~0.05–0.25 mmHg×μL, or Wₑ ~7–35 nJ) was less than mouse, rat or human left ventricles by factors of ~10⁴, 10³ and 10¹, respectively. Contractile work in our ventricle models was measured using conductance catheters and was smaller than reported for cardiac organoid chambers based on NRVMs or pluripotent hES2 human embryonic stem cell-derived cardiomyocytes, where volume changes were indirectly assessed from video recordings. Both types of model cardiac chambers generated similarly shaped elliptical PV loops (Fig. 3b and Supplementary Fig. 14), which are expected from ventricles contracting in the absence of valves and show similarities to PV loops obtained from patients with severe valvular insufficiencies. Our use of conductance catheters was advantageous for direct volume readouts but electrical pacing during catheterization was not recommended by the catheter manufacturer and therefore not performed in these studies. Thus, the positive inotropic effects of isoproterenol observed by others during electrical pacing was not observed by us when isoproterenol was administered to spontaneously beating model ventricles (Fig. 3b, right panels). One solution to this challenge may be the use of optogenetics for optical pacing.

Scaling of chamber contractile strength with cardiomyocyte number is expected and it is encouraging to note that contractile strength increased in our preliminary extended culture period experiments (Supplementary Fig. 14). Lastly, our structural arrhythmia disease model reaffirmed the importance of building native-like anisotropic tissues that are important for both normal propagation and arrhythmogenesis. Precise anatomical defects applied to our tissue-engineered model ventricles generated stable pinned rotors and spiral waves (Fig. 5, Supplementary Fig. 11, and Supplementary Videos 12 and 13) that have so far not been demonstrated in engineered cardiac chambers.

In summary, our results demonstrate the feasibility of engineering functional scale models of the heart chambers, where tissue assembly is guided by a nanofibrous culture substrate, with implications for multiscale in vitro cardiology assays and regenerative medicine research. We used primary rat cardiomyocytes and human-stem-cell-derived cardiomyocytes because rat is the current industry standard for the study of heart disease in vitro and in vivo, yet the use of human models will be key for personalized medicine. In this respect, we believe it is important to include use of pre-/clinical grade measurement technologies to provide a more seamless transition between in vitro and in vivo models. This will extend patient-specific in vitro assays from individual cells to complete organ models, and our design rules provide a path towards engineering heart chambers with functional performance increasingly comparable to native organs. Taken together, the capability to tissue-engineer functional model ventricles, and the modular bioreactor technologies designed for in situ instrumentation and functional assessment, provide a suitable system for in vitro cardiology studies that increasingly translate to clinical outcomes.

**Methods**

**Scaffold fabrication.** **Polymer solutions.** Ventricle scaffold material precursors were PCL (Sigma-Aldrich 440744) and gelatin type A (Sigma-Aldrich G2500) dissolved...
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Ventricle scaffold fabrication. Ventricle scaffolds were produced by pull spinning (Fig. 1b and Supplementary Fig. 1a) PCL/gelatin nanofibres onto ellipsoidal collection mandrels (half-ellipsoid with radii: a = b = 4.5 mm, c = 9 mm; Supplementary Fig. 1b). Pull spinning incorporates a high-speed (up to 45,000 r.p.m.) rotating bristle that dips into a fixed, continuous polymer source and pulls the polymer column into an anistropic network of non-woven nanofibres. The solution was injected through a needle (18G flat tip; BD Biosciences) at a rate of 0.2 ml min⁻¹ for a total duration of 5 min. Bristle rotation rate was 30,000 r.p.m. Ventricle mandrels were connected to a DeWALT DC 720 1/2 cordless drill driver operating at 300 r.p.m. and rotated at a rate of 300 r.p.m. to 30 cm from the polymer source. All pull-spinning fabrication was performed within a chemical fume hood and the relative humidity was between 10 and 20%. Scaffolds were stored in de-ionized water for 48 h and sterilized by overnight exposure to ultraviolet radiation within a tissue culture hood before use. Overnight exposure to ultraviolet radiation was sufficient to prevent bacterial contamination but future work with thicker scaffolds may benefit from more thorough sterilization procedures.

Scaffold structural and biochemical analysis. X-ray microcomputed tomography. Microcomputed tomography was performed at Harvard University’s Center for Nanoscale Science (CNS). We used an X-Tek HMXST225 system (Nikon Metrology) equipped with a 225 kV microfocus X-ray source with a 3 µm focal spot size. We used an aluminum target and 115 kV accelerating voltage. Image acquisition and reconstruction was performed using the following software suites: InspetX (X-ray imaging and computed tomography acquisition), CT Pro 3D (volume reconstruction), VG Studio MAX 2.2 (3D volume visualization, render and analysis) and Amira (3D volume visualization, rendering and analysis)

Scanning electron microscopy. All SEM imaging was done using a field-emitting electron microscope (FESEM Ultra Plus, Zeiss) at a voltage of 15 kV to image scaffold and tissue fibre alignment. Before imaging, all samples were sputter coated with 5 nm of platinum/palladium (Pt/Pd) using a Quorum Sputter Coater (EMS 300T D, Quorum Technologies) to reduce charge accumulation and tissue decomposition during imaging. Decellularized human tissue was prepared using a previously published SDS (sodium dodecyl sulfate) profusion protocol80 and dehydrated in serial ethanol washes. Dehydrated tissues were then dried using a SAMDRI critical point drier (931 Series SAMDRI, Tousimis) before sputter coating to ensure complete removal of interstitial fluids.

Mechanical testing. To measure elastic modulus, we produced sheets of nanofibrous material (Supplementary Figs 1 and 2), collected on flat glass coverslips, using the same pull-spinning conditions as for ventricle chamber production. Square samples were laser cut (10 × 10 mm) from these sheets and loaded onto 5 × 5 mm mounting tines for biaxial tensile testing (2.5 N load cells; Biotest, CellScale). A pre-stress of 5 mN was applied before running four biaxial preconditioning cycles at 5% strain rate to 20% strain. Using the original dimensions of the nanofibrous samples, the strain-strain curve was then calculated (same strain rate and strain were used as preconditioning). To replicate in vitro conditions, tensile measurements were performed in PBS at 37 °C.

X-ray photoelectron spectroscopy. A K-Alpha X-ray photoelectron spectrometer (XPS) and Advantage software (K-Alpha XPS, Thermo Scientific) was used to evaluate fresh spun and wetted scaffold composition in time. Pieces of 75/25 PCL/gelatin scaffold (5 × 5 mm) were wetted in 11 of ultra-pure water and stored in an incubator at 37 °C for up to 1 week. Fresh-spun and wetted samples removed from the water bath daily were dried for 12 h under vacuum and their composition evaluated using the XPS system (s = 3 scaffold pieces and XPS measurements per time point). Each sample was scanned at 20000× and all peaks were deconvoluted. Surface debris and was survey scanned over a 400 µm spot size. Gelatin content was estimated based on the measured presence of nitrogen in the sample and the amount of solvent (HFIP) was estimated based on the measured presence of fluorine, each normalized to the element's percentage within their respective molecule.

Fourier transform infrared spectroscopy. We used a Bruker FTIR Microscope (Lumos, Bruker) in attenuated total reflection mode to measure the infrared spectra of the nanofibers. Data plotting was conducted with custom software written in MATLAB (MathWorks).

Experimental animals. NRVMs were isolated from two-day-old neonatal CRL: CD (SD) rats, and left ventricle histological preparations were obtained from adult female CRL: CD (SD) rats. All procedures were approved by the Harvard Animal Care and Use Committee and all research personnel handling animals were appropriately qualified and trained by Harvard’s Office of Animal Resources, under the direction of the Attending Veterinarian. Pups were euthanized using a method consistent with the recommendations of the 2013 American Veterinary Medical Association (AVMA) Guidelines on Euthanasia for rodents. Care and use of the animals used in this study comply with the ‘US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training’, the Guide for the Care and Use of Laboratory Animals, and the Animal Welfare Act/Regulations. Harvard University, Faculty of Arts and Sciences (FAS) maintains an Institutional Animal Care and Use Committee (IACUC) as required by the US National Health Service (PHS) Policy on Human Care and Use of Laboratory Animals. All animal protocols must be approved by the IACUC before animals can be ordered.

Cell and tissue culture. NRVMs. NRVMs were isolated from two-day-old neonatal CRL: CD (Sprague-Dawley, 3D) rats using published methods81. All procedures were approved by the Harvard Animal Care and Use Committee. Cells were seeded at a density of three million cells per well, following procedures described below. Standard culture media were used (M199 culture medium supplemented with 0.1 mM MEM nonessential amino acids, 10% heat-inactivated FBS, 10 mM HEPES, 3.5 g l⁻¹ glucose, 2 mM l-glutamine, 2 mg l⁻¹ vitamin B-12 and 50 U ml⁻¹ penicillin). Samples were incubated under standard conditions at 37 °C and 5% CO₂. At 48 h post seeding the media was exchanged with maintenance media (M199 media supplemented as above but with 2% FBS) and was exchanged again every 48 h until use.

hiPSC-CMs. hiPSC-CMs were acquired commercially (lot numbers CB169CL_V1_1M, CB301_CL_V1_1M, CB319CL_V1_1M, CB324CL_V1_1M, CS313CL_V1_4M) and cultured according to manufacturer’s instructions with slight modifications. Briefly, for each cryovial containing one million viable hiPSC-CMs, three wells of a six-well tissue culture plate were coated with 0.01 µ g ml⁻¹ fibronectin (BD Biosciences) for 4 h at 37 °C incubator. According to the manufacturer’s protocol, Cor.4U cells showed significant pre-adhesion of hiPSC-CMs, which we found to be critical for efficient plating. To get good coverage of hiPSC-CMs in our nanofibrous scaffolds, we performed preliminary experiments using the human iPSC line PG1-P1-iPSC, which we have used previously for monolayer muscle thin-film assays81. hiPSC-CMs were seeded on the surface of nanofibrous sheets at a density of 10⁶ cells cm⁻² and proliferated to confluence within four days of culture in mESR culture medium (Supplementary Fig. 13a, left panels). hiPSC-CM differentiation was then induced by changing media from mESR to RPMI/1640+B27 minus insulin with 8 µM CHIR99021, incubating for two days, and subsequently changing media to RPMI/1640+B27 minus insulin with 5 µM IWR-endo-1, and incubated for another two days. We then maintained the culture with fresh RPMI/1640+B27 media every other day. Tissue contraction was observed following 8–12 d of differentiation, concomitant with the expression of sarcomeric and assembly of myofibrillar actin and α-sarcomeric actinin as previously described64. Briefly, washed samples were fixed in 4% paraformaldehyde for 20 min. Four biaxial preconditioning cycles at 5% strain rate to 20% strain. Using the original dimensions of the nanofibrous samples, the strain-strain curve was then calculated (same strain rate and strain were used as preconditioning). To replicate in vitro conditions, tensile measurements were performed in PBS at 37 °C.

Ventricle seeding. We sterilized ventricle scaffolds by exposure to ultraviolet radiation in a biosafety hood overnight. The following morning, we incubated the ventricle scaffolds with 100 µg ml⁻¹ fibronectin (human natural fibronectin, BD Biosciences) in PBS for 90 min, then transferred cells to the scaffold at high density (8000 cells/cm²) and incubated for 1 h. Full media (2 ml) was then added to each well and incubated overnight. Ventricles were then transferred to larger wells, each well containing 5 ml full media and one ventricle, with media refreshed every 48 h until use.

Histochemical staining. Cardiomycyte infiltration and orientation within tissue-engineered ventricles was visualized by fluorescent staining of actin and sarcomers. We stained for F-actin fibres and sarcomeric α-actinin as previously described46. Briefly, washed samples were fixed in 4% paraformaldehyde for 20 min. To prepare ventricle cross-sections, we first cast gelatin moulds of the ventricular volume by pouring dissolved gelatin (20% w/v) into ventricle-shaped moulds, followed by cooling and removal of the solid gelatin from the mould. We then pulled fixed ventricles over the gelatin mould to ensure that thin-walled ventricles maintained an ellipsoidal shape during cryopreservation. Ventricles (with gelatin interior) were stored in PBS+30% sucrose solution overnight at 4 °C, then transferred to 50% sucrose/50% optimal cutting temperature (OCT) water-soluble blend of glycols and resins for 24 h at 4 °C. They were then transferred...
to cryosectioning containers in 100% OCT and stored at −4°C for 48 h. Samples were frozen by partial immersion in 2-methylbutanone which was, itself, partially immersed in liquid nitrogen. Frozen ventricles were stored at −80°C until cryosectioning (by Leica). We obtained cryosections that were transferred to glass microscope slides (Superfrost microscope slides, Sigma) and maintained at room temperature for 2 h prior to storage at −80°C until staining.

Staining and imaging. Ventricle surfaces or cross-sections were permeabilized in 0.5% Triton-X100 for 20 min in PBS at 37°C, followed by 2 h incubation with 1:200 dilutions of mouse anti-sarcomeric α-actinin monoclonal primary antibody (Sigma-Aldrich, clone EA-53, catalogue number A7811-11UL).

Samples were then washed and concurrently incubated with 1:200 dilutions of DAPI (Sigma-Aldrich), phalloidin conjugated to Alexa Fluor 488 (Invitrogen) and goat anti-mouse secondary antibody conjugated to Alexa Fluor 594 (Invitrogen) for 2 h at room temperature. Imaging was performed using a Zeiss LSM 5 LIVE confocal microscope with a Plan-Neofluar 40x/1.3 oil objective. For long-range myocardium tissues, samples were imaged using an Olympus IX83 microscope with an attached Andor spinning disk confocal system, on LUCPlanFLNPh 20 × objective and recorded on a Hamamatsu Orca Flash 4.0 Ci III 1440 at 16-bit depth. Z-stacks were collected over the height of a single myocardium wall, and then sections were spliced together using a standard deviation projection followed by a pairwise stitching algorithm in National Institutes of Health ImageJ. We quantified cell alignment using a metric known as the orientational order parameter (OOP) that ranges from zero (random organization) to one (perfect alignment)16,17, applied to immunostained F-actin.

Optical mapping experiments. Calcium propagation was monitored using a modified tandem-lens microscope (Scimedia) equipped with a high-speed camera (MiCAM Ultima, Scimedia), a plan APO 0.63 × objective, a plan APO 0.63 × modified tandem-lens macroscope (Scimedia) equipped with a high-speed camera (MiCAM Ultima, Scimedia), a plan APO 0.63 × objective, a plan APO 0.63 × modified tandem-lens macroscope (Scimedia) equipped with a high-speed camera (MiCAM Ultima, Scimedia), a plan APO 0.63 × objective, a plan APO 0.63 × modified tandem-lens macroscope (Scimedia) equipped with a high-speed camera (MiCAM Ultima, Scimedia), a plan APO 0.63 × objective, a plan APO 0.63 × modified tandem-lens macroscope (Scimedia) equipped with a high-speed camera (MiCAM Ultima, Scimedia), a plan APO 0.63 × objective, a plan APO 0.63 × modified tandem-lens macroscope (Scimedia) equipped with a high-speed camera (MiCAM Ultima, Scimedia), a plan APO 0.63 × objective, a plan APO 0.63 × modified tandem-lens macroscope (Scimedia) equipped with a high-speed camera (MiCAM Ultima, Scimedia), a plan APO 0.63 × objective, a plan APO 0.63 × modified tandem-lens macroscope (Scimedia) equipped with a high-speed camera (MiCAM Ultima, Scimedia), a plan APO 0.63 × objective, a plan APO 0.63 × modified tandem-lens macroscope (Scimedia) equipped with a high-speed camera (MiCAM Ultima, Scimedia), a plan APO 0.63 × objective, a plan APO 0.63 × modified tandem-lens macroscope (Scimedia) equipped with a high-speed camera (MiCAM Ultima, Scimedia), a plan APO 0.63 × objective, a plan APO 0.63 × modified tandem-lens macroscope (Scimedia) equipped with a high-speed camera (MiCAM Ultima, Scimedia), a plan APO 0.63 × objective, a plan APO 0.63 × modified tandem-lens macroscope (Scimedia) equipped with a high-speed camera (MiCAM Ultima, Scimedia), a plan APO 0.63 × objective, a plan APO 0.63 × modified tandem-lens macroscope (Scimedia) equipped with a high-speed camera (MiCAM Ultima, Scimedia), a plan APO 0.63 × objective, a plan APO 0.63 × modified tandem-lens macroscope (Scimedia) equipped with a high-speed camera (MiCAM Ultima, Scimedia), a plan APO 0.63 × objective, a plan APO 0.63 × modified tandem-lens macroscope (Scimedia) equipped with a high-speed camera (MiCAM Ultima, Scimedia), a plan APO 0.63 × objective, a plan APO 0.63 × modified tandem-lens macroscope (Scimedia) equipped with a high-speed camera (MiCAM Ultima, Scimedia), a plan APO 0.63 × objective, a plan APO 0.63 × modified tandem-lens macroscope (Scimedia) equipped with a high-speed camera (MiCAM Ultima, Scimedia), a plan APO 0.63 × objective, a plan APO 0.63 × modified tandem-lens macroscope (Scimedia) equipped with a high-speed camera (MiCAM Ultima, Scimedia), a plan APO 0.63 × objective, a plan APO 0.63 × modified tandem-lens macroscope (Scimedia) equipped with a high-speed camera (MiCAM Ultima, Scimedia), a plan APO 0.63 × objective, a plan APO 0.63 × modified tandem-lens macroscope (Scimedia) equipped with a high-speed camera (MiCAM Ultima, Scimedia), a plan APO 0.63 × objective, a plan APO 0.63 × modified tandem-lens macroscope (Scimedia) equipped with a high-speed camera (MiCAM Ultima, Scimedia), a plan APO 0.63 × objective, a plan APO 0.63 × modified tandem-lens macroscope (Scimedia) equipped with a high-speed camera (MiCAM Ultima, Scimedia), a plan APO 0.63 × objective, a plan APO 0.63 × modified tandem-lens macroscope (Scimedia) equipped with a high-speed camera (MiCAM Ultima, Scmia...
Data availability. All data generated and analysed during this study are included in the paper and its Supplementary Information.

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References

1. Benam, K. H. et al. Engineered in vitro disease models. *Annu Rev Pathol.* **10**, 195–262 (2015).

2. Tzatzalos, E., Abilez, O. J., Shukla, P. & Wu, J. C. Engineered heart tissues and induced pluripotent stem cells: macro- and microstructures for disease modeling, drug screening, and translational studies. *Adv Drug Deliv Rev.* **96**, 234–244 (2016).

3. Pacher, P., Nagayama, T., Mukhopadhyay, P., Batkai, S. & Kass, D. A. Measurement of cardiac function using pressure–volume conductance catheter technique in mice and rats. *Nat. Protoc.* **3**, 1422–1434 (2008).

4.Ram, R., Mikkelsen, D. M., Theodorescu, C. & Blaxall, B. C. New approaches in small animal echocardiography: imaging the sounds of silence. *Am. J. Physiol. Heart Circ. Physiol.* **301**, H1765–1780 (2011).

5. Bakermans, A. J. et al. Small animal cardiovascular MR imaging and spectroscopy. *Prog. Nucl. Magn. Reson. Spectrosc.* **88–89**, 1–47 (2015).

6. Chandrasheker, P. C. & Pippin, J. J. The human subject: an integrative animal model for 21st century heart failure research. *Am. J. Transl. Res.* **7**, 1636–1647 (2015).

7. Glocstat, C. R. et al. Arrhythmogenic and metabolic remodelling of failing human heart. *J. Physiol.* **594**, 3963–3980 (2016).

8. Karakikes, I., Ameen, M., Termglinchan, V. & Wu, J. C. Human induced pluripotent stem cell-derived cardiomyocytes: insights into molecular, cellular, and functional phenotypes. *Circ. Res.* **117**, 80–88 (2015).

9. Feric, N. T. & Radiscic, M. Maturing human pluripotent stem cell-derived cardiomyocytes in human engineered cardiac tissues. *Adv. Drug Deliv. Rev.* **96**, 110–134 (2016).

10. Eder, A., Vollert, I., Hansen, A. & Eschgenhen, T. Human engineered heart tissue as a model system for drug testing. *Adv. Drug Deliv. Rev.* **96**, 214–224 (2016).

11. Wang, G. et al. Modeling the mitochondrial cardiomyopathy of Barth syndrome with induced pluripotent stem cell and heart-on-chip technologies. *Nat. Med.* **20**, 616–623 (2014).

12. Lind, J. U. et al. Instrumented cardiac microphysiological devices via multimeter three-dimensional printing. *Nat. Mater.* **16**, 303–308 (2017).

13. Boudou, T. et al. A microfabricated platform to measure and manipulate the mechanics of engineered cardiac microtissues. *Tissue Eng. Part A* **18**, 910–919 (2012).

14. Nunes, S. S. et al. Biowire: a platform for maturation of human pluripotent stem cell-derived cardiomyocytes. *Nat. Methods* **10**, 781–787 (2013).

15. Thavandiran, N. et al. Design and formulation of functional pluripotent stem cell-derived cardiac microtissues. *Proc. Natl Acad. Sci. USA* **110**, E4698–E4707 (2013).

16. Mannhardt, I. et al. Human engineered heart tissue: analysis of contractile force. *Tissue Cell Rep.* 7, 29–42 (2016).

17. Huebsch, N. et al. Miniaturized iPS-cell-derived cardiac muscles for physiologically relevant drug response analyses. *Sci. Rep.* **6**, 24726 (2016).

18. Mathur, A. et al. Human iPS/CPC-based cardiac microphysiological system for drug screening applications. *Adv. Drug Deliv. Rev.* **96**, 203–213 (2016).

19. Pacher, P., Nagayama, T., Mukhopadhyay, P., Batkai, S. & Kass, D. A. Measurement of cardiac function using pressure–volume conductance catheter technique in mice and rats. *Nat. Protoc.* **3**, 1422–1434 (2008).

20. Burkhoff, D., Mirsky, I. & Suga, H. Assessment of systolic and diastolic ventricular properties via pressure–volume analysis: a guide for clinical, translational, and basic researchers. *Am. J. Physiol. Heart Circ. Physiol.* **289**, H501–H512 (2005).

21. Lee, E. J., Kim do, E., Azeleolu, E. U. & Costa, K. D. Engineered cardiac organoid chambers: toward a functional biological model ventricle. *Tissue Eng. Part A* **14**, 215–225 (2008).

22. Gonen-Wadmany, M., Gepstein, L. & Seliktar, D. Controlling the cellular architecture and three-dimensional systolic mechanics in canine ventricular myocardium. *Am. J. Physiol. Heart Circ. Physiol.* **280**, H2222–H2229 (2001).

23. Rohr, S., Scholly, D. M. & Kleber, A. G. Patterned growth of neonatal rat heart cells in culture. Morphological and electrophysiological characterization. *Circ. Res.* **68**, 114–130 (1991).

24. Kleber, A. G. & Rudy, Y. Basic mechanism of cardiac impulse propagation and associated arrhythmias. *Physiol. Rev.* **84**, 431–488 (2004).

27. Gonen-Wadmany, M., Gepstein, L. & Seliktar, D. Controlling the cellular architecture and three-dimensional systolic mechanics in canine ventricular myocardium. *Am. J. Physiol. Heart Circ. Physiol.* **280**, H2222–H2229 (2001).

28. Rohr, S., Scholly, D. M. & Kleber, A. G. Patterned growth of neonatal rat heart cells in culture. Morphological and electrophysiological characterization. *Circ. Res.* **68**, 114–130 (1991).

29. Kleber, A. G. & Rudy, Y. Basic mechanisms of cardiac impulse propagation and associated arrhythmias. *Physiol. Rev.* **84**, 431–488 (2004).
55. Akselrod, S. et al. Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. Science 213, 220–222 (1981).
56. Fenske, S. et al. Comprehensive multilevel in vivo and in vitro analysis of heart rate fluctuations in mice by ECG telemetry and electrophysiology. Nat. Protoc. 11, 61–86 (2016).
57. Barrett, A. M. & Carter, J. Comparative chromotopic activity of beta-adrenoceptor antagonists. Br. J. Pharmacol. 40, 373–381 (1970).
58. Brito-Martins, M., Harding, S. E. & Ali, N. N. Beta(1)- and beta(2)-adrenoceptor responses in cardiomyocytes derived from human embryonic stem cells: comparison with failing and non-failing adult human heart. Br. J. Pharmacol. 153, 751–759 (2008).
59. Simpson, P. & Savion, S. Differentiation of rat myocytes in single cell cultures with and without proliferating noncardiac cell cross-striations, ultrastructure, and chromotopic response to isoproterenol. Circ. Res. 50, 101–116 (1982).
60. Moretti, A. et al. Patient-specific induced pluripotent stem-cell models for long-QT syndrome. N. Engl. J. Med. 363, 1397–1409 (2010).
61. Koglin, J., Bohm, M., Vonscheidt, W., Stabilein, A. & Erdmann, E. Antiadrenergic effect of carbachol but not of adenosine on contractility in the intact human ventricle in vivo. J. Am. Coll. Cardiol. 23, 678–683 (1994).
62. Lim, Z. Y., Maskara, B., Aguel, F., Emokpae, R. J. & Tung, L. Spiral wave attachment to millimeter-sized obstacles. Circulation 114, 2113–2121 (2006).
63. Ogle, B. M. et al. Distilling complexity to advance cardiac tissue engineering. Sci. Transl. Med. 8, 342p313 (2016).
64. Feinberg, A. W. et al. Muscular thin films for building actuators and powering devices. Science 317, 1366–1370 (2007).
65. Novosel, E. C., Kleinhaus, C. & Kluger, P. J. Vascularization is the key challenge in tissue engineering. Adv. Drug Deliv. Rev. 63, 300–311 (2011).
66. Lumbscheid, M. S., Baldwin, J. T. & Buxton, D. B. Building a bioartificial heart: obstacles and opportunities. J. Thorac. Cardiovasc. Surg. 153, 748–750 (2017).
67. Chaturvedi, R. R. et al. Passive stiffness of myocardium from congenital heart disease and implications for diastole. Circulation 121, 979–988 (2010).
68. Quinn, K. P. et al. Optical metrics of the extracellular matrix predict compositional and mechanical changes after myocardial infarction. Sci. Rep. 6, 35823 (2016).
69. Gonzalez, G. M. et al. Production of synthetic, para-aramid and biopolymer nanofibers by immersion rotary jet-spinning. Macromol. Mater. Eng. 302, 1600365 (2017).
70. Gladman, A. S., Matsumoto, E. A., Nuzzo, R. G., Mahadevan, L. & Lewis, J. A. Biomimetic 4D printing. Nat. Mater. 15, 413–418 (2016).
71. Zimmermann, W. H. et al. Three-dimensional engineered heart tissue from neonatal rat cardiac myocytes. Biotechnol. Bioeng. 68, 106–114 (2000).
72. Germanguz, I. et al. Molecular characterization and functional properties of cardiomyocytes derived from human inducible pluripotent stem cells. J. Cell. Mol. Med. 15, 38–51 (2011).
73. Ronaldsson-Bouchard, K. et al. Advanced maturation of human cardiac tissue grown from pluripotent stem cells. Nature 556, 239–243 (2018).
74. Endoh, M. Force–frequency relationship in intact mammalian ventricular myocardium: physiological and pathophysiological relevance. Eur. J. Pharmacol. 500, 73–86 (2004).
75. Bai, S. L., Campbell, S. E., Moore, J. A., Morales, M. C. & Gerdes, A. M. Influence of age, growth, and sex on cardiac myocyte size and number in rats. Anat. Rec. 226, 207–212 (1990).
76. Ferric, N. T. & Radisc, M. Strategies and challenges to myocardial replacement therapy. Stem Cells Transl. Med. 5, 410–416 (2016).
77. Pech, S., Eschenhagen, T. & Reichenspurner, H. Myocardial tissue engineering for cardiac repair. J. Heart Lung Transplant. 35, 294–298 (2016).
78. Khubchandani, R. E. Cardiovascular Physiology Concepts 2nd edn (Lippincott Williams & Wilkins/Wolters Kluwer, Philadelphia, 2012).
79. Park, S. J. et al. Phototactic guidance of a tissue-engineered soft-robotic ray. Science 353, 158–162 (2016).
80. Guyette, J. P. et al. Bioengineering human myocardium on native extracellular matrix. Circ. Res. 118, 56–72 (2016).
81. Laughner, J. L., Ng, F. S., Sulkun, M. S., Arthur, R. M. & Efinov, I. R. Processing and analysis of cardiac optical mapping data obtained with potentiometric dyes. Am. J. Physiol. Heart Circ. Physiol. 303, H753–H765 (2012).
82. Pearce, J. A., Porterfield, J. E., Larson, E. R., Valvano, J. W. & Feldman, M. D. Accuracy considerations in catheter based estimation of left ventricular volume. Conf. Proc. IEEE Eng. Med. Biol. Soc. 2010, 3556–3558 (2010).
83. Baan, J. et al. Continuous stroke volume and cardiac output from intra-vascular dimensions obtained with impedance catheter. Cardiovasc. Res. 15, 328–334 (1981).
84. Baan, J. et al. Continuous measurement of left ventricular volume in animals and humans by conductance catheter. Circulation 70, 812–823 (1984).
85. Raghavan, K. et al. Electrical conductivity and permissivity of murine myocardium. IEEE Trans. Biomed. Eng. 56, 2044–2053 (2009).
86. Clark, J. E. & Marber, M. S. Advancements in pressure–volume catheter technology—stress remodelling after infarction. Exp. Physiol. 98, 614–621 (2013).

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Author contributions
L.A.M. and K.K.P. conceived the ideas and designed the experiments. L.A.M., S.P.S., C.O.C., J.F.Z., F.S.P., X.L., J.A.G., P.H.C., G.M.G., S.-J.P., A.K.C., J.P.F. and T.F.K conducted the experiments and analysed the data. L.M. derived the scaling laws. L.A.M., W.T.P. and K.K.P interpreted the data. L.A.M., S.P.S. and K.K.P. wrote the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
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Experimental design

1. Sample size
Describe how sample size was determined.

Given the absence of prior data on human tissue-engineered ventricles, a statistical determination of sample size was not possible. Instead, we chose a sample size (n=4) compatible with existing datasets relative to contractility assays powered by the same commercially available line of hiPS-CMs. We confirmed this choice by power analysis, which indicated that a sample size of N=4 was sufficient, assuming a 30% increase in beat rate across all groups and a 10% variance.

2. Data exclusions
Describe any data exclusions.

Data was included for analysis if pressure or volume recordings were discernible at each isoproterenol dose (total experiment duration, ~20-30 minutes). Pressure–volume loops and stroke-work analysis required that both pressure and volume recordings were discernible at each isoproterenol dose. Approximately 50% of cultured ventricles (rat or human) produced pressure or volume differences that were measurable by catheterization. Of these, approximately 50% contracted with sufficient stability to conduct isoproterenol dose–response experiments. In total, we built 20 rat (neonatal rat ventricular myocyte) and 10 human (Cor.4U) model ventricles, from which we obtained pressure–volume data as a function of isoproterenol from 8 rat and 4 human model ventricles.

3. Replication
Describe whether the experimental findings were reliably reproduced.

We observed the expected biological difference in baseline recordings from the ventricles, that is, a distinct spontaneous beating rate. However, the pharmacological response was repeatable. Following isoproterenol exposure, seven out of eight rat ventricles showed positive chronotropy, whereas one ventricle showed a negligible response. For human ventricles, three out of four showed positive chronotropy, whereas the fourth showed mild negative chronotropy.

4. Randomization
Describe how samples/organisms/participants were allocated into experimental groups.

We built a total of 20 rat (neonatal rat ventricular myocyte) and 10 human (Cor.4U) model ventricles. Of these, 12 rat and 6 human ventricles were selected for catheterization on the basis of the synchrony and amplitude of contraction observed in vitro by eye and microscope. We report data for 8 rat and 4 human ventricles, for which pressure or volume recordings were discernible at each of 7 isoproterenol doses (0.1 nM to 0.1 mM; total experiment duration, ~20-30 minutes). These data include 3 human and 4 rat ventricles, for which both pressure and volume were recorded at each isoproterenol dose.

For arrhythmia injury models, we built 4 NRVM model ventricles: 2 healthy and 2 pre-injured. Calcium mapping of pre-injured ventricles was done on day 11 but tissue had grown into the injury site, preventing stable-rotor pinning. We repeated calcium mapping experiments on day 12 using the two uninjured ventricles. Both showed evidence of spontaneously generated calcium plane-wave propagation but one had significantly greater homogeneity; this favorable sample is reported in our revised manuscript. Injuries were applied to this model ventricle following pre-injury recordings.
5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Blinding was not performed because experiments were performed in a single setting, and data analysis was automated using commercial software.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

| n/a | Confirmed | n/a |
|------|-----------|-----|
| ☒    | X         | ☒   |
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| ☒    | X         | ☒   |
| ☒    | X         | ☒   |
| ☒    | X         | ☒   |

- The exact sample size \( (n) \) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
- A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- A statement indicating how many times each experiment was replicated
- The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
- A description of any assumptions or corrections, such as an adjustment for multiple comparisons
- A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
- Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

7. Software

Describe the software used to analyze the data in this study.

Statistical analysis was performed using SigmaPlot (v13.0, Systat Software Inc.). Pressure–volume data was acquired using manufacturer-supplied acquisition software (LabChart v7.3, ADInstruments) and was exported for post-processing with Matlab import routines (Matlab R2016a, MathWorks). We used InspectX and CT Pro 3D (Nikon metrology) for X-ray imaging, CT acquisition and volume reconstruction. We used VG Studio MAX 2.2 (Volume Graphics) and Amira 6.0 (ThermoFisher Scientific) for 3D-volume visualization, rendering and analysis. Calcium imaging and analysis was done using MiCAM imaging software (BV_Ana, SciMedia) and a custom MATLAB-based optical mapping analysis package (Rhythm2014b), publicly available at https://code.google.com/archive/p/rhythm-analysis-software

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

8. Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

No unique materials were used.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

We used standard antibodies validated by the manufacturers. Monoclonal anti-α-actinin (sarcomeric) antibodies produced in mouse were obtained from Sigma-Aldrich (clone EA-53, catalog number A7811-100UL). For secondary antibodies, we used goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 488 (ThermoFisher Cat#A32723) and Alexa Fluor Plus 647 (ThermoFisher Cat#A32728).
10. Eukaryotic cell lines
   a. State the source of each eukaryotic cell line used. Neonatal rat ventricular myocytes were isolated from 2-day old Sprague-Dawley using published methods. Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) were acquired commercially (Cell-type: Cor.4U, Lot numbers CB169CL_V1_1M, CB301_CL_v1_1M, CB319CL_V1_1M, CB324CL_V1_1M, CB331CL_V1_4M; Axiogenesis, Cologne, Germany). For preliminary in situ hiPSC-CM differentiation, we used cell-type PGP1-iPSC (GM23338; Church lab/Coriell Institute GM23338).
   b. Describe the method of cell line authentication used. Cell viability, sterility, and behavior were authenticated by the vendor.
   c. Report whether the cell lines were tested for mycoplasma contamination. Cells tested negative for mycoplasma contamination as specified by the provider.
   d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use. No commonly misidentified cell lines were used.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals
   Provide details on animals and/or animal-derived materials used in the study.
   Neonatal rat ventricular myocytes were isolated from 2-day old Sprague-Dawley using published methods. For histology, we obtained hearts from adult female Sprague-Dawley rats. All procedures were approved by the Harvard Animal Care and Use Committee.

Policy information about studies involving human research participants

12. Description of human research participants
   Describe the covariate-relevant population characteristics of the human research participants.
   The study did not involve human research participants.