Engineering a living cardiac pump on a chip using high-precision fabrication

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Biomimetic on-chip tissue models serve as a powerful tool for studying human physiology and developing therapeutics; however, their modeling power is hindered by our inability to develop highly ordered functional structures in small length scales. Here, we demonstrate how high-precision fabrication can enable scaled-down modeling of organ-level cardiac mechanical function. We use two-photon direct laser writing (TPDLW) to fabricate a nanoscale-resolution metamaterial scaffold with fine-tuned mechanical properties to support the formation and cyclic contraction of a miniaturized, induced pluripotent stem cell–derived ventricular chamber. Furthermore, we fabricate microfluidic valves with extreme sensitivity to rectify the flow generated by the ventricular chamber. The integrated microfluidic system recapitulates the ventricular fluidic function and exhibits a complete pressure-volume loop with isovolumetric phases. Together, our results demonstrate a previously unexplored application of high-precision fabrication that can be generalized to expand the accessible spectrum of organ-on-a-chip models toward structurally and biomechanically sophisticated tissue systems.

INTRODUCTION

Biomimetic tissue models are rapidly evolving toward elaborate, next-generation in vitro culture models to study organ-level function during development, adulthood, regeneration, and disease (1, 2). The development of such models can be attributed to progress in induced pluripotent stem cell (iPSC) technology (3), as well as innovative methods to assemble cells into aggregates and functional structures, from organoids (4) to different types of three-dimensional (3D) organ-on-chip models (5). In recent years, staggering advances have been made in differentiating stem cells into a variety of lineages and in triggering them to do so in multicellular organoids, largely built on insights from recapitulating developmental signals (1–5). Despite these advances, the lack of approaches to generate higher-order structural and anatomical features at the micrometer scale has made it difficult to model organ-level functions in vitro. For instance, while cardiac, skeletal, and smooth muscle cells have been derived from iPSCs (6–9), attempts to organize these cells to recapitulate the many different muscular architectures that give rise to different biomechanical functions have been sparse.

The ability to build more complex structures has been hindered partly by the poor adaptation of fabrication approaches with the nanometer-to-millimeter fabrication length scales relevant to tissue engineering. Here, we explore the use of two-photon direct laser writing (TPDLW) (10), a high-resolution fabrication method, to build biological systems requiring specific architectures and functions. As a challenge, we engineered a microfluidic cardiac chamber powered by human iPSC-derived cardiomyocytes (hiPSC-CM), aiming to replicate the ventricular function on a chip. While current state-of-the-art cardiac tissue models are well suited to measure contractile forces generated by cardiomyocytes (11–13), modeling the cardiac pumping function using engineered cardiac tissues has been challenging. Here, we harness the high precision of TPDLW to implement a miniaturized metamaterial scaffold that supports the anisotropic contraction of an in vitro cardiac ventricle, as well as functional miniaturized valves featuring sufficient sensitivity to respond to the cardiac chamber’s flow. The integration of the chamber and valves within a microfluidic device recapitulates the unidirectional volumetric output of the human ventricle and exhibits a complete ventricular pressure-volume (PV) loop. Our cardiac miniaturized precision-enabled unidirectional microfluidic pump (miniPUMP) system sets a precedent for a general approach to the assembly of sophisticated tissue structures that leverages the benefits of high-precision manufacturing to recapitulate whole-organ function.

RESULTS

To emulate cardiac output, we envisioned a microfluidic system that would replicate the main features of the human ventricle, namely, a contracting myocardial chamber that generates flow, cardiac valves that rectify the generated flow, and a pressure gradient between the venous blood supply and the arterial output vessel of said flow (Fig. 1A). We first set out to build a microscopic, hollow, cylindrical scaffold (approximately 1-mm inner diameter and 1-mm height) that would guide the assembly of hiPSC-CMs in a chamber format (Fig. 1B) within the miniPUMP device (figs. S1 and S2). To mimic the auxetic compression of the cardiac chambers for maximum ejection volume, we first fabricated chamber scaffolds composed of auxetic inverted hexagon unit cells (Fig. 1, C and D) using...
TPDLW to achieve submicrometer resolution of the individual beams. However, structural instabilities (fig. S3; see the Supplementary Text) ultimately caused asymmetric collapse of the structures under contractile forces generated by seeded hiPSC-CMs (Fig. 1, E and F, and movie S1). To address this instability, we restricted chamber deformability to the axial direction using an improved scaffold design. The improved design consisted of two sets of fine flexible concentric helices that were connected to each other in the transverse plane through thick rigid horizontal rings (Fig. 1, G and H). The rings were narrowly spaced in the axial direction to prevent radial constrictions of the scaffold that could compromise its stability. Importantly, compressive mechanical tests indicated that the axial stiffness of the helical scaffolds could be repeatably controlled by varying the helix cross-sectional thickness (Fig. 1, I to K, and movie S2), for example, increasing from 41.7 ± 1.9 to 71.3 ± 1.5 N/m when the thickness increased from 8.3 ± 0.2 to 10.1 ± 0.3 μm (fig. S4 and table S1). Therefore, cardiac chambers based on this helical scaffold (Fig. 1L and figs. S5 and S6, A to C) allowed us to rapidly and empirically identify a helix design that could sustain substantial tissue contractions and allowed generation of flow without collapsing over the span of a week (movie S3).

Although the scaffold exhibited a largely linear stiffness with slight strain softening (Fig. 1K) that does not mimic the strain stiffening of native contracting myocardium (14), the hiPSC-CMs remained viable, spread on the scaffolds, and formed thick multilayered tissues with dense sarcomere networks, suggesting that the scaffold did not exhibit major cytotoxic effects (fig. S7 and movie S4).

To evaluate the pump function of a helical cardiac chamber, helical chambers were embedded in the miniPUMP device, generating bidirectional flow in the device’s channels (Fig. 2A), and microbeads were suspended in the media to calculate the volumetric flow rate within the channels using particle image velocimetry. To assess the ability of the miniPUMP chambers to generate pressure, we built a finite element model (FEM) of the microfluid network consisting of a 3D network of rigid interconnected pipes (Fig. 2B) filled with water at 37°C, where the measured flow rate was introduced as the laminar flow input of the model. The fluid velocity was extracted as a validation metric (Fig. 2C), and the pressure between the outlet to the cardiac chamber and the outlets to the wells was estimated as the output (Fig. 2D). This approach allowed for a concurrent estimation of the volumetric flow rate (Fig. 2E), the ejection volume (Fig. 2F), and the pressure (Fig. 2G) of the cardiac chambers.
The suspension valve allowed us to refine the parameters of the entire valve. A rapid iteration of computational simulations and testing of the suspension valve enabled us to reliably rectify flow efficiently at low pressures and flow rates. The open and the closed state (Fig. 3, G and H), could be fabricated to achieve a suspension valve that faithfully opened and closed as designed (Fig. 3, I and J, and movie S5). By subjecting suspension valves to various flow patterns in a separate microfluidic device (fig. S10), we validated that the valve’s microfluidic resistance increased by two orders of magnitude in the closed state (Fig. 3K), which translated to flow rectification (movie S6). The valve could reliably switch to the open and closed states under 0.25 ± 0.06 Pa and 0.11 ± 0.03 Pa transvalvular pressure respectively, substantially lower than those generated by our cardiac chambers (Fig. 3L). Furthermore, we approximated the rapid flow reversals of our cardiac chambers using oscillatory pressure and observed that the valve rectification ratio was a decreasing function of the pressure frequency, exceeding 0.80 ± 0.02 below 2 Hz (Fig. 3M and movie S7). Therefore, the valve should readily rectify flow oscillating at a frequency faster than 1.4 Hz, which is equivalent to a pulse with 350-ms half period, same as the duration of the cardiac chamber’s contraction. The addition of suspension valves in the miniPUMP’s channels indeed rectified the flow that was generated by the cardiac chamber (movie S8) and resulted in net volume displacement (~4.14% ejection fraction) from the supply well to the output well (Fig. 3, N to Q, and fig. S6D) that depended on the beating frequency (fig. S11). Thus, implementing the suspension valve in the miniPUMP system allowed us to convert the small-scale mechanical work of the cardiac microchamber into directed unidirectional flow.

To further simulate the pumping function of the native cardiac ventricles, we next sought to replicate the pressure gradient between the venous supply (preload) and the arterial output vessel (afterload) of the heart, which gives rise to the ventricular PV curves. The PV curves and their PV loop summarize the ventricular mechanical outputs.
work of generating flow against the pressure gradient and are a valuable clinical metric for the assessment of cardiac health that would greatly enhance the modeling power of in vitro models (15). To introduce an afterload in the absence of external control in our system, we exploited the miniPUMP’s unidirectional flow. We introduced a glass column in the output well (Fig. 4A) and partly obstructed the escape of liquid out of the column using a TPDLW-fabricated microfluidic flow constrictor (Fig. 4B) to mimic the resistance of the arterial circulation. The liquid ejected by these cardiac chambers into their output well accumulated in the column, raising the filling level of the column and increasing the hydrostatic pressure. To estimate the afterload, we calculated the fluidic resistance of the constrictor channel with FEM fluid simulation and measured the flow in the channel containing the constrictor using particle image velocimetry after 1 hour of electrical stimulation with the inserted glass column and flow constrictor. The afterload pressure increased in the presence of the constrictor and was a function of the constrictor inner diameter, e.g., rising from 13.1 ± 1.5 Pa (0.0983 ± 0.0113 mmHg) to 28.6 ± 1.7 Pa (0.2145 ± 0.0128 mmHg) when the constrictor inner diameter was reduced from 90 to 70 μm (Fig. 4C), indicating indirect control over the afterload pressure. Interestingly, the unidirectional flow was sustained despite the presence of the afterload in all tested devices, suggesting that the loaded miniPUMP exhibits a complete PV loop.

To assemble a complete PV loop, we needed to computationally derive the pressure dynamics within the cardiac chamber during...
the filling and ejection phases. We separated the microfluidic device (Fig. 4D) into two computationally inexpensive 2D FEM models (Fig. S12), one representing the flow route during ejection and one representing the flow route during filling, both comprising two serially placed independent simulations (see the Supplementary Text). As anticipated, the resulting simulations show that implementation of unidirectional flow and a pressure gradient using the suspension valves and the flow constrictor sufficed for the emergence of the two isovolumetric phases in the volume trace (~4.06% ejection fraction; Fig. 4E and movie S9). The isovolumetric phases were accompanied by abrupt pressure transitions between the preload and afterload pressures [e.g., 0 and 28.6 Pa (0.2145 mmHg), respectively] and were followed by pressure overshoots past the preload and afterload (Fig. 4F), peaking at ~6.35 Pa (~0.0476 mmHg) and 42.77 Pa (0.3208 mmHg). Similarly, the PV loops were characterized by two distinct filling and ejection phases that are separated by rapid vertical isovolumetric phases (Fig. 4G), as it is observed in the classic PV loop of the native ventricle (13). Interestingly, the pressure briefly overshoot in the pressure trace, and the PV loop featured spike noise caused by the transient motion of the opening valves, while the delayed closing of the valves caused a minor volume change in the volume trace and the PV loop before the isovolumetric phases. These observations were consistent across multiple devices and were accompanied by similar trends across all devices in representative PV metrics (figs. S13 and S14 and table S2). In testing the afterload response, we largely observed a decrease in stroke volume with increased afterload, a trend reminiscent of the end-systolic PV relationship (fig. S15; see the Supplementary Text). Together, the complete TPDLW-enabled miniPUMP system recapitulates the main principles of the ventricular volumetric function and exhibits the key features of the complete PV loop.

**DISCUSSION**

iPSC-derived cardiac models have progressively evolved from 2D isotropic monolayers to anisotropically aligned layers using adhesive or topological cues (11), 3D organoids using cell-mediated self-assembly (16, 17), and linear tissue microbundles using directed self-assembly between anchoring points (12, 13). While current models have provided insight into the importance of tissue load in cardiac biomechanics, maturation, and pathophysiological remodeling (18–20), the auxotonic loading in these architecturally simple models substantially differs from the loading experienced during the filling and emptying of a cardiac chamber (15). Recent efforts have extended self-assembly toward a cardiac chamber format by using innovative fabrication approaches to build centimeter-scale chambers that fill and eject fluid through their cyclic contractile activity (21, 22). While a major step forward, the lack of sufficiently
sensitive, functional valves in these models did not allow for the recapitulation of the fluid and loading dynamics of the cardiac cycle without external intervention. In addition, the scale of these chambers requires a large number of cells to be assembled and does not exploit the degree of miniaturization that has tremendously facilitated scaled-up production for disease modeling (12, 17, 20) and predictive pharmacological testing (23). Here, we used high-precision fabrication to recreate miniaturized analogs of the ventricular anatomical features that are necessary to replicate the ventricular functional output. We fabricated and connected a microscopic cardiac chamber and functional microvalves to produce an on-chip platform, the miniPUMP, that exposes the tissue to a physiologically shaped PV loop, with isovolumetric phases and separate preload and afterload pressures. We have thus demonstrated the mechanical functionality of this system and anticipate future iterations to further improve scaffold materials and mechanics to better replicate myocardial mechanics and allow for structural remodeling (i.e., myocardial hypertrophy and dilation) in response to acute or chronic interventions. Further investigation is required to understand the electrophysiological characteristics of our system, but we would anticipate that the benefits of miniaturizing a cardiac chamber might also be accompanied by an inability to model behaviors that are typically enhanced by increasing size, such as the arrhythmic propagation of the cardiac action potential. In the future, our design-based methodology can be harnessed to enrich the chamber with additional cell types and functional structures, such as a fluidically insulating endothelium. Ultimately, this work not only provides an avenue for building functional cardiac chamber models but also illustrates the rapid design-build-test cycles critical to engineering of complex systems.

Radical advances in miniaturization are redefining the boundaries of many fields, such as materials (24) and robotics (25), but have yet to fully manifest in the engineering of tissues despite the promise of such miniaturization for organ-on-chip applications. Propelled by its increasing accessibility and throughput (26–28), as well as its potential in patterning materials to guide cell adhesion (29), spatial arrangement (30), and extracellular stiffness (20), high-precision fabrication is well poised to advance small-scale in vitro models to begin to explore fundamental insights into how tissue structures, complex mechanics, and physiologic functions are linked. Here, the use of microscale precision-designed structures to guide tissue formation and mechanical functionality showcases an approach to translate architecture and mechanics to enable the replication of mechanically complex physiological behaviors in an unprecedentedly small scale. By embracing such a paradigm, we anticipate that high-precision fabrication will provide the means to further elucidate and incorporate on-chip the critical role of structure, mechanics, and function in human health and ultimately motivate a new generation of more predictive and more physiologically relevant in vitro models.

MATERIALS AND METHODS

Polydimethylsiloxane device fabrication

The polydimethylsiloxane (PDMS) device used in the assembly of the miniPUMP system (fig. S2A) was made using PDMS molding and an outsourced (Protolabs) mold (fig. S1A). The mold has small 410-μm-diameter holes across its width and large 1100-μm-diameter holes across its length that serve as guides for placing the needles. Plastic 1050-μm-diameter blunt dispensing needles were placed through the large holes (fig. S1B, step 1); then, metal 400-μm-diameter sharp needles were placed through the small holes (fig. S1B, step 2). The metal needles pierced though the plastic dispensing needles, forming a network of needles that served as the negative of the microfluidic channels of our PDMS device. The entire mold was encased between two frames (fig. S1B, step 3) that were designed to tightly fit the PDMS mold and fabricated using stereolithography (Form 2; Formlabs). The encased mold was filled with PDMS (fig. S1C) made with a 1:10 ratio of cross-linker and prepolymer (SYLGARD 184, Dow). If air bubbles were observed in the PDMS, the encased mold was left in a vacuum desiccator for at least 30 min at 2.0-mbar pressure to ensure the removal of any air that was trapped in the liquid PDMS. The filled mold was left in an oven at 60°C for a minimum of 2 hours until the PDMS completely polymerized (fig. S1D). After polymerization, the metal and plastic needles and the 3D-printed case were removed, and the molded PDMS part was extracted from the mold (fig. S1E). Given that each mold was designed to make four PDMS devices, an additional case that served as a cutting guide was fabricated to separate the individual devices (fig. S1, F and G). Each device (fig. S1H) was then used in the device assembly protocol as described in the “Device assembly, seeding and preparation for experiment” section below.

Particle image velocimetry protocol

The following protocol was used to process any flow recordings that were used to characterize the microfluidic valves or derive the reported metrics of the cardiac chambers’ function. The flow recordings feature the motion of fluorescent beads within microfluidic channels, and the details on the acquisition and experimental conditions are described in the respective sections. The fluorescent beads in the flow recordings were tracked using the TrackMate plugin in ImageJ. To compensate for the difference in fluorescence between devices and imaging sessions, the brightness and contrast of all videos were automatically adapted to the same range using frames where the beads are stationary (e.g., during the late diastole of the cardiac chamber). The bead motion was recorded at a sufficient distance from any channel outlets to ensure that the flow was fully developed. To estimate the average velocity and the flow rate in the microfluidic channels, the tracking data produced by TrackMate were imported into MATLAB, and the velocity as a function of the channel radius was calculated for each frame through a least squares fit on the frame-to-frame displacement of the microbeads in each frame. The average velocity in the channel was then calculated per frame using the velocity function, and the flow rate per frame was calculated as the average velocity times the cross-sectional area of the channel. Where necessary, the flow rate was then integrated in time to derive the displaced volume or imported into FEM simulations to derive the corresponding pressure in the microfluidic device.

Valve fabrication

The valves were designed using computer-aided design (CAD; SolidWorks, Dassault Systèmes) and printed using TPDLW (Photonic Professional GT, Nanoscribe) and the commercially available photoresist IP-dip (Nanoscribe). The printing process per valve lasted for 45 min, enabling rapid generation of multiple copies of the valve. To remove the excess unpolymerized resist, the 3D-printed valves were submerged in poly ethyl glycol mono ether acetate (PGMEA) for 1 hour on an orbital shaker, then were moved...
to fresh PGMEA for 15 min on a shaker, 99.5% isopropanol (IPA) for 30 min on a shaker to remove the PGMEA, and, lastly, moved and stored in deionized water for up to 2 days. Because of the hydrophobic nature of the valve materials, it was impossible to remove any air bubbles entering the valves; therefore, it was imperative that the interior of the valve remained wetted and completely occupied with water until use. Before use, the valves were sonicated in water for 10 s to ensure full separation of the free-floating plate from the cylindrical frame of the valve. Defective valves were identified through visual inspection under a stereoscope (ZEISS SteREO Discovery.V20) and discarded. Through gradual optimizations of the fabrication and selection protocols, the yield of functional valves was approximately 60%.

**Valve characterization**

To acquire scanning electron microscope (SEM) images, the valves were sputter coated with gold (Cressington 108 Manual Sputter Coater, Ted Pella), and the images were acquired using the SEM microscope Zeiss SUPRA 55-VP. To estimate the valve sensitivity to pressure (Fig. 3L), a separate microfluidic device was made (fig. S10A). This microfluidic device featured two parallel channels of 400-µm diameter and 5-mm length that connected two glass columns. The valve was added in one channel, while the second channel was left empty. The device was filled with phosphate-buffered saline (PBS) containing 1:1000 dilution of 1-µm red fluorescent beads (FluoSpheres carboxylate, 1.0 µm red 580/605; F8821, Invitrogen). To ensure that the device was leak-proof at the junction with the glass columns, the outer surface of the glass columns was covered with vacuum grease. To mitigate any hydrostatic instability induced by the capillary effect in the glass column, 10 µl of paraffin oil was added in both glass columns after insertion. The results of the valve simulation (fig. S9) indicated that the valve sensitivity would be lower than the precision of most commercially available pumps; therefore, we opted to control the pressure across the valve indirectly. A syringe pump (PHD 2000 Programmable; Harvard Apparatus) was used to successively infuse and retract fluid from one glass column, generating a common pressure gradient across the two channels and making the valve open and close cyclically. The flow in the empty channel and the motion of the valve were monitored and recorded at a frame rate of 206 Hz using the inverted confocal raster-scanning microscope Zeiss Axiovert 200M with a 561-nm laser and a 4× and a 10× objective. Our goal was to estimate the pressure across the empty channel when the valve began to move between the open and the closed states. To achieve that, both channels were first concurrently imaged using the 4× objective to estimate when the valve begins to move relative to the flow changes in the empty channel. Specifically, the average time lag (for \( n = 13 \) flow cycles) between the flow reversal in the empty channel and the ensuing beginning of the valve transition between its states was estimated. Then, the flow in the empty channel was recorded using the 10× objective and processed (particle image velocimetry protocol) to estimate the flow rate in the empty channel at the estimated time lags relative to the flow reversal in the recordings. To calculate the pressure across the empty channel, the flow rate in the empty channel was then averaged across \( n = 5 \) flow cycles and multiplied with the theoretical hydraulic resistance of the hollow microfluidic channel. Because of the parallel arrangement of the empty channel and the valve channel, the calculated pressure was equated to the pressure across the valve.

To estimate the valve resistance (Fig. 3K), we used a second version of the microfluidic device with two parallel microfluidic channels (fig. S10B). The channel containing a valve was filled with PBS containing 1:1000 dilution of 2-µm polystyrene beads (Polysciences, PS-COOH 2.0 µm), and a controlled pressure gradient across the channels was applied using a microfluidic pressure control system (Elveflow OB1-MK3 with a 0- to 20-kPa channel, ±0.5-Pa pressure stability, 35-ms settling time, and 10-ms response time). To ensure a hermetic connection at the junction of the device and the tubing to the pump, the exterior of the tubing was covered with vacuum grease. Flow recordings were acquired in an inverted microscope (Axio Observer, Carl Zeiss) using a 20× objective, an AxioCam 503 mono camera (Carl Zeiss), bright-field transillumination, and 67-Hz frame rate. The system was allowed to passively equilibrate until the polystyrene beads stopped flowing. The pressure was then increased at specified increments, and videos of the flow through the valve channel were recorded at a sufficient distance from the valve and the channel ends to ensure that the flow was fully developed. Following the particle image velocimetry protocol described previously, TrackMate and MATLAB were used to calculate the flow rate across the valve channel.

To estimate the rectification ratio (Fig. 3M), the same microfluidic device with one valve was used with the same pressure controller and imaging system as described for the valve resistance measurement (fig. S10B). A square wave pulse train between 25 Pa (valve in open state) and −25 Pa (valve in closed state) with 50% duty cycle was applied at 1 to 4 Hz in 1-Hz increments. The flow in the valve channel was recorded for 8 s at 65.2-Hz sampling rate for pulse frequency between 1 and 3 Hz and for 12.8 s at 82.47-Hz sampling rate for pulse frequency at 4 Hz. The flow rate was estimated using TrackMate and MATLAB following the particle image velocimetry protocol described previously, and the volume displaced through the valve was estimated by integrating the flow rate over time. The rectification ratio was defined as \( \frac{(V_{\text{open}} - V_{\text{closed}})}{V_{\text{open}}} \) where \( V_{\text{open}} \) is the volume of fluid crossing through the valve during the positive phase of the pressure pulse and \( V_{\text{closed}} \) is the volume that regurgitates through the valve during the negative phase of the pressure pulse. The reported rectification ratio was the average across all the cycles within the 8 s of recording. The number for cycles was \( n = 7 \) for 1 Hz, \( n = 15 \) for 2 Hz, \( n = 25 \) for 3 Hz, and \( n = 50 \) for 4 Hz.

**Scaffold fabrication**

The scaffolds were designed using CAD (SolidWorks, Dassault Systèmes) and printed using TPDLW (Photonic Professional GT, Nanoscribe) and the commercially available photosensitive IP-S (Nanoscribe). To facilitate the design, we identified the scaffold unit cell (Fig. 1, D and G) and constructed the complete scaffold by patterning the unit cell along a cylindrical face. Likewise, the scaffold was printed in a step-and-repeat process of individual unit cells that could be easily automated. The printing process for the entire scaffold (Fig. 1L) lasted for approximately 1.5 hours per scaffold. To remove the excess unpolymerized resist, the 3D-printed scaffolds were submerged in 99.5% IPA for 30 min. While remaining submerged in liquid, the scaffolds were then moved to clean 99.5% IPA for 5 min, then moved again to clean 99.5% IPA, and, lastly, dried using critical point drying (Samdri-PVT-3D, Tousimis). The dried scaffolds were stored in ambient air and shielded from light until they were added into the microfluidic device. Critical point drying was necessary because of the intentionally low mechanical
stiffness of the unit cells. When critical point drying was omitted and the scaffolds were directly removed from the IPA, the scaffolds were destructively deformed by the surface tension of the liquid IPA. Consecutive optimizations of the scaffold fabrication protocol enabled a yield exceeding 90%.

**Scaffold characterization**

Static SEM images of the scaffold were acquired by affixing the scaffolds on a silicon substrate (University Wafers), coating the scaffolds and substrate with gold (Cressington 108 Manual Sputter Coater, Ted Pella), and imaging them with the SEM Zeiss SUPRA 55 VP (Zeiss). The reported thickness of the scaffold helix was estimated as the average of the helix thickness at \( n = 7 \) locations on the scaffolds in the SEM images.

To perform the mechanical characterization, the scaffolds were fixed on a silicon substrate to ensure stability during the mechanical testing. The mechanical testing of the scaffold was performed inside a different SEM (JEOL JSM-6330F, Japan) using an instrumented in situ indenter (Picolndenter, Hysitron PI 87, Bruker, USA). The scaffolds were sputtered with a thin coating of gold to prevent charging of the specimen surface. The compressive displacement was applied with a titanium cylindrical flat punch with a diameter of 500 \( \mu \)m. A peak compressive displacement of 150 \( \mu \)m was imposed at a rate of 5 \( \mu \)m/s for all tests, and the corresponding load response was recorded throughout the compression cycle. Real-time SEM videos capturing scaffold deformation were acquired at low acceleration voltages (≤5 kV) to prevent electron beam–induced damage of scaffolds.

The reported instances of the load response and stiffness (fig. S4) were calculated using the data from the aforementioned compressive test. The stiffness was calculated as the derivative of the force-displacement curve. To illustrate that the stiffness was a function of displacement, we calculated the load response and stiffness at two separate displacement values. We used data at 50-\( \mu \)m displacement as the first displacement value to avoid the transiently unstable response at lower displacements. We used the peak compression displacement (150 \( \mu \)m) as the second displacement value. The reported load response and stiffness were calculated as the averages of \( n = 3 \) scaffolds for the thick (10.1 ± 0.3 \( \mu \)m) and thin (8.3 ± 0.2 \( \mu \)m) helix designs.

**Flow constrictor fabrication**

The flow constrictors were designed using CAD (SolidWorks, Dassault Systèmes) and printed using TPD LW (Photonic Professional GT, Nanoscribe) and the commercially available photoresist IP-S (Nanoscribe). The printing process per constrictor lasted for 20 min. To remove the excess unpolymerized resist, the 3D-printed flow constrictors were washed twice in 99.5% IPA for 30 and 5 min, respectively, on an orbital shaker, then rinsed in the highly volatile solvent Novec 7100 for 1 min to remove the IPA, and were left to dry in the residual Novec 7100. The constrictor inner diameter was verified optically using an inverted microscope (Axio Imager.A2m, Zeiss) with a \( \times 20 \) objective and an AxioCam 305 color camera (Zeiss). The yield of the constrictor fabrication protocol was 100%.

**Cell culture and cell preparation for seeding**

The cardiomyocytes (hiPSC-CMs) were derived from human iPSCs from the Harvard Personal Genome Project line 1 (GM23338; PGP1). Stem cells were maintained in Matrigel-coated six-well plates and fed daily with mTeSR1 media (85850, STEMCELL Technologies) until they reached sufficient (80 to 100%) confluency to initiate differentiation (day 1). The mTeSR1 media were aspirated, washed with PBS (10010023, Thermo Fisher Scientific), and then replaced with RPMI + GlutaMAX media (61870, Thermo Fisher Scientific) with 2% B27 minus insulin (B27−) supplement (A1895601, Thermo Fisher Scientific) and 10 \( \mu \)M CHIR 99021 (4423, Tocris). At precisely 24 hours (day 2), the media were aspirated, washed out with PBS, and replaced with RPMI B27− media. After precisely 48 hours (day 4), the media were aspirated and replaced with RPMI B27− media with 5 \( \mu \)M IWP-4 (5214, Tocris). After 48 hours (day 6), the media were aspirated and replaced with RPMI B27−. On day 8, the media were replaced again with RPMI B27−, and on day 10 with RPMI media with 2% B27 with insulin (B27+) supplement (17504-044, Thermo Fisher Scientific). On days 12 and 14, metabolic selection was used to deplete most nonmyocyte cells. The media were washed out with PBS and replaced with RPMI without glucose (11979, Gibco) and supplemented with 60% (w/w) sodium DL-lactate solution (L1375, MilliporeSigma) diluted by a 3:4000 factor and 1% GlutaMAX Supplement (35050-061, Gibco). On day 16, the media were replaced with RPMI B27+. On day 18, the hiPSC-CMs were replated in six-well plates coated with fibronectin (356009, Thermo Fisher Scientific) in RPMI B27+ supplemented with 2% fetal bovine serum (FBS; F0926, MilliporeSigma) and 5 \( \mu \)M Y-27632 (1254, Tocris). Starting at day 19, the media were replaced with fresh RPMI B27+ every other day until 14 days later, when the cells were used for seeding.

To replate the cells on fibronectin, the cell plates were washed with PBS and incubated in 0.5 ml per well with 0.25% Trypsin-EDTA (25200072, Thermo Fisher Scientific) solution with deoxyribonuclease I (DNase; 10 \( \mu \)g/ml; 7469, STEMCELL Technologies) solution for 10 min. The cells were then moved to 50% FBS in PBS in 15-ml conical tubes to neutralize the proteases and centrifuged with a relative centrifugal force (RCF) of 90 for 4 min. Following that, the supernatant was aspirated, the cells were resuspended in 2 ml of day 18 media per seeding well, and distributed in the wells.

Primary human bone marrow stromal cells (hMSCs) were used as a support cell population in the in vitro cardiac tissues. The hMSCs were maintained using Dulbecco’s modified Eagle’s medium (DMEM) + GlutaMAX media [glucose (1 g/liter); 10567014, Thermo Fisher Scientific], supplemented with 10% FBS and penicillin and streptomycin (P/S; 100 U/ml; 15140418, Life Technologies). On the day of seeding, the hMSCs were treated with mitomycin-C (10 \( \mu \)g/ml; HY-13316, MedChemExpress) for 2 hours and 50 min in 5 ml of their regular media, then washed twice with PBS, and returned to their regular media until they are needed for seeding.

To harvest the hiPSC-CMs for seeding, the cell plates were washed with PBS and incubated at 37°C in 0.25% Trypsin-EDTA and DNase (10 \( \mu \)g/ml) solution for 10 min. The cells were then moved to 50% FBS in RPMI B27+ with 5 \( \mu \)M Y-27632 in 15-ml conical tubes to neutralize the proteases and centrifuged with a relative centrifugal force (RCF) of 90 for 4 min. Following that, the supernatant was aspirated, the cells were resuspended in 2 ml of day 18 media per seeding well, and distributed in the wells.

To harvest the hMSCs for seeding, the hMSCs were washed with PBS and incubated at 37°C in 0.25% Trypsin-EDTA solution for 7 min. The cells were moved to 50% FBS in DMEM + GlutaMAX media in a 15-ml conical tube and counted using a hemocytometer. The conical tube was left on ice until the cells were used for seeding.
Device assembly, seeding, and preparation for experiment

The miniPUMP’s assembly is illustrated in fig. S2. In detail, the scaffold was inserted manually in one of the channels of the PDMS device (fig. S2, A to C). For all reported tissue data, the thin helix (8.3 ± 0.2 μm) scaffold of Fig. 1K was used. A blunt needle was inserted in the device to occupy the internal cavity of the scaffold, and the second channel leading to the well with the scaffold was sealed (fig. S2D). To enhance surface wetting in the microfluidic wells and channels in the following steps, the entire devices were rendered hydrophilic through exposure to ambient air plasma (EMS 1050X, EMS Quorum) for 1 min under 0.6-mbar pressure. The devices were then submerged in 70% ethanol for 1 min, and the ethanol was then aspirated from all wells of the devices. To ensure that no air would remain in the microfluidic channels after the devices are submerged in subsequent liquids in the following steps, the devices were placed in a vacuum desiccator for at least 1 hour at 2.0-mbar pressure to degas their solid PDMS bodies. The devices were then immediately submerged in 2% F-127 Pluronic (P2442, MilliporeSigma) sterile aqueous solution for 1 hour at room temperature, ensuring that there was no air remaining in the wells and minimizing the air remaining in the channels. The devices were then removed from the Pluronic solution, the solution was aspirated out of the wells (but not the channels), and the devices were submerged in PBS for 1 hour. Meanwhile, the ECM solution and the cells were prepared. hiPSC-CMs (170,000) and hMSCs (4000) were seeded in each device in 14 μl of the ECM solution, which consisted of fibrinogen (4 mg/ml; F3879, MilliporeSigma), 10% Matrigel (CB-40230, Corning), 5 μM Y-27632, aprotinin (33.3 μg/ml; A1153, MilliporeSigma), and 0.2 U of thrombin (T7201, MilliporeSigma) per milligram of fibrinogen. To prevent unwarranted gelation of the ECM solution before seeding, the cells and all the ECM solution constituents except for the fibrinogen were mixed together and kept on ice, and the fibrinogen was added to the solution right before seeding. The seeded devices (fig. S2E) were incubated at 37°C for 10 min to ensure gelation of the seeding solution and were then placed in a standard six-well plate submerged in warm H1 media, consisting of DMEM media [glucose (4.5 g/liter); 10-013-CV, Corning], 10% FBS, P/S (100 U/ml), 1% GlutaMAX, aprotinin (33.3 μg/ml), and 1% MEM Non-essential Amino Acid Solution (11140, Gibco), and supplemented with 5 μM Y-27632. During the first 24 hours of incubation, the degassed PDMS served as a sink for any air present in the channels after the seeding process, ensuring total removal of all air bubbles and complete wetting of the entire microfluidic device. Through this continuous improvement of the seeding protocol, the seeding process yield exceeded 90%.

The following day (day 2), the media were replaced with H1 media without Y-27632. The tissues were left to compact for 2 days (fig. S5) and form an even, thick layer on the scaffold (fig. S2F), and then, on day 4, the media were washed out with PBS and replaced with the metabolic selection media supplemented with aprotinin (33.3 μg/ml) and P/S (100 U/ml). The selection media were replenished on day 6 and replaced with H1 media on day 8. Starting at day 8, the H1 media were replenished every other day.

Experimental procedure for flow recording in the assembled miniPUMP device

Because of the low scale of the tissue output, we anticipated that use of external equipment to measure the tissue flow rate and pressure would disturb the tissue function and degrade the measured metrics. To avoid affecting our measurements, we opted to determine the flow rate indirectly, as suggested in previous work (31), using particle
image velocimetry on video recordings of fluorescent particles in the channels of the microfluidic device. We then calculated the pressure in the channels using finite element analysis (see the “Pressure calculation in an assembled chamber device using FEM simulations” section). All video recordings were acquired using a Zeiss Axiovert 200M inverted confocal raster-scanning microscope using an iXon Ultra EMCCD (Andor) camera and white light transillumination or a 561-nm laser. The devices were placed in an integrated microscope chamber at 37°C and 5% CO₂, remained submerged in H1 media, and were stimulated at 0.66 Hz and 20 V (stimulator C-Pace EP and six-well circular electrodes, IonOptix) for 1 hour before the collection of any data. The 0.66-Hz frequency was selected because it was the lowest frequency at which no spontaneous beating was observed in any of the tissues during preliminary experimental efforts. To monitor and record the flow, the 561-nm laser illumination was used to excite the fluorescent beads that served as traceable fluorescent indicators of the flow. The flow videos that were used to calculate the flow rate were acquired at an increased 206-Hz frame rate to capture the rapid flow transitions. All remaining videos were acquired at a lower 30-Hz frame rate. We presented the derived metrics of only one representative device in Figs. 2 to 4. Similar traces resulted from multiple experiments performed on different occasions (n ≥ 3).

**Experimental procedure for the stimulation frequency sweep**

For the frequency sweep experiments in fig. S11, we used miniPUMP devices containing a cardiac chamber and valves. The tissues were initially stimulated at 0.6 Hz for 1 hour. Following the 1 hour and starting with the 0.6-Hz frequency, flow recordings of approximately 20 s were acquired for each device in the current frequency, and then the frequency was incremented by 0.1 Hz. Videos at the new stimulation frequency were recorded immediately after the increment, resulting in a total of 5 min of stimulation per stimulation frequency. This process was repeated iteratively until the tissues were unable to beat at the pace of the stimulation frequency. To acquire a control measurement, once all the tissues failed to beat on tempo, the stimulation frequency was reset to 0.6 Hz and the flow was recorded again.

The flow videos were processed as described in the “Particle image velocimetry protocol” section to calculate the flow rate. The flow rate was then integrated to calculate the ejected volume time trace of each device (similar to Fig. 2F). For each cycle, the ejection volume was calculated as the difference between a peak relaxation (minimum time trace value in the cycle) and the subsequent peak contraction (maximum time trace value in the cycle). The presented ejection volume data (fig. S11A) were calculated as the average and SD of the ejection volumes across all cycles in the 20 s of the recording. Therefore, the sample size increased as the stimulation frequency increased, from n = 11 at 0.6 Hz up to n = 49 at 2.6 Hz. In addition, the data of each device were normalized separately, relative to the ejection volume of the device at 0.6 Hz at the beginning of the experiment. The ventricular output data (fig. S11B) were calculated as the projection of the ejection volume to a 60-s interval using the formula 60 × (ejection volume) × (frequency). The ventricular output was then normalized to the output of each tissue during the first 0.6-Hz stimulation.

**Fluorescent staining and imaging**

Fluorescent images of the tissues were acquired using the confocal multiphoton microscope Leica TCS SP8 MP, operated in single-photon mode, or the Zeiss Axiovert 200M inverted confocal raster-scanning microscope. To perform a viability stain, we used a membrane permeability and esterase activity assay based on calcein and ethidium homodimer (LIVE/DEAD Viability/Cytotoxicity Kit for mammalian cells, Invitrogen). The stains were diluted to a final concentration of 4 μM for calcein and 2 μM for ethidium homodimer in the H1 media where the tissues were already submerged in, and the devices were incubated for 30 min. The media were then aspirated, washed with PBS once, and replaced with fresh H1 media. The cardiac chambers were imaged immediately afterward.

To fix the tissues, the media were aspirated out of the wells, and the devices were washed once in PBS and then submerged in a 4% paraformaldehyde aqueous solution for 15 min. The solution was aspirated, and the tissues were submerged in fresh PBS three times for 5 min each time and then stored in PBS.

To fluorescently stain the cardiac chambers, the miniPUMP devices were first permeabilized in a PBS solution containing 0.3% Triton X-100 (T8787, MilliporeSigma) and 2% bovine serum albumin (BSA; A4503, MilliporeSigma) for 30 min. The solution was washed out by submerging in fresh PBS three times for 5 min each time. The miniPUMP devices were then moved to a PBS solution with 1% BSA and an anti-sarcomeric α-actinin primary antibody (10 μg/ml; ab9465, Abcam) at 4°C overnight. The solution was washed out with fresh PBS three times for 5 min each time, and the devices were moved to a PBS solution with 1% BSA and all the fluorescently labeled antibodies and small molecules and stored at 4°C overnight. We used DAPI (5 μg/ml; 3571, Invitrogen) to stain for the nuclei, 0.22 μM phalloidin (A12379, Thermo Fisher Scientific) for F-actin, and 1:100 for the secondary antibody (A11030, Invitrogen) for α-actinin. The devices were washed in PBS three times for 5 min and were stored in PBS until imaging.

**Pressure calculation in an assembled chamber device using FEM simulations**

All simulations of the assembled miniPUMP device were run on COMSOL and assumed that the device is filled with pure water at 37°C. The flow was assumed to be globally laminar, which was verified by estimating the Reynolds number (Re < 1) and by observing the motion of the microbeads at various locations of the microfluidic device. Therefore, we assumed that the laminar, time-dependent formulation of the Navier-Stokes equation applied to the entire microfluidic system

\[
\frac{\partial \mathbf{u}}{\partial t} + (\mathbf{u} \cdot \nabla) \mathbf{u} = \nabla \cdot (-p \mathbf{I} + \mathbf{K}) + \mathbf{F}
\]

(1)

\[
p \nabla \cdot \mathbf{u} = 0
\]

(2)

\[
\mathbf{K} = \mu (\nabla \mathbf{u} + (\nabla \mathbf{u})^T)
\]

(3)

where \( \mathbf{u} \) is the local velocity vector, \( p \) is the local pressure, \( \mu \) is the density of water at 37°C (993.33 kg/m³), and \( \mu \) is the dynamic viscosity of water at 37°C (6.90 × 10⁻⁵ Pa·s).

Equations 1 to 3 were applied to all the time-dependent simulations described in this section. As for the boundary conditions, we assumed that all walls in contact with the fluid (i.e., the walls of the microfluidic channels, the inner walls of the valve and the valve plate) were completely rigid and exhibited a no-slip condition with the outer laminae of the fluid.
To estimate the pressure in the miniPUMP device without valves (Fig. 2, A and G), the flow rate was measured in the channel leading to the cardiac chamber using the particle image velocimetry protocol described previously. A 3D model replicating the structure of the microfluidic network in the PDMS device was used, and the measured flow rate was imported as input into a time-dependent simulation at the same location where it was measured experimentally (Fig. 2B). The pressure at the outlets leading to the supply and output wells was assumed to be equal, and the pressure difference between the outlet to the cardiac chamber and the outlet to the output well was reported as the average pressure in the cardiac chamber.

To estimate the afterload pressure in the output well within the assembled miniPUMP device with the valves and the afterload (Fig. 4, A and C), the flow rate in the channel containing the flow constractor was measured using particle image velocimetry as described in the particle image velocimetry protocol. The fluidic resistance of the channel with the constractor was estimated using a FEM fluid simulation of the channel, and the afterload pressure was calculated as the product of the flow rate and the channel resistance.

To estimate the pressure in the channels of assembled miniPUMP device with the valves and the afterload (Fig. 4, A and F), the microfluidic network was converted into a simplified set of three microfluidic models (Fig. S12; see Supplementary Text). The measured flow was used as input in each model, and the pressure across each model was estimated as described in the Supplementary Text. The estimated pressures were added to determine the pressure at the outlet to the cardiac chamber, which was reported as the average pressure in the cardiac chamber. The pressure rate was calculated as the time derivative of the pressure curve from the simulation. To acquire the valve position that was used to validate the suspension valve simulation, the moving valve plate was recorded during the experiment at an increased frame rate of 206 Hz. The valve position was measured through frame-by-frame manual tracking.

Figures 2 and 4 present the pressure data from representative devices. Similar traces resulted from multiple experiments performed on different occasions (Fig. S13).

**SUPPLEMENTARY MATERIALS**

Supplementary material for this article is available at https://science.sciencemag.org/content/sciadv/abm3791.

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Micha et al., *Sci. Adv.* 8, eabm3791 (2022) 22 April 2022

11 of 12
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