Microfluidics-enabled 96-well perfusion system for high-throughput tissue engineering and long-term all-optical electrophysiology

Lai Wei\textsuperscript{1,2}, Weizhen Li\textsuperscript{1,2}, Emilia Entcheva\textsuperscript{1*} and Zhenyu Li\textsuperscript{1*}

**ABSTRACT:**

This work demonstrates a novel high-throughput (HT) microfluidics-enabled uninterrupted perfusion system (HT-µUPS) and validates its use with chronic all-optical electrophysiology in human excitable cells. HT-µUPS consists of a soft multichannel microfluidic plate cover which could button on a commercial HT 96-well plate. Herein, we demonstrate the manufacturing process of the system and its usages in acute and chronic all-optical electrophysiological studies of human induced pluripotent stem-cell-derived cardiomyocytes (iPSC-CMs) and engineered excitable (Spiking HEK) cells. HT-µUPS perfusion maintained functional voltage and calcium responses in iPSC-CM and Spiking HEK cells under spontaneous conditions and under optogenetic pacing. Long-term culture with HT-µUPS improved cell viability and optogenetically-tracked calcium responses in Spiking HEK cells. The scalability and simplicity of this design and its compatibility with HT all-optical electrophysiology can empower cell-based assays for personalized medicine using patient-derived cells.

Cell-based assays for personalized medicine use precious patient-derived human stem cells, differentiated in cardiac, neural or other cell types. Specifically, cardiac induced pluripotent stem-cell-derived cardiomyocytes (iPSC-CMs) are optimized for disease modelling, drug testing, and heart repair\textsuperscript{1,3}. There is a great need for high throughput (HT) characterization and measurements of function in these cells within their multicellular environment. Recent all-optical electrophysiology approaches\textsuperscript{4,5}, including the OptoDyCE platform developed in our lab\textsuperscript{6,7}, use non-contact optogenetics-based methods for stimulation and optical mapping of voltage and calcium. They offer a key HT-enabling technology for conducting cellular functional assays in standard HT microplates, e.g. 96-well format or higher. In many cases, chronic functional probing is desirable, with continuous or repeated measurements within the same samples. For example, maturation strategies\textsuperscript{8} of human iPSC-CMs to increase their utility in heart regeneration or drug discovery necessitate such chronic probing. Static cultures are inadequate for highly-metabolically-active samples over longer periods of time\textsuperscript{9} because standard lab solutions in the absence of mass transport fail to provide proper oxygenation under increased (work)load\textsuperscript{10}. Currently, there is no commercial solution for microperfusion that can directly be applied to standard HT microwell plates, for which industrial-level robotics and automation for sample and solution handling exist. Such microperfusion solution should allow for seamless integration with all-optical electrophysiology for chronic/long-term monitoring using genetically-encoded or small-molecule actuators and sensors, within a standard incubator or in customized on-stage systems.

Recently, several microperfusion solutions have been proposed and applied to non-myocytes to create HT dynamic cell culture\textsuperscript{11-13}. Specifically, Kim\textsuperscript{14} and Parrish\textsuperscript{15}, developed sophisticated designs using custom-built 96 well arrays, albeit with high complexity and difficult to replicate or adopt. Furthermore, in designing such a perfusion for cardiomyocytes or other excitable cells, special considerations need to be taken to protect them from high shear stresses\textsuperscript{16} (e.g. ≥ 2.4 dyn/cm\textsuperscript{2}) as in their native environment they do not have direct exposure to blood flow.

We developed a simple yet robust microperfusion system, compatible with standard HT-format; suitable for excitable cells, which do not tolerate high shear rates; and miniaturizable for incubator use and integration with all-optical electrophysiology. We present validation of this high-throughput uninterrupted perfusion system (HT-µUPS), operating with standard 96-well microplates, by applying it to human cell constructs of iPSC-cardiomyocytes or engineered excitable cells over prolonged periods with repeated all-optical functional measurements.

**Results**

**Design and fabrication of the microfluidic cover.** The key part of the developed HT-µUPS perfusion system is the soft multichannel microfluidic plate cover which delivers liquid reagents into the standard 96-well microplate. The microfluidic plate cover has a two-layered structure made of polydimethylsiloxane (PDMS) as shown in Fig. 1a. The top layer has fluidic channels which connect to the liquid inlet and outlet ports embedded in the bottom layer (Fig. 1b,g). The bottom

\textsuperscript{1} Department of Biomedical Engineering, The George Washington University, Washington DC, USA.

\textsuperscript{2} These authors contributed equally: Lai Wei, Weizhen Li.

\* Corresponding authors: e-mail: entcheva@gwu.edu, zhenyu@gwu.edu
layer has “button”-like features which can snap into individual wells in standard 96-well microplates (Fig. 1e,f). The diameter of the button is designed to be 0.2mm larger than the diameter of the well in a standard 96-well microplate. When pushed into the well, the deformed elastomeric PDMS button forms a water-tight seal with the sidewall. The inlet and outlet ports embedded in the button enable culture media and other liquid reagents to perfuse in and out of each well, as shown in Fig. 1e. The size of the ports and the depth of the buttons can vary based on the requirements of different experiments.

We designed and fabricated different sized microfluidic covers, matched to the desired experimental conditions: Fig. 1c shows a cover designed to perfuse 8 wells in series on a standard 96-well microplate, as well as a cover for the entire standard 96-well microplate. In the latter case, 8 wells in each column are perfused in series and the 12 columns are perfused in parallel as
shown in Fig. 1d. Both microfluidic covers are fabricated as shown schematically in Fig. 1h. (Design files for the cover are available in the Supplementary Information.)

The microfluidic plate cover was made of polydimethylsiloxane (PDMS) using standard soft lithography\(^7\). However, the most widely used PDMS for microfluidic devices, Sylgard 184, has very low tear strength\(^18\) and is not durable enough for repeated use. Dragon Skin, another commercial silicone elastomer, has much higher tear strength but is very soft and cannot be plasma bonded\(^18\). In order to improve the durability of the cover and enable a water-tight seal with standard 96-well microplate, we mixed these two materials with 1:1 volume ratio which gave the resulting PDMS material both high tear strength and plasma bonding capability. The cover can be sterilized by immersing it in pure ethanol for 1 hour and rinsing it with pure water afterward.

The dimension of the channel in the cover was designed to match the diameter of the inlet/outlet ports in the button feature to avoid trapped bubbles. The width and height of the channels were chosen to be 1.5mm and 0.5mm respectively. The diameter of the inlet/outlet port was designed to be 1.5mm and the distance between the centres of the inlet and the outlet ports is 2.75mm. The diameter and the height of the button structure was fixed at 7.1mm and 1.5mm after empirical tests. The distance between the centres of two adjacent buttons is 9mm.

**Computational fluid dynamics (CFD) simulation and perfusion test results.** Cardiomyocytes live in a low shear stress environment because they are shielded by endothelial cells from direct contact with blood\(^16,19\). *In vitro*, both studied cell types here - cardiomyocytes and genetically-engineered excitable cells - form confluent layers that are sensitive to shear forces. Computational fluid dynamics simulations of shear rate and streamlines were used as a guide to estimate the shear stress applied on the cells in the HT-µUPS system.

CFD simulations were done using COMSOL Multiphysics 5.4 software. The 3D model used in the CFD simulation was designed to match the dimensions of the HT-µUPS cover and a standard 96-well microplate as shown in Fig. 2a. A well was simulated to have a bottom radius of 3.2mm, height of 9.4mm, and top radius of 3.48mm; the inlet/outlet ports in a “button” were simulated by two cylinders with radius of 1mm and height of 1.5mm located 2.75mm apart from each other on the top of the well. The boundary condition for the inlet and outlet was set as fully-developed flow with volume flow rate \(V_c=3.33\times10^{-4} \text{ m/s} \) (i.e. 0.2mL/min). (COMSOL simulation file is available in the Supplementary Information.)

The CFD simulation result for shear rate is shown in Fig. 2b. The highest shear rate at 10µm distance above the bottom of the well is smaller than 10×10\(^{-4}\) (1/s), which corresponds to a shear stress of ~8.9×10\(^{-4}\) dyn/cm\(^2\) for a liquid viscosity of 0.89 mPa·s (water). The simulated streamline distribution is shown in Fig. 2c.

A video camera was used to record the liquid flow inside a 96-well microplate perfused by the HT-µUPS system. By observing the colour change (using a food dye) inside each well in the standard 96 well microplate, we obtained the time required to finish perfusing all eight wells in series. The perfusion test result at 0.4mL/min is shown in Fig. 2b. Five minutes after the dye solution was introduced all eight wells were sufficiently perfused. This result shows that the HT-µUPS is capable of providing media exchange of 8 wells in a series perfusion configuration within 5 minutes; in contrast, manual media exchange typically is done once every two days. (A video of the perfusion test is available in the Supplementary Information).

**General HT-µUPS system setup.** The automated HT-µUPS perfusion system for standard 96-well microplates includes: a computer with pump control software, a piezo pump system and a miniature/portable perfusion set with a fluidic unit (ibiidi) connected to the HT-µUPS cover, and a standard glass-bottom 96-well microplate.

The ibiidi pump and fluidic unit system provide continuous unidirectional flow to the HT-µUPS system and consist of a piezo pump, a computer, a fluidic unit, a...
drying bottle, and the perfusion set. The connection schematic diagram is shown in Fig. 3. The computer connected with the pump by a USB cord enables the user to control the operating pressure and the flow rate of the perfusate. The drying bottle is connected to the inlet of the pump and the fluidic unit is connected to the outlet of the pump.

The piezo pump has an air inlet connected to the drying bottle, an air outlet, and an electric cable connected to the fluidic unit. The fluidic unit holds the perfusion set and uses pinching valves to maintain the flow into the microfluidic cover unidirectional. The perfusion set is connected via microbore tubing to the microfluidic plate cover which buttons on the 96-well plate.

The compact HT-μUPS can be deployed in on-stage microscope incubators as well as in standard cell culture incubators. The computer and the pump remain outside the incubator; they are connected to the incubator-located fluidic unit, perfusion set, microfluidic cover, and 96-well plate through pressure lines and electrical cables.

**Integration of the HT-μUPS with on-stage all-optical electrophysiology system (OptoDyCE).** Excitable cells were used in the testing of the HT-μUPS platform. These were differentiated human iPSC-cardiomyocytes and engineered “Spiking” HEK cells that represent a convenient experimental model to test fluorescent probes for tracking action potential and calcium dynamics. The latter remain proliferative but have been made “excitable” by genetically modifying them with a sodium ion channel and an inward-rectifying potassium ion channel to be able to generate rudimentary action potentials. Both cell types were virally transduced to express light-sensitive ion channel actuator (Channelrhodopsin-2, ChR2) and to respond to optical stimulation. HT-μUPS was combined with automated all-optical electrophysiology – the OptoDyCE platform - that provides contact-less optical pacing and optical recording of voltage and calcium through the glass bottom of the plate.

The microfluidic cover sealed a 96-well microplate with the cell assemblies, connected with the fluidic unit, positioned in a temperature-controlled on-stage incubator on an inverted microscope Nikon Eclipse Ti2, as shown in Fig. 4a. The controlling computer and the pump remained outside and were connected through the side port of the incubator. The pump operated under set fluid pressure, and flow rate was calculated based on the volume change in the liquid reservoirs (Fig. 3).

**HT-μUPS is compatible with all-optical cardiac electrophysiology studies in human iPSC-CMs.** Cardiomyocytes are sensitive to stress and were needed to experimentally validate HT-μUPS’ utility for use with iPSC-CMs.

After fluorescent dye labeling of the cell samples for live optical imaging and positioning of the microfluidic perfusion cover (Fig. 4c) onto the microwell plate, fresh Tyrode’s solution was manually flooded and de-bubbled. The covered microwell plate was then set inside the on-stage incubator (Fig. 4a). The transparent window of the on-stage incubator allows monitoring of the liquid volume in the fluidic unit. During the functional tests, samples were perfused with 10ml of Tyrode solution over three hours while optogenetic pacing (470nm) was applied and optical measurements were taken periodically using fast fluorescent small molecules, spectrally compatible with the optogenetic actuator. Cells remained spontaneously
active (at rates <0.5Hz) for 5 hours during this perfusion test. Overdrive optical pacing was able to provide stimulation at higher frequency – responses to 0.8Hz pacing shown in Fig. 4d. Intracellular calcium transients were measured using Rhod-4 (ex. 540nm) and voltage was measured with a near-infrared probe, BeRST123 (ex. 660nm), acquired on a single camera using temporal multiplexing as described previously. Since all-optical interrogation is from the bottom of the plate, the optical pacing, the optical measurements and the automated x-y-z positioning of the system did not disturb the HT-µUPS operation.

Preserved electrophysiological responses (membrane voltage and intracellular calcium) to pacing were confirmed in the perfused samples after 3h, Fig. 4d. Compared to the non-perfused group, the perfused samples had only a slightly worse signal-to-noise ratio, likely due to dye dilution over time. Similar results, indicating preserved functionality and dye response, were obtained using the engineered generic excitable cells (Fig. 5). These acute-test results indicate that HT-µUPS can safely be deployed in pharmacological and toxicological experiments, where stimulation and measurements with small fluorescent molecules can be applied, while the cells are perfused with varying drug doses over the course of several hours. Such dosing will likely reduce variation between wells normally present due to manual pipetting.

**Deployment of HT-µUPS for long-term cell culture in a standard incubator.** Long-term (chronic) experiments with perfusion are particularly valuable to address the metabolic demands of continuously stimulated or spontaneously active excitable cells. Maturation protocols for human iPSC-CMs often involve stimulation over multiple days24-27. In HT-format small samples, depletion of oxygen is a real constraint to apply such chronic stimulation. Other cell-assay applications also require perfusion over days, e.g. studies of chronic drug effects28.

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**Fig. 4** | HT-µUPS perfusion integrated with an on-stage incubator and all-optical electrophysiology (OptoDyCE) in human iPS-cardiomyocytes. 

- **a,** Experimental setup with on-stage incubation, automated all-optical electrophysiology measurements (OptoDyCE) and HT-µUPS perfusion in a 96-well plate. 
- **b,** Human iPS-cardiomyocytes immunolabeled for alpha-actinin (red), nuclei (blue) and genetically-modified with ChR2 (green) for optical pacing; scale bar is 20μm. 
- **c,** a row configuration of microfluidics-enabled perfusion in a 96-well plate. 
- **d,** functional data from non-perfused vs. continuously-perfused hiPS-CMs, optically paced at 0.8Hz using 470nm LED, with simultaneous optical recordings of voltage (red) and calcium (green) using fluorescent probes as indicated.

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**Fig. 5** | On-stage HT-µUPS and functional data from ChR2-Spiking-HEK cells using OptoDyCE.

- **a,** ChR2-Spiking HEKs: an excitable cell line with spontaneous electrical activity due to expression of Nav1.3 and Kir2.1, was transduced with an optogenetic actuator, ChR2. Fluorescent dyes for optical imaging of voltage and calcium were used as indicated. 
- **b,** ChR2-Spiking HEKs, non-perfused or continuously perfused with HT-µUPS maintained spontaneous electrical (voltage) and calcium (green) activities in 2 hours of on-stage functional tests.

The compact HT-µUPS format allows its straightforward deployment in standard cell culture incubators for longer-term studies. Here, proof-of-principle chronic experiments were conducted over 5 days using the Spiking HEK cells in combination with genetically-encoded optical actuator (ChR2) and optical sensor of calcium (R-GECO)29, so that repeated probing of function over multiple days was possible, Fig. 6. The study period was limited due to the proliferative nature of the engineered excitable cell combined with the 96-well microplate format.
For chronic perfusion, the fluidic unit and the perfusion cover with the microplate were placed inside the incubator, while the controlling computer and the ibidi pump were set outside and connected with the fluidic unit through incubator’s back opening. A row of eight samples were buttoned by the customized microfluidic cover and subjected to constant perfusion. The non-perfused control wells were plated in another row of wells and were also covered (Fig. 6c). For perfused wells, 10ml of culture medium was loaded in the fluidic unit for 5 days of culture. And non-perfused wells got manual medium exchange every other day by lifting the edge of perfusion cover. Optogenetic stimulation and optogenetic records of calcium were performed every day.

Brightfield images after five days in culture (Fig. 6d) revealed that cells in the non-perfused group aggregated into subgroups and the monolayer was sometimes disrupted. Cells in the perfused wells had healthier appearance. Gradual increase of intracellular calcium signal amplitude was observed in the perfused samples (Fig. 6e). Both groups remained responsive to optical pacing.

**Long-term perfusion with HT-µUPS improves cell viability and calcium responses of proliferating engineered excitable cells.** The overall healthier appearance of the HT-µUPS perfused Spiking HEK cells was further corroborated by cell viability quantification using propidium iodine (PI) and quantification of functional parameters in the paced calcium responses (Fig. 7). The perfused cell samples proliferated more to form confluent layers and also had less PI-positive cells with leaky cell membranes (Fig. 7a-b). Examining trends of intracellular calcium parameters over the 5 days of culture, we noted similar behaviour between perfused and non-perfused samples at the beginning (days 0 to 2) and distinct deviation between the two groups at later days. Specifically, only the perfused samples showed a monotonic increase in maximum rate of rise, calcium peak amplitude and maximum fall rate over time in culture, Fig. 7d. These parameters relate to more functional cells contributing to the measured signals in the perfused samples, as well as more “agile” calcium responses, which are typically an indicator of health in excitable cells. Both cell groups experienced shortening of the calcium transients over time in culture.

These proof-of-principle longer-term experiments demonstrated that HT-µUPS perfusion created favourable conditions for the proliferating excitable cells and promoted their healthy growth. It was possible to use genetically-encoded optical actuators and sensors to monitor cell function repeatedly over time without interrupting their perfusion. HT-µUPS operation did not require supervision and saved time in cell culture maintenance.

**Discussion**

In summary, this work presents a novel high-throughput microfluidic perfusion system, HT-µUPS, that can integrate with commercially-available standard microwell plates in a non-obstructive manner. HT-µUPS microplate perfusion can be combined with acute or chronic all-optical electrophysiology studies on a microscope or during a long-term cell/tissue culture in a standard incubator. The soft microfluidic perfusion cover, made of high-strength composite PDMS, is mechanically robust, biocompatible, sterilizable and reusable. The tube-free design minimized the size of the device and made it easy to clean for re-use. We demonstrated the design and
Several technological improvements can be considered in future work. Currently, the flow rate in the system is controlled by the perfusion pump pressure. In the future, a flow rate monitor can be added for better control of flow rate and shear stress. The current HT-μUPS cover is made of PDMS, which is porous and hydrophobic, and can lead to non-polar molecule adsorption and other material compatibility issues that may interfere with drug testing studies. Surface functionalization techniques can be utilized to partially address these problems. Alternatively, other elastomer materials such as polyurethane and fluoroelastomers can be explored to overcome material related limitations. The HT-μUPS can be extended by integrating microfluidic concentration gradient generators and on-chip valves and multiplexers for individual well addressability and programmable combinatorial drug/reagent condition generation.

Beside personalized medicine applications, leveraging patient-derived stem cells and tissue engineering, as an integrated platform technology, HT-μUPS and all-optical electrophysiology can also be deployed in future applications with organs-on-chip and microphysiological systems, albeit at a lower scalability compared to the cellular studies. Furthermore, this robust scalable perfusion solution can help in-vitro pharmacokinetics and pharmacodynamics studies of pharmaceuticals, including antibiotic resistance, microbioreactors, and automated molecular/digital pathology among others.
Methods

Materials and equipment for the manufacturing of HT-μUPS. Polydimethylsiloxane (PDMS) (Sylgard 184) and high-tear strength Silicone Rubber (Dragon Skin 10 Fast) were purchased from Ellsworth and Smooth-on respectively. Acrylic sheets (12”x12”x0.5”) Clear Sealant and UV, compatible with the 3D printer (Stainless Steel) were purchased from McMaster-Carr. PDMS glue (Gorilla Sealant clear, Clear Sealant, Silicone) was purchased from Grainger. Microtubes (Tygon® ND-100-80 Microube, 0.030” ID x 0.090” OD) was purchased from Corning Perimeter. Standard 96 well microplates (P96-1-N) were obtained from In Vitro Scientific.

Fabrication of HT-μUPS. Each layer of the microfluidic plate cover was made by soft lithography from acrylic moulds designed in SolidWorks and fabricated by laser cutting and CNC micro-milling (MDS-50, Roland). After cutting and milling, the moulds were cleaned using an ultrasonic cleaner with pure water for 30 minutes. The PDMS was used as a mixture with 1:1 volume ratio between Sylgard-184 and Dragon Skin. After thorough mixing, the PDMS mixture was poured onto the mould carefully to avoid air bubbles. The mould was placed in a vacuum chamber to de-bubble for 30 minutes and then in a 60°C convection oven to cure.

After curing, the two PDMS layers were peeled off the mould, treated with 0.5% perhydrol in ultra-pure water for 5 minutes to eliminate all air bubbles. Both layers were then baked at 100°C for six hours. After cooling, the bonded device was baked in a 60°C convection oven over night. The main inlet and outlet ports were connected with two 18G blunt needles, and the gaps between the channel and needles were sealed with a PDMS glue (Gorilla Clear Silicone Sealant).

Perfusion tests. A video camera (iPhone 8 back camera, 30fps) was used to record, from a 90-degree angle, the liquid flow inside a 96-well microplate perfused by the HT-μUPS system. The pressure used for the perfusion test was 5mbar and the average flow rate of the media is 0.2mL/min. First, pure water was run through the HT-μUPS system for 5 minutes to eliminate all air bubbles. Then artificial food dye solution was introduced and used to determine the flow rate.

Computational fluid dynamics (CFD) simulations. CFD simulations were conducted using COMSOL Multiphysics 5.4 software. The simulation was based on Reynolds-averaged Navier-Stokes equations (Turbulent Flow K-ω interface) and the boundary condition was No-Slip. The mesh had an extra fine element size (minimum element quality < 0.002). The simulations were done on an Intel® 64bit CPU (Intel® Core™ i7-9900KF CPU @3.60GHz, Family 6, Model 158S, Stepping 12, 8 cores with 64G RAM) running a Windows® 10 operating system. The simulation ran for one hour and 20 minutes with a pre-defined extra fine mesh (total degrees of freedom were ~3.6 million).

Excitable cell culture. Human induced pluripotent stem-cell derived cardiomyocytes (iPS-CMs) were purchased from Fujifilm/Cellular Dynamics International (iCell Cardiomyocytes® CMC-100-012-001) and handled according to the manufacturer’s instructions, growing them in CDS cell culture medium. A genetically-engineered excitable HEK cell line (“Spiking HEK®”), capable of generating action potentials, was a gift from Adam Cohen. These cells were grown in DMEM/Ham’s F-12 cell culture medium (Caisson Laboratories, Smithfield, UT). For both cell types, glass 96-well plates (Cellvis, Mountain View, CA) were pre-covered with 50g/ml fibronectin (Cytiva, NY) and then plated to form confluent monolayers in an incubator at 37°C, 5%CO₂.

Optogenetic transductions and optical sensors. Five days after thawing, the iPS-CMs were genetically modified to express Channelrhodopsin-2 (ChR2) to enable optical pacing. The viral infection was done using adenoviral vector Ad-CMV-1hChR2(H134R)-EYFP (Vector Bioslabs, Malvern, PA), as described previously, at multiplicity of infection (MOI) 50 for 2 hours, after which standard culture medium was added. ChR2 expression was confirmed by the eYFP reporter, 5 minutes after transfection.

In acute functional experiments, cells were dual-labeled with small-molecule fluorescent dyes (3,4-dihydropyridine receptor sensor CIB1, ChR2. Rhod-4AM at 10μM (AAT Bioquest, Sunnyvale, CA) was used to measure calcium, and the NIR voltage-sensitive dye BeRST1272 (gift from Evan W. Miller) was used at 1μM for fast measurements of membrane potential.

Automated all-optical dynamic cardiac electrophysiology (OptoDyCE). The Opto-DyCE platform66 is built around an inverted microscope Nikon Eclipse Ti2 (Nikon Instruments, Melville, NY) with a temperature-controlled cage incubator (Okolab, Ambridge, PA). It is a “CMOS” optical system that combines simultaneous excitation and emission to achieve all-optical interrogation with a single photodetector, iXon Ultra 897 EMCCD camera (Andor-Oxford Instruments, Oxford, UK), ran at 200fps, 4x4 binning. Positioning control (x-y) and autofocus (z) were done using Nikon LIS-Elements system using a programmable stage (Prior Scientific, Rockland, MA). Optical actuation was triggered by short (5 ms) pulses of blue light (470nm, approx. 1mW/mm²) using a LED connected to a digital micromirror device (DMD) Polygon400 (Mightex, Toronto, ON, Canada), with the capability to pattern the light. Additionally, green (535nm) and red (660nm) LEDs Lumen 1600 (Prior Scientific) were used for excitation of the calcium and voltage optical sensors, respectively. Emission was measured at 605nm (for Rhod-4AM and R-GECO) and filtered with a 750nm-long-pass-pass filter (Thorlabs, USA), using a series of PDMS-designed dichroic mirrors and filters from Chroma Technology, Bellows Falls, VT and Semrock Rochester, NY, to achieve on-axis operation, as described in detail previously12. Temporal multiplexing (interlaced frames) was used to record both calcium and voltage onto the same camera. In house written and Matlab software was used to pre-process the data and to extract shape parameters. Data processing included baseline correction, artifact removal and temporal filtering using a Savitzky-Golay polynomial filter (2nd order, 3 frame window) and normalization13.

Experiments with the integrated HT-μUPS and OptoDyCE platform. Acute-perfusion experiments with human iPS-CMs and with Spiking HEK cells were performed in fresh Tyrode’s solution (in mM): NaCl, 135; MgCl₂, 1; CaCl₂, 2; NaH₂PO₄, 0.33; glucose, 5.1; and HEPES, 5 adjusted to pH 7.4 with NaOH, after dual-labeling the cells with the voltage and calcium small-molecule fluorescent sensors. Chronic-perfusion experiments were performed directly in cell culture medium using the optogenetic actuator (ChR2) and sensor (R-GECO). For these experiments, samples were taken out of the standard incubator and measurements were taken once a day for five days. In both cases, spontaneous activity and optogenetically-paced activity was recorded. Pacing frequency was chosen based on the observed spontaneous rate, to overdrive-the-paces the samples. All measurements were performed in glass-bottom 96-well microplates on the inverted Nikon Eclipse Ti2 microscope with the cage incubator at 37°C.

During functional experiments with OptoDyCE, HT-μUPS covered the 96-well plate. It was connected with the fluidic unit, placed inside the cage incubator and the inverted microscope. The controlling computer and the ibidi pump were set to be outside of the on-stage incubator and connected with the fluidic unit inside. Similar arrangement was used for the long-term (chronic perfusion) experiments in the standard cell culture incubator. Pumping was set to 3mL/min and the perfusion flow rate was approximately 0.2mL/min. Before experiments, the HT-μUPS microfluidic cover was sterilized using a syringe and pushing 70% ethanol three times through the cover (positioned on top of sterile microplate), followed by five pure water rinses. The process not only sterilized the device but also tested it for possible leaks. The tubes and the pump components were sterilised by running through 20mL ethanol three times, followed by 20mL pure water five times to completely rinse the ethanol. These procedures were done inside a sterile laminar-flow hood before buttoning-on of the inverted microscope. The controlling computer and the ibidi pump were set to be outside of the on-stage incubator and connected with the fluidic unit inside. To remove potential air bubbles, the system was pre-filled by gently and slowly pushing through Tyrode’s solution or culture media using a syringe.

Cell viability and structural fluorescence imaging. For cell viability testing, cells were labeled with propidium iodide (PI, ThermoFisher Scientific, Waltham, MA) at 2mg/mL in PBS for 3 min, followed by PBS wash. Fluorescent images were acquired with Nikon Eclipse Ti2 (Ex 530nm, Em 610nm). PI labels unhealthy cells with compromised (“leaky”) membranes. For structural imaging, human iPS-CMs were fixed with 10% formalin and permeabilized with 0.2% Triton X-100 in 3% PBS in 1x PBS for 10 minutes. Anti mouse anti-alpha actinin (AAT-781, Sigma-Aldrich, MO) and 1:1000 goat anti-mouse Alexa 647 (ab150115, Abcam, Cambridge, UK) were used for alpha-actinin immunolabeling. Hoechst 33342 dye (ThermoFisher Scientific) was applied for nuclei labeling. Samples were
Statistical analysis. Sample size of all experiments were indicated in each section. Bar plot in Fig.7b was presented as mean±SE. Unequal two-tailed T-test was performed, P<0.01 was statistically significant.

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Author contributions
E.E. and Z.L. conceived the project and designed the experiments. Z.L. and L.W. designed the microfluidic cover and performed the CFD simulations. L.W. built and tested the microfluidic cover and set up HT-µUPS. W.L. developed the excitable cell line cells and performed all live cell experiments using...
OptoDyCE. L.W. and W.L. planned and executed all experiments with HT-µUPS and analysed the data. E.E. and Z.L. provided reagents and financial support. L.W., W.L., E.E. and Z.L. wrote the manuscript and all authors provided valuable feedback and revisions on the manuscript.

**Competing interests**

The George Washington University has filed a patent application related to this technology. L.W., W.L., E.E. and Z.L. are co-inventors of this patent application.

**Additional information**

Supplementary information is available for this paper on bioRxiv.

Correspondence and requests for materials should be addressed to E.E. and Z.L.