Supplementary Information

Compartmentalized perfusion for temporal control of chemical microenvironment of iPSC derived cardiac cells

Kaisa Tornberg,*[a] Wolfram Grötsch,[a] Niina Ritari,[a] Saara Haikka,[a] Lassi Sukki,[a] Katriina Aalto-Setälä,[a] Mari Pekkanen-Mattila[a] and Pasi Kallio[a]

*Corresponding author: kaisa.tornberg@tuni.fi

[a]Tampere University, Faculty of Medicine and Health Technology, Tampere, Finland

1 CHIP STRUCTURE

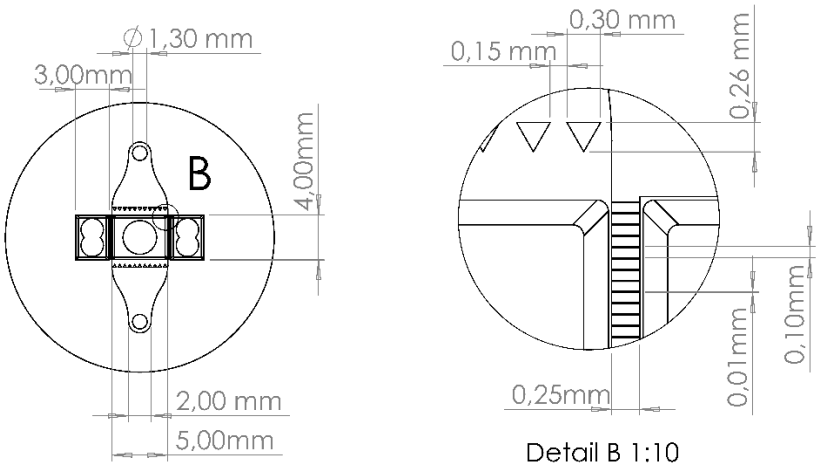

Fig. 1 Dimensions of the compartmentalized chip structure introducing perfusion channel to the middle cell culture compartment. 1.3 mm wide inlets and outlets lead to 200 µm high and 17 mm long perfusion channel that passes through cell culture compartment with 2.2 mm high and 5 mm wide and 4 mm long cell culture area. The middle cell culture area is separated from the perfusion channel by array of 0.3 mm x 0.26 mm triangular pillars with 0.15 mm spacing and maintain connection to neighboring culture compartments via array of 40 microtunnels with height of 3.5 µm, width of 10 µm and length of 250 µm with 100 µm spacing.

2 MODEL GEOMETRIES, BOUNDARY CONDITIONS, ASSUMPTIONS AND PARAMETERS

2.1 MODEL GEOMETRIES

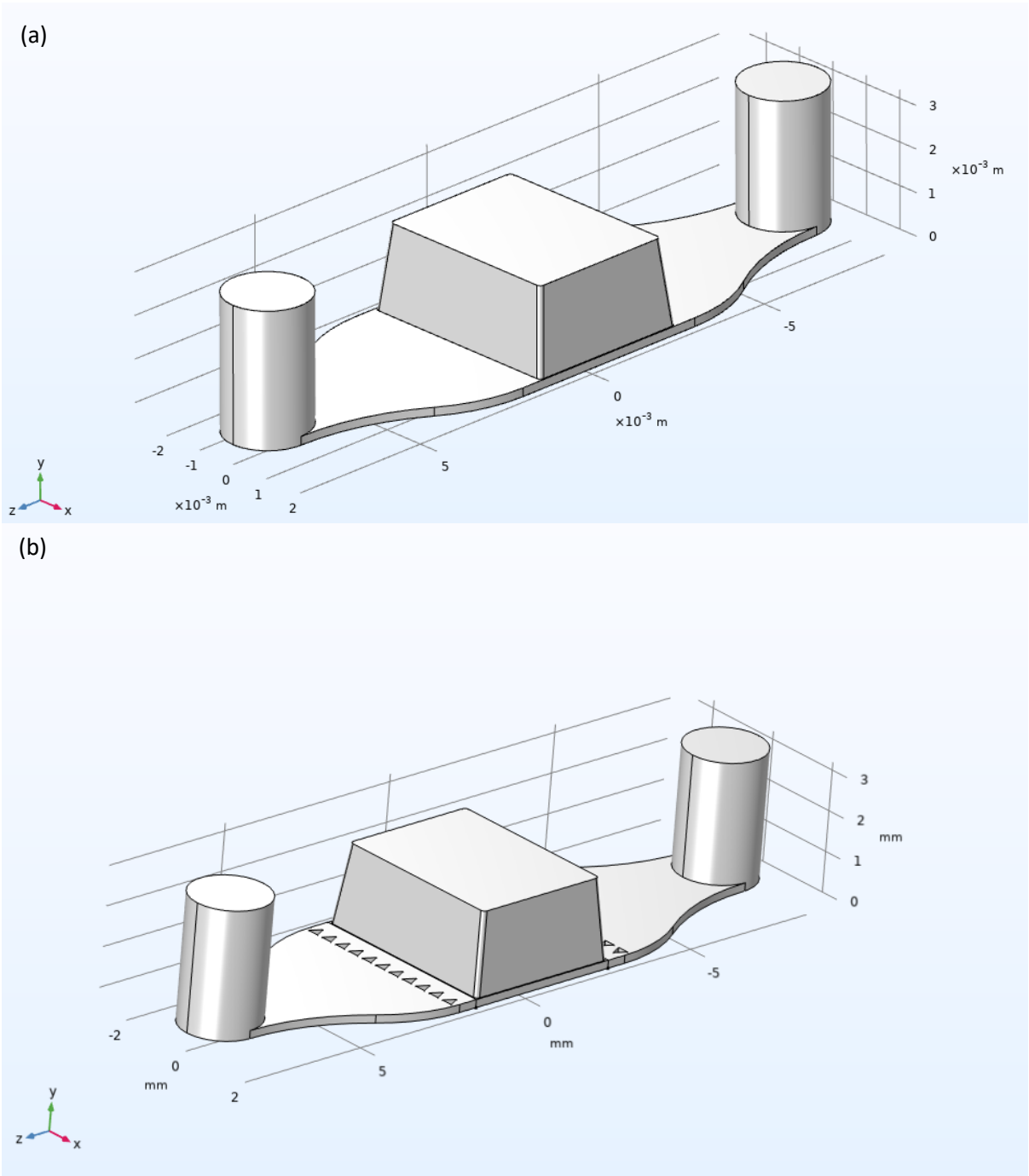

Fig. 2 Chip geometry (a) without the pillars and (b) with pillars.

2.2 BOUNDARY AND INITIAL CONDITIONS

In the Figure 2, the two circular pillars at the side represented the punched inlet and outlet to the cell culture chip, where for all models the left one was defined as inlet and the right one as outlet. They were defined as inlets and outlets for the laminar flow, particle tracing, and transport of diluted species nodes. All other surfaces were modeled as the channel walls. These were assigned with no slip and no flux conditions. The initial condition was fully developed flow without drug or particles.

Most of the model parameters were defined based on the needs of the cell culture and are listed in Table 1.

Table 1: The parameters used to build the numerical model.

Parameter	Value	Unit	Source
Q_in	5	µl/min	defined
Diffusion coefficient of adrenalin	1.03e-9	m^2/s	(Madhavan 2022)
Adrenalin concentration	0.001	Mol/m^3	defined
Experiment temperature	37	°C	defined
Experiment duration	90	min	defined
Injection duration	15	min	defined
Temperature	37	°C	defined

3 SIMULATION RESULTS OF FLOW RATES EFFECT ON SHEAR STRESS

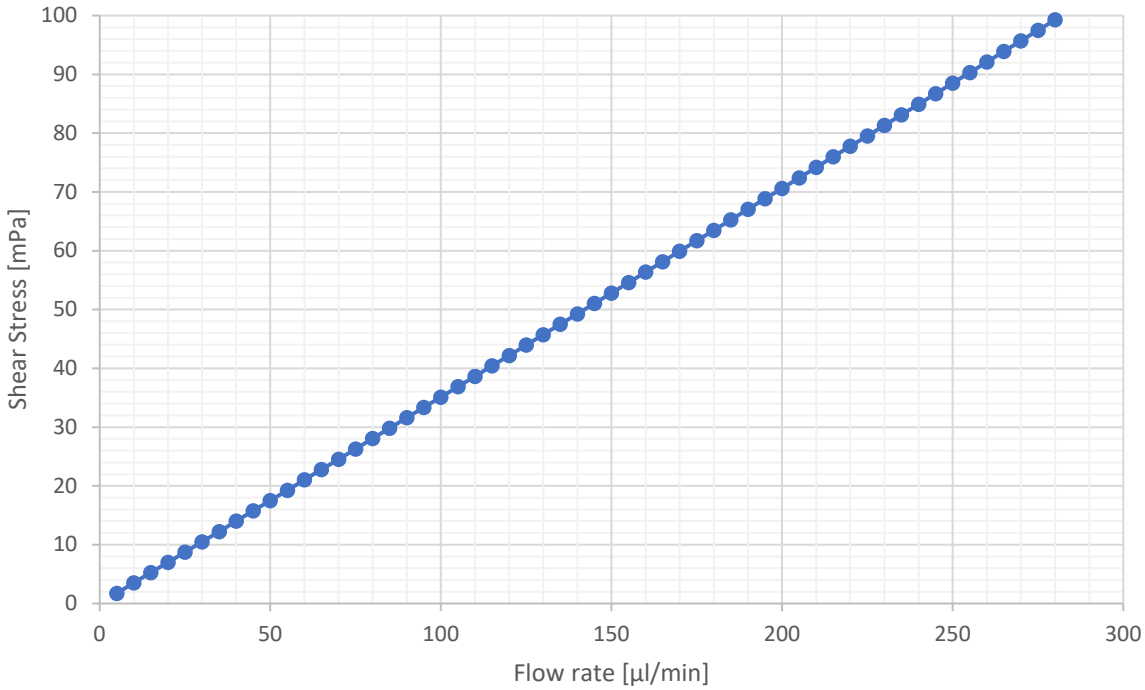

Fig. 3 Relationship between maximum shear stress and flow rate in the perfusion channel. Graph depicts maximum shear stress values in the cell culture area determined through numerical simulation, illustrating a linear dependence on flow rate. The shear stress increases with the flowrate and the maximum approaches threshold of 100 mPa at 280 µl/min.

4 FLOW AND VELOCITY FIELD WITH CHIP GEOMETRIES WITH AND WITHOUT PILLARS

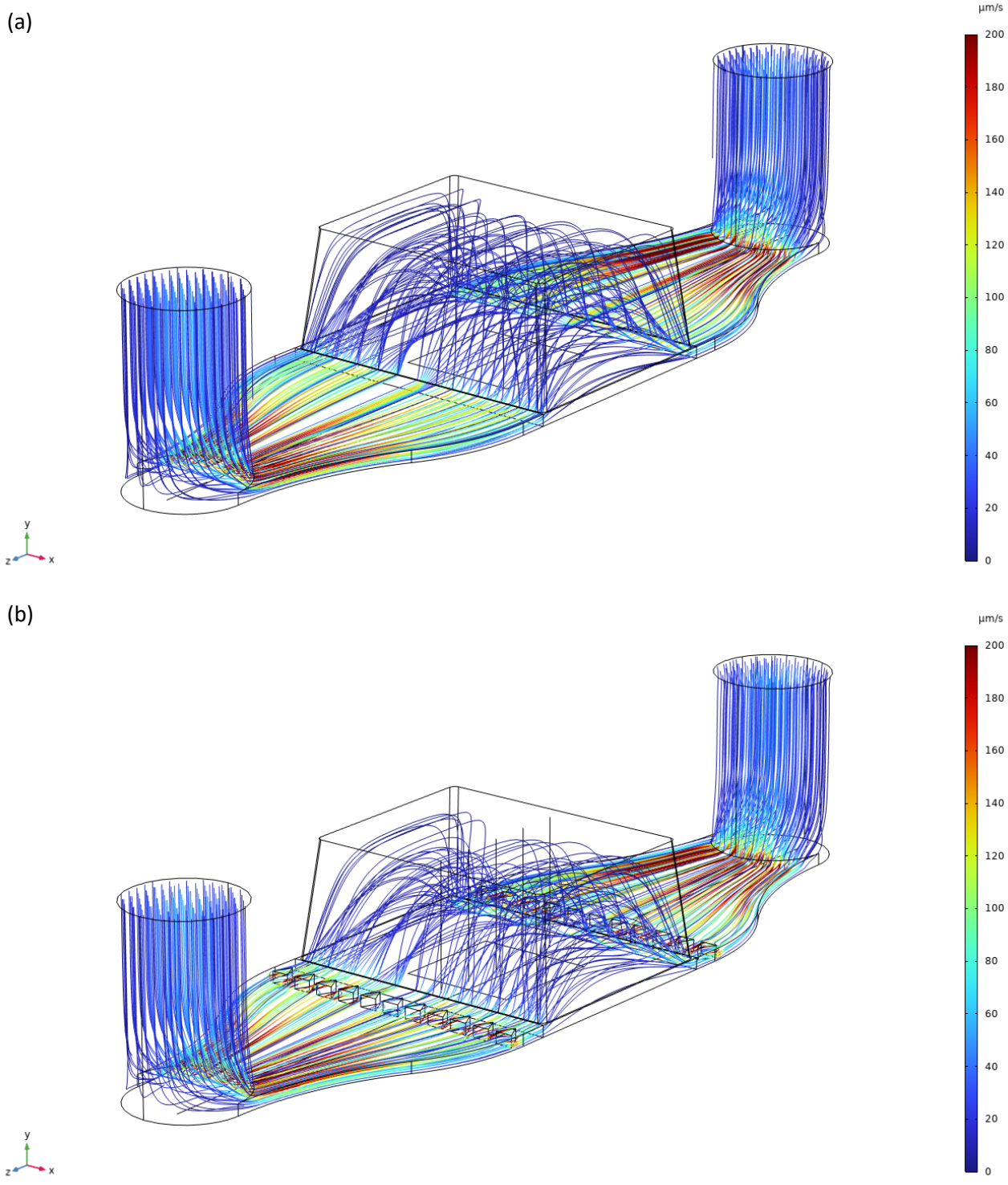

Fig.4 Simulated velocity field in chip geometries (a) without the pillars and (b) with the pillars. Figures show that the pillars have minimal effect on the development of the flow.

5 PARTICLE TRACKING ANALYSIS

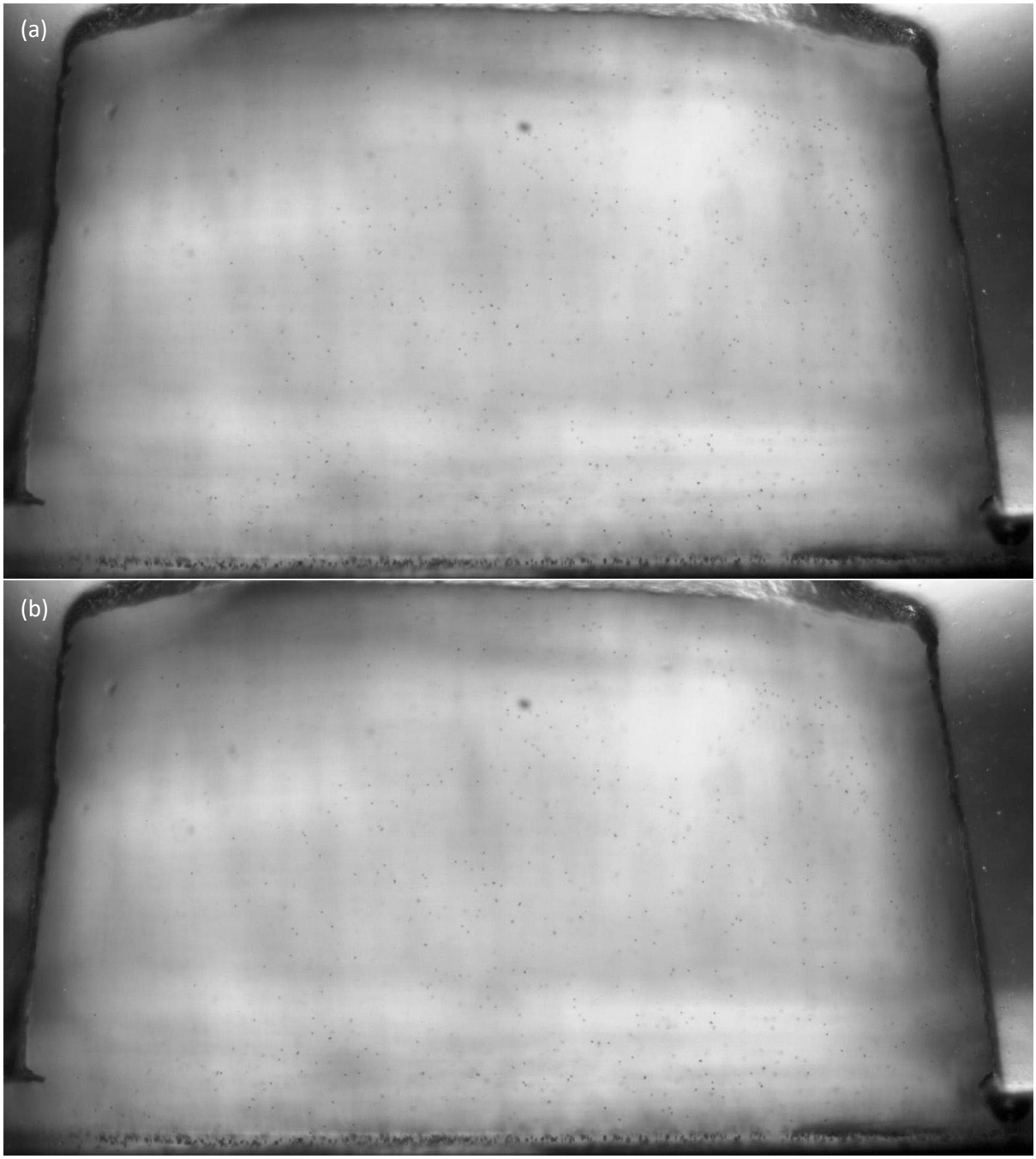

Fig. 5 Frames chosen for particle tracking analysis. (a) First frame chosen for analysis and (b) second frame chosen for analysis.

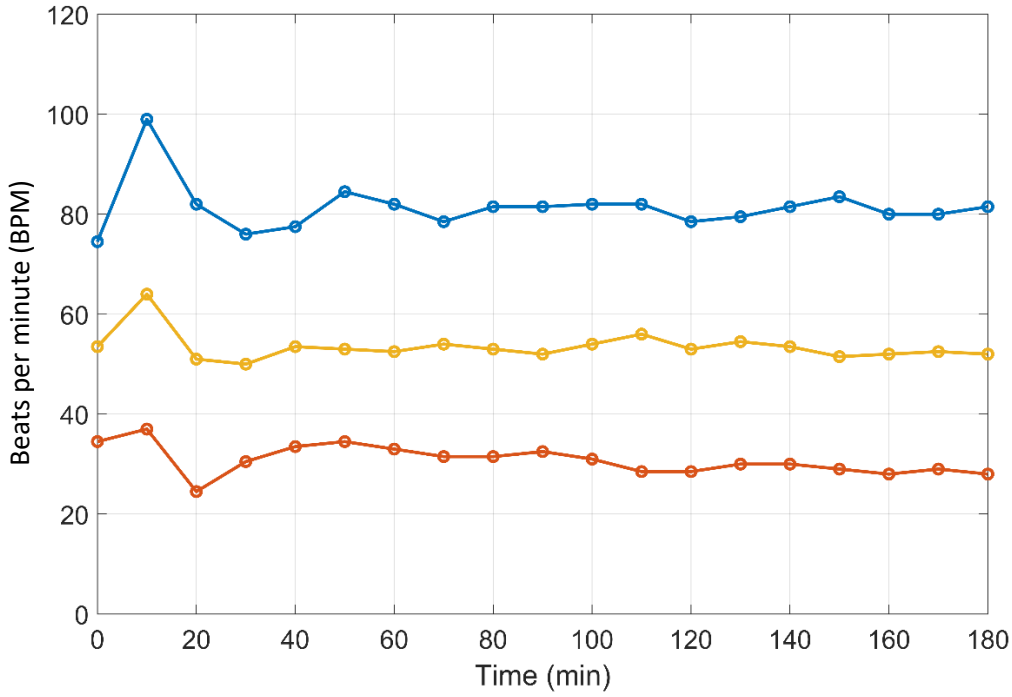

Fig. 6 Beat rates of iPS-CMs within the same chip. Three separate regions of interests (ROIs) measured for 180 minutes while the flow is on. Timepoint 0 indicates beating rate before starting the flow and each time point after that represent beating rate of hiPS-CMs under flow conditions without any stimulating molecules. Each cell responded to the start of the flow with elevated beating rate for the first 10 minutes and after that beating rate stabilized.

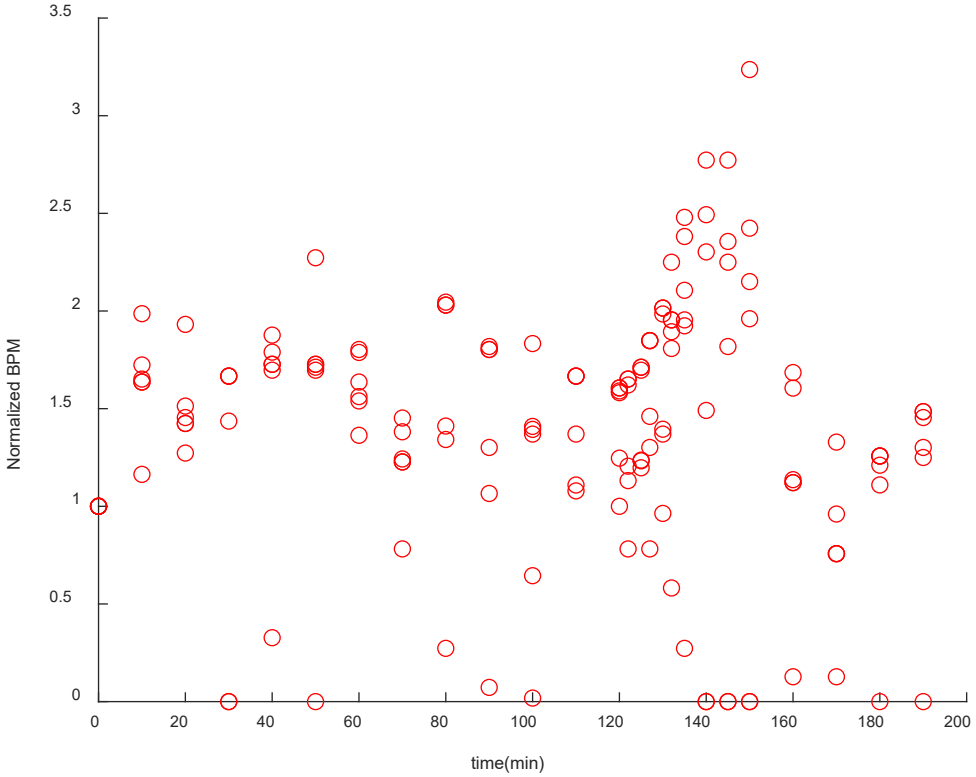

Fig. 7 All MUSCLEMOTION detected and normalized beat rates of iPS-CMs in all ROIs across three separate chips in adrenaline stimulation experiment. Cells were let to accommodate to the flow environment for the first 90 minutes. After that the measurement for the stimulation experiment was started. The transient stimulation with adrenaline molecules start at 120 minutes and all adrenaline molecules are washed off by time point 160 minutes.

6 SAFETY AND HAZARDS WORKING WITH TRICHLORO(1H,1H,2H,2H-PERFLUOROOCTYL)SILANE

Working and storing trichloro(1H,1H,2H,2H-perfluorooctyl)silane involves serious hazards and risks. If it comes to contact with water it reacts violently, it is toxic if inhaled, causes severe skin burns and eye damage. See safety data sheet for additional hazards and safety risks that are not listed here. Ensure that all personnel handling trichloro(1H,1H,2H,2H-perfluorooctyl)silane are properly trained in its safe use and emergency procedures.

Always wear appropriate personal protective equipment, including tightly fitting safety goggles, protective clothing, and thick nitrile gloves. Ensure that the work area is well-ventilated and use vacuum desiccator inside fume hood. Ensure that in case of fume hood malfunction, wear a ABEK2 filter equipped mask and ensure no other people are exposed to toxic gases during the process.

The SU-8 molds were coated with trichloro(1H,1H,2H,2H-perfluorooctyl)silane by Room Temperature Chemical Vapor Deposition (RT-CVD). Total of 15 µl trichlorosilane was vaporized together with the molds in water vacuum operated desiccator for 2 hours. All trichloro(1H,1H,2H,2H-perfluorooctyl)silane residues and contaminated plastic ware were neutralized with tap water (containing calcium).

7 SOURCES

Sethu Madhavan A, Kakkaraparambil Vijayan J, Rajith L. A Layered Electrochemical Sensor for Epinephrine Based on a Nitrogen-Doped Reduced Graphene Oxide-ZnFe2O4/β-Cyclodextrin-Modified Platinum Electrode. ChemistrySelect. 2022;7(44):e202203252. doi:10.1002/slct.202203252