Supplementary Information

Measuring both pH and O_2 with a single on-chip sensor in cultures of human pluripotent stem cell-derived cardiomyocytes to track induced changes in cellular metabolism

Esther Tanumihardja*[a], Rolf H. Slaats[b], Andries D. van der Meer[b], Robert Passier [b], Wouter Olthuis[a], and Albert van den Berg [a]

[a] BIOS Lab on a Chip group, MESA+ Institute for Nanotechnology, Max Planck Centre for Complex Fluid Dynamics and Technical Medical Centre, University of Twente, Enschede 7500 AE, The Netherlands

[b] Applied Stem Cell Technologies group, Technical Medical Centre, University of Twente, Enschede 7500 AE, the Netherlands

---

S1. pH response of bare Pt

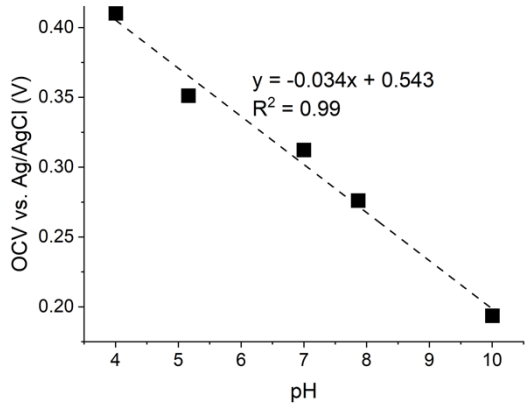

Figure S1. pH response of bare, unmodified platinum electrode

S2. Cl^- sensitivity of AgCl electrode

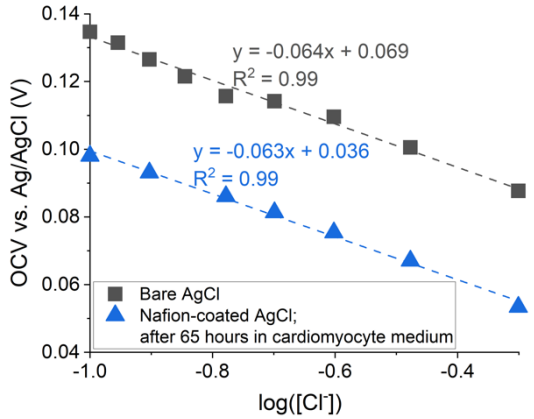

Figure S2. Cl^- response of bare and Nafion-coated AgCl electrode after prolonged exposure to used CM-TDI medium.

S3. Oxygen sensing calibration in CM-TDI medium

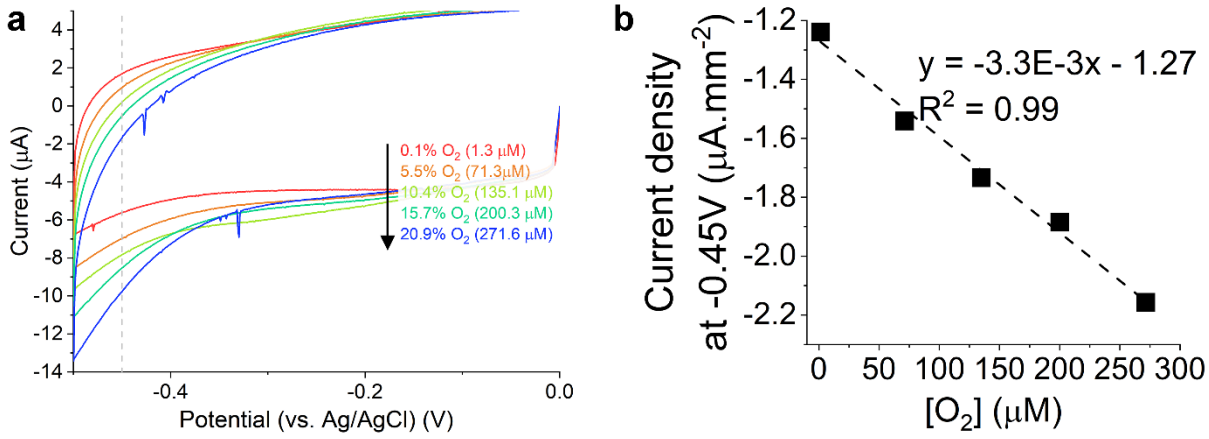

Figure S3 (a) Cyclic voltammetry (SR = 100 mV/s) recorded on RuOx electrode in CM-TDI medium with different aeration. (b) The reductive current measured at -0.45 V (normalised to the electrode's geometric surface area) is plotted against oxygen concentration, which shows a highly linear correlation.

S4. Microscopy images of hPSC-CMs

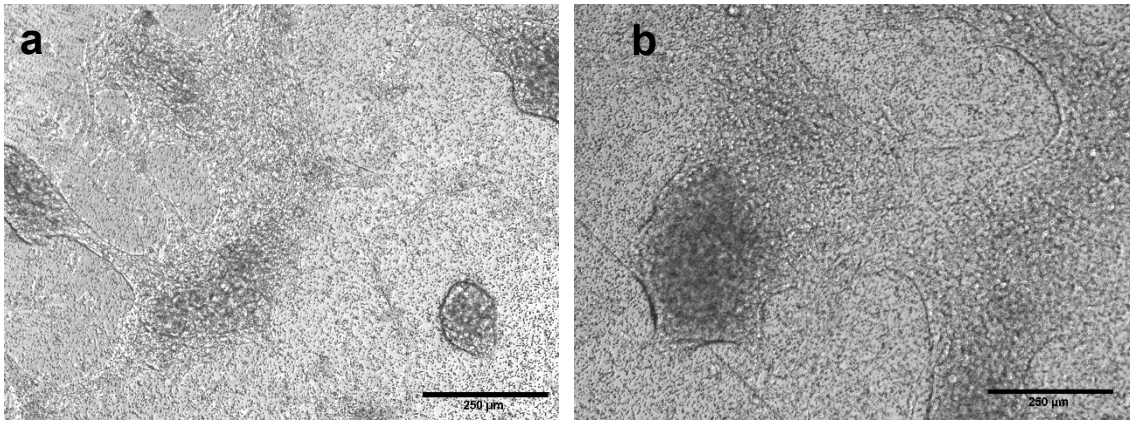

Figure S4. Images of hPSC-CMs (seeding density of 600k per well) in the 12 mm Transwell insert, pictured using EVOS through the polyester membrane. (a) hPSC-CMs after two days cultured in glucose-rich medium. (b) hPSC-CMs after two days cultured in galactose-rich medium.

S5. Blank pH measurement

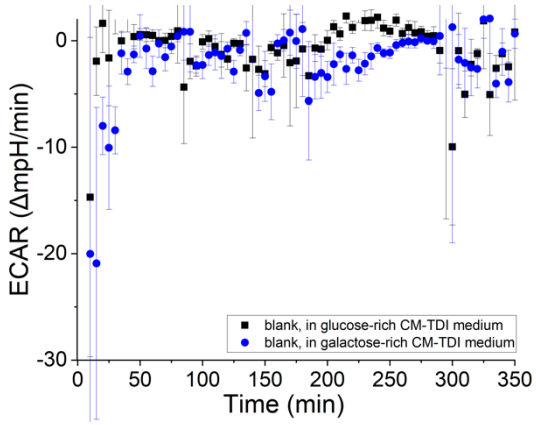

Figure S5. Calculated ECARs from pH measurements in the different CM-TDI media in the absence of hPSC-CMs in an incubator (5% CO_2, 37°C). The two setups showed comparable ECARs throughout the measurement.

S6. Images at the end of ECAR measurements

Buffering cell medium

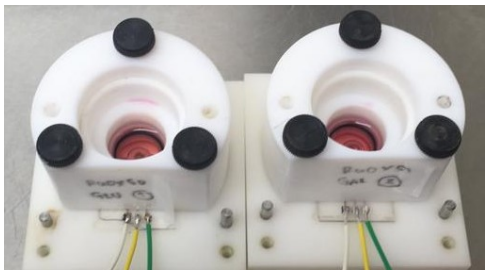

Figure S6. Image of the setups (without cells) in the experiment with buffering cell medium, taken at the end of day 2, seeding density 300k per well. The colour of the pH indicator (phenol red) did not show a clear difference between the setup with glucose-rich medium (left) and galactose-rich medium (right).

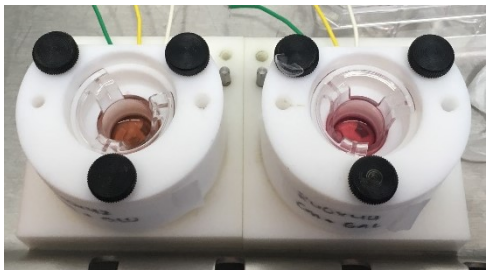

Figure S7. Image of the setups in the experiment with buffering cell medium, taken at the end of day 2, seeding density 600k per well. The colour of the pH indicator (phenol red) showed distinguishable difference between the setups. The medium in glucose-rich group (left) showed a more acidic environment (more orange colour) compared to the more red/purple galactose-rich medium (right).

Low-buffering cell medium

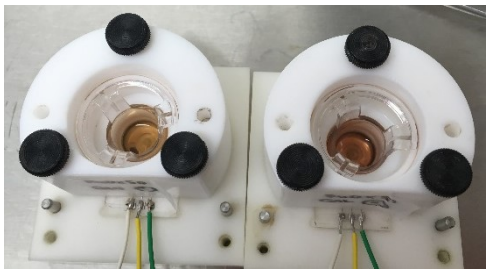

Figure S8. Image of the setups in the experiment with low-buffering cell medium, taken at the end of day 2. The colour of the pH indicator (phenol red) showed a clear difference between the setup with the glucose-rich medium (left) and the galactose-rich medium (right).

S7. pH calibration in CM-TDI medium

Table S1. Summary of RuOx electrodes calibration curve in CM-TDI medium.

	Sensitivity (mV/pH)	***Offset (mV)***	***Rsq***
Electrode #1	-53.7	609.2	0.9970
Electrode #2	-56.9	623.2	0.9988
Electrode #3	-52.2	581.6	0.9983