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Isolation of cardiac myocytes and measurement of myocyte shortening

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Prepare solutions

- 1 Prepare 400 mL of buffer solution. Adjust the pH to 7.4 with NaOH.

	Concentrat ion [mM]	Molar Mass [g/mol]	Mass [mg]
NaCl	134	58,44	3132,38
KCl	4,0	74,56	119,30
NaH ₂ PO ₄	1,2	156,01	74,88
HEPES	10	238,30	953,20
MgSO ₄	0,5	120,40	24,08
D- Glucose	11	180,16	435,99

Buffer solution

- 2 Prepare 10 mL of 500 mM CaCl₂ solution.

	Concentrat ion [mM]	Molar Mass [g/mol]	Mass [g]
CaCl ₂	500	147,02	0,74

500 mM CaCl₂ solution

- 3 Prepare 50 mL of 100 mM CaCl₂ solution.

	Concentrat ion [mM]	Molar Mass [g/mol]	Mass [g]
CaCl ₂	100	147,02	0,74

100 mM CaCl₂ solution

- 4 Separate 200 mL of buffer solution and add 0,5 mL of 500 mM CaCl₂ solution to obtain a buffer solution with 1,25 mM of Ca²⁺.
- 5 Separate 50 mL of buffer solution and add 25 µL of 100 mM CaCl₂ solution to obtain a buffer solution with 50 µM of Ca²⁺.
- 6 Separate 15 mL of buffer solution and add 15 µL of 100 mM CaCl₂ solution to obtain a buffer solution with 100 µM of Ca²⁺.
- 7 Separate 15 mL of buffer solution and add 30 µL of 100 mM CaCl₂ solution to obtain a buffer solution with 200 µM of Ca²⁺.
- 8 Separate 15 mL of buffer solution and add 75 µL of 100 mM CaCl₂ solution to obtain a buffer solution with 500 µM of Ca²⁺.

- 9 Separate 15 mL of buffer solution and add 150 μ L of 100 mM CaCl_2 solution to obtain a buffer solution with 1 mM of Ca^{2+} .

On the day of the experiment

- 10 Separate 100 mL of buffer solution with 1,25 mM of Ca^{2+} , oxygenate the solution and keep it in the fridge at 4 °C.
- 11 Heat and maintain the remaining 100 mL of buffer solution with 1,25 mM of Ca^{2+} at 37 °C.
- 12 Prepare the digestion buffer. Add 10 mg of type II collagenase in 40 mL buffer solution with 20 μ M of Ca^{2+} . Maintain the digestion buffer in the fridge at 4 °C.
- 13 Heat and maintain the buffers solutions with different Ca^{2+} concentrations (50 μ M, 200 μ M, 500 μ M, 1 mM) at 37 °C.

Experimental setup

- 14 Assemble a constant flow Langendorff apparatus using a peristaltic pump, tubing, cannula and a heat exchanger coil. Connect the exchanger coil to a circulating water bath temperature set at 37 °C.
- 15 Connect a thermometer to the outflow of the Langendorff apparatus, to measure the temperature of the heated solution.
- 16 Fill the Langendorff apparatus with pre-heated buffer solution with 1,25 mM of Ca^{2+} . Eliminate possible air bubbles. Adjust the peristaltic pump to a flow rate of 5,5 mL/min at outflow from the

cannula.

Myocyte isolation

- 17 Inject mouse with heparin solution (5000 U/Kg), via intraperitoneal injection.
- 18 After 30 min, anesthetize rat with a single intraperitoneal injection of sodium thiopental (50 mg/kg). When fully anesthetized (no response to strong foot pinch), euthanize the animal by cervical dislocation.
- 19 Open the chest and identify the aorta. Excise the heart, being careful not to damage the aorta. Place the heart in 4 °C, pre-oxygenated buffer solution with 1,25 mM Ca^{2+} .
- 20 Cannulate the heart by the aorta in the cannula attached to the Langendorff system. Secure the aorta to the cannula by tying a loop of silk suture.
- 21 Perfuse the heart with a buffer solution with 1,25 mM Ca^{2+} at a flow of 5,5 ml/min for 10 min.
- 22 Switch the solution to Ca^{2+} free buffer and perfuse the heart with this solution at a flow of 7,5 ml/min for 5 min.
- 23 Switch the solution to digestion buffer and perfuse the heart with this solution at a flow of 7,5 ml/min for 5 min. Recirculate the solution with the enzyme. Keep the perfusion until the heart become flaccid and pale (20 – 30 minutes approximately).
- 24 Remove heart from cannula and place in petri dish with buffer solution with 50 μM Ca^{2+} . Cut the

heart into small chunks and pipette several times with a transfer pipette to further disperse cells.

- 25 Filter the cell suspension into two 15 ml conical tube through a 250 μm nylon mesh filter.
- 26 Centrifuge the cell suspension for 3 minutes, 35g.
- 27 Aspirate the supernatant, and resuspend the pellet to buffer solution with 100 μM Ca^{2+} .
- 28 Allow cells to pellet for 10 min. Aspirate the supernatant, and resuspend the pellet to buffer solution with 200 μM Ca^{2+} .
- 29 Repeat last step, until cells are in the buffer solution with 1 mM Ca^{2+} .

Measuring contractility

- 30 Add some drops of buffer solution with cells to C-Stim chamber (IonOptix, USA).
- 31 Adjust syringe pump to perfuse (1 ml/min) buffer solution with 1 mM Ca^{2+} into the chamber. Adjust the temperature controller to 37 °C to pre-heat the solution in the chamber inlet and attach vacuum aspiration to chamber outlet.
- 32 Use 40X objective to locate desired cell. A healthy cell should be rod shaped and not

spontaneously contracting.

- 33** Set the electrical stimulator for bipolar pulses with 5 ms pulse duration, 25 V pulse amplitude and 1 Hz frequency.
- 34** Adjust camera rotation so cell is aligned horizontally on the screen.
- 35** In the software Ionwizard (IonOptix, USA), align edge detection bars to each end of myocyte and adjust threshold for contractility measurement with myocyte length variation.
- 36** Turn the electrical stimulator on and pace cells. Record tracing for posterior analysis.
- 37** Still in the software, align sarcomere detection on an area of cell with uniform sarcomeres. Choose a longer bar in the longitudinal axis to include as many sarcomeres as possible and improve contractility measurement with sarcomere length variation.