# Isolation of adult cardiomyocytes from mouse heart by a novel, semiautomated perfusion technology

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## Background and Aims

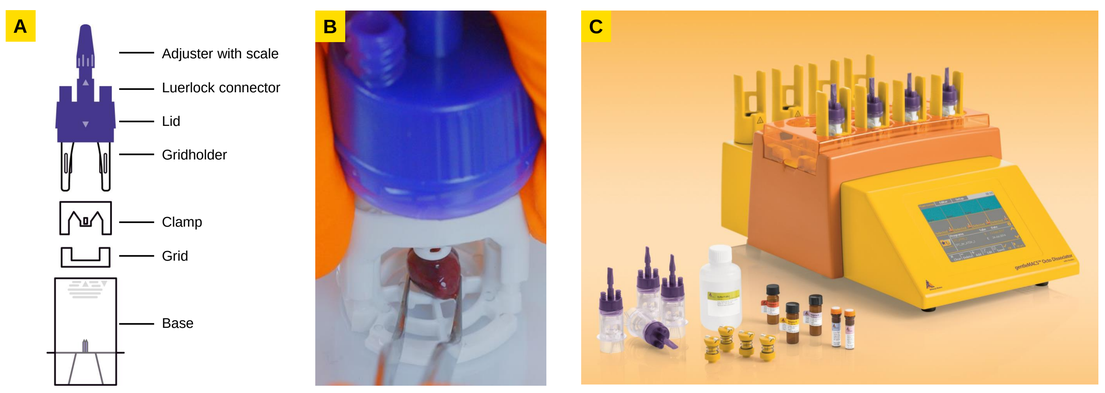
Primary adult cardiomyocytes represent a major tool in biomedical research. To date, different protocols for cardiomyocyte isolation, such as the Langendorff perfusion system, have been introduced, all requiring highly trained staff to avoid considerable fluctuations in quality and yield.

We aimed at establishing an easy and user-independent workflow for the isolation of primary cardiomyocytes.

## Methods

We recently introduced a new semi-automated perfusion technology, which is suitable for the gentle, rapid, and efficient generation of single-cell suspensions from rodent liver tissue [1]. We have now adapted this procedure for adult mouse heart with focus on C57Bl/6 and BALB/c mice. Tissue is clamped into an adjusted disposable and enzymatically digested using optimized reagents (fig. 1, A-C). Afterwards, single cells are liberated by a short mechanical disruption of the perfused tissue and the sample is loaded onto a strainer (MACS SmartStrainer, 100µm) to remove any remaining larger particles from the single-cell suspension. Cardiomyocytes are then enriched by a low spin centrifugation step. Yield and viability of mouse cardiomyocytes were determined, and cells were analyzed by flow cytometry as well as microscopy. In addition, functionality tests were performed.

**Figure 1**

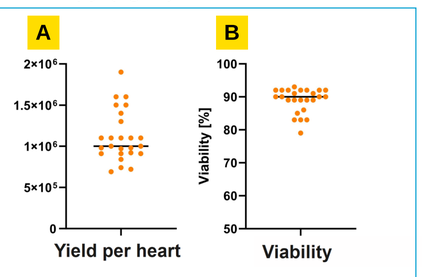



## Results

### 1 Yield and viability of adult mouse cardiomyocytes

The new perfusion technology is efficiently generating single-cardiomyocyte suspensions from mouse heart. We have designed a new tube format that is operated in combination with optimized reagents and newly designed perfusion sleeves on the instrument (gentleMACS Octo Dissociator with Heaters, Miltenyi Biotec) for optimal isolation of cardiomyocytes. Nested processing allows simultaneous handling of up to 8 samples. The protocol does not require inconvenient steps such as ligation of the aorta to a syringe needle under a microscope which is part of the Langendorff procedure. The low spin fraction of isolated cardiomyocytes from C57Bl/6 mice (n = 25) yielded 1 x $10^6$ cardiomyocytes (fig. 2A) with 89% viability (fig. 2B) and was further analyzed by flow cytometry (fig. 3) and microscopy (fig. 4).

**Figure 2**




### 2 Flow cytometry analysis of adult mouse cardiomyocytes

Samples were analyzed (fig. 3) on the MACSQuant with pre-gating on high scatter parameters (A). For analysis of viability, cells were stained with propidium iodide (B). For intracellular staining, cells were fixed and stained with following antibody fluorochromes (C): Connexin 43 (REAL935)-PE, α-Actinin (REA402)-VioR667, Cardiac Troponin T (REA400)-APC, MLC2v (REA401)-APC, MLC2a (REA398)-APC and Myosin Heavy Chain (REA399)-APC. Typical cardiomyocyte markers have been successfully stained. Note the different autofluorescence (much stronger in PE than APC).

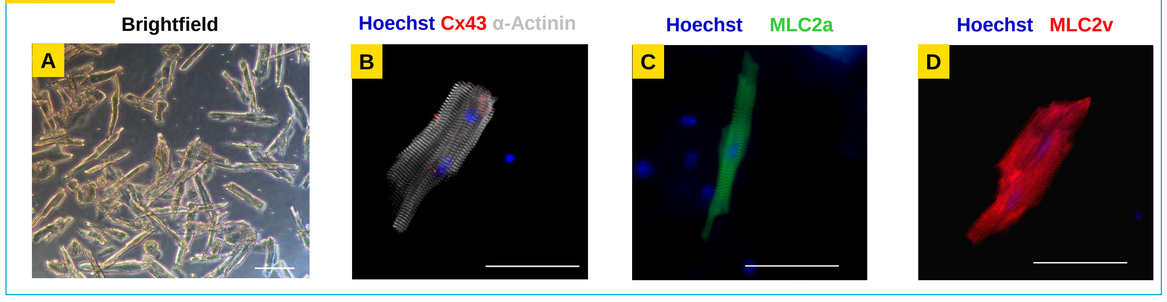
**Figure 3**




### 3 Microscopic Immunofluorescence Analysis

Microscopic analysis (fig. 4) revealed that the cells show the characteristic rod-shaped appearance (A). Perfusion-isolated cardiomyocytes from 6 weeks old mice were stained for α-Actinin, Cx43, MLC2a and MLC2v. Cell nuclei were stained with Hoechst (blue). Representative fluorescence images show α-Actinin (white) and Cx43 positive (red) ventricular cardiomyocytes (B). Staining for MLC2a (green) and MLC2v (red) distinguished atrial (C) from ventricular (D) cardiomyocytes. Scale bars are 50 µm.

**Figure 4**

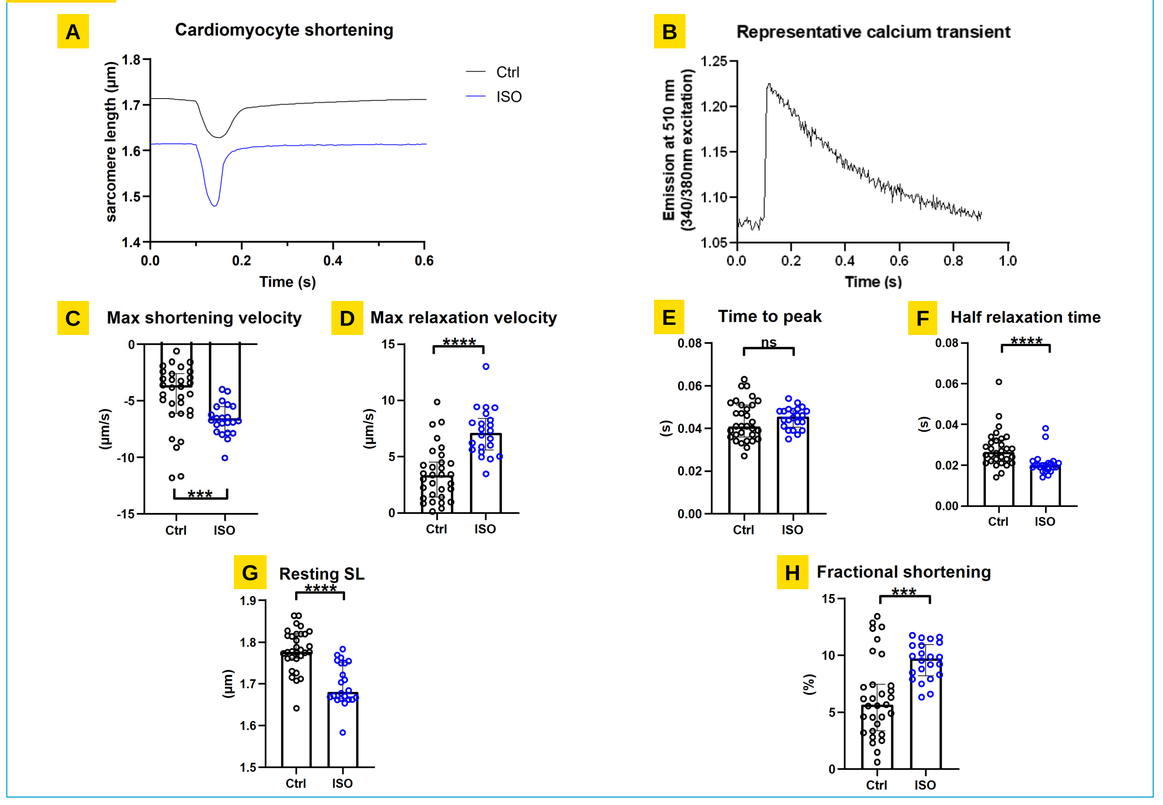



### 4 Single-Cell Sarcomere Contraction and Relaxation and Intracellular Calcium Transient Analysis

Cardiomyocytes isolated from 3 mice were taken for functional analysis. Three hours after attachment the coverslip with adherent cardiomyocytes was placed into home-made perfusion chamber under constant recirculation with an isotonic electrolyte solution containing (in mmol/L) NaCl 117, KCl 5.7, $NaH_2PO_4$ 1.2, $CaCl_2$ 1.25, $MgSO_4$ 0.66, glucose 10, sodium pyruvate 5, creatine 10, and HEPES 20 (pH 7.4) and cardiomyocytes were electrically stimulated at 37±0.5°C. For each cell, a rectangular region of interest including 15–20 sarcomeres was defined and changes in sarcomere length were registered with a variable-rate CCD video camera (MyoCam-S, IonOptix) connected to an inverted microscope (Olympus IX71). The Fast Fourier Transform (FFT) algorithm was used to record changes in sarcomere length during electrically paced contractions. 100 nM isoprenaline was used to evaluate the reaction of the cardiomyocytes to the drug. Data from 20–30 twitches per cell were averaged (fig. 5). Contraction amplitude, maximum shortening velocity (C), maximum relaxation velocity (D), time to peak (E) and half relaxation time (F) were analyzed with IonWizard 6.5.

Three mice were taken for functional analysis. Upon stimulation with isoprenaline, resting sarcomere length (G) was shorter and fractional shortening of cardiomyocytes (H) increased while the maximum shortening (C) and relaxation velocities (D) were faster and half relaxation time (F) decreased. These observations are in line with inotropic and lusitropic effects of isoprenaline and suggest intact β-adrenergic signaling cascade after cardiomyocyte isolation. For detection of intracellular calcium transients (B), cardiomyocytes were loaded with Fura-2 1.5 µM and emission ratio at 510 nm after alternating excitation with 340 and 380 nm was measured using a dual excitation fluorescence photomultiplier system (IonOptix).

**Figure 5**




## Conclusions

- The novel perfusion technology allows for the simple and reliable isolation of primary adult mouse cardiomyocytes (from up to 8 mice in parallel) which are functional, showed the expected rod-shaped morphology and were successfully stained with specific markers.
- Disposable (gentleMACS Perfusers 2, Miltenyi Biotec) and appropriate reagents (Heart Perfusion Kit, mouse, Miltenyi Biotec) have been optimized for mouse cardiomyocytes but can be adapted to non-parenchymal cells.
- The semi-automated workflow enabling heart perfusion in a closed system is easy to apply and helps to implement the 3R principle of animal experimentation as the failure rate is drastically reduced.
- A video of the developed protocol can be found using the QR code:

**References**

[1] Poggel, Carsten 2022, 'Isolation of Hepatocytes from Liver Tissue by a Novel, Semi-Automated Perfusion Technology', *Biomedicines*, 10, 2198, https://doi.org/10.3390/biomedicines10092198