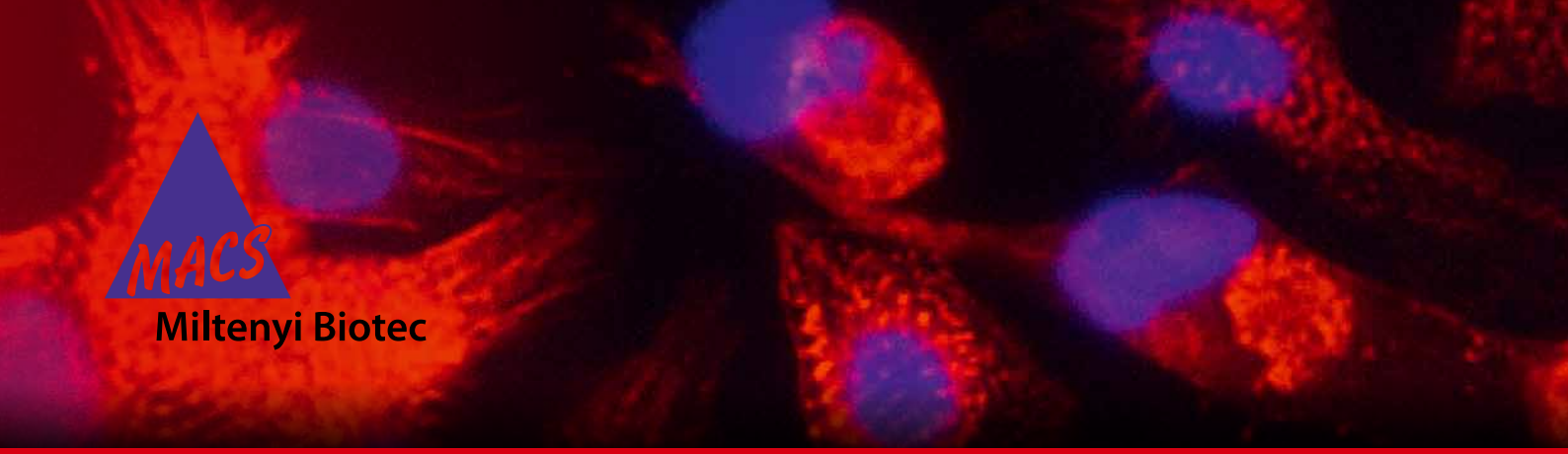

Sample preparation

Neonatal rat ventricular myocytes

Functional analysis of neonatal rat ventricular myocytes: comparison of manually isolated cells and cells isolated automatically using the gentleMACS™ Dissociator

Rasheda Chowdhury
Myocardial Function, NHLI, Imperial College London

Background

Manual preparation of neonatal rat ventricular myocytes (NRVM) for *in vitro* studies is time consuming and prone to variability as it involves numerous manual handling steps. To ensure reproducible results it is desirable to use an automated, standardized procedure. The gentleMACS Dissociator in combination with the Neonatal Heart Dissociation Kit, mouse and rat offers a high level of standardization as the protocol is based on a fully automated mechanical dissociation process and optimized, ready-to-use reagents.
This application note describes the functional analysis of NRVM isolated automatically using the gentleMACS Dissociator or manually with an established protocol[1].

Materials and methods

Preparation of NRVM

NRVM were isolated automatically using the gentleMACS Dissociator and the Neonatal Heart Dissociation Kit, mouse and rat, according to the manufacturer's protocol. Alternatively, cells were prepared manually using an established procedure[1]. Dissociated single-cell suspensions were pre-plated for 1 hour to remove any non-myocyte cells.

Functional analysis of NRVM

A drop containing 50,000 NRVM cells was placed on a microelectrode array (MEA) (Multichannel Systems) containing 60 electrodes (30 µm diameter, 200 µm spacing in an 8×8 array).

Cells were maintained in M199 media supplemented with 10% or 5% neonatal calf serum, 1.4 mM L-glutamine, 2 mg/mL vitamin B12 and 50 U/mL penicillin/streptomycin. After 3 days cells were transferred to Hanks buffered salt solution (HBSS) containing 1.5 mM calcium chloride. Prior to stimulation, cells were incubated with 0.05 mM di-8-ANNEPS (voltage-sensitive dye) for 5 minutes. After washing the cells, the MEA system was used to pace the cells at 1 Hz at 120% of the threshold (70 µA) and to record extracellular electrograms and measure conduction propagation.
A fast-acquisition optical mapping camera (Red Shirt Imaging) was used to measure cellular action potentials.

Results

Conduction velocity (CV) and propagation

Extracellular electrograms of cells prepared automatically or manually are shown in figure 1. Electrogram morphology was similar in both preparations. CV was determined from the MEA system by calculation of activation times at each electrode. Although CV in cells from the gentleMACS Dissociator was slower than in those isolated manually (fig. 2), these values were still acceptable as they were within the range previously published. Concentric isochronal lines in the activation maps show smooth propagation (fig. 2), indicative of a homogenous cell population with no areas of conduction block.

Action potentials

Cellular action potentials were recorded simultaneously with MEA electrograms. Action potential morphology was similar for both types of cells (fig. 3). Rise time was calculated as time from first deviation from the baseline to maximum of the upstroke. APD50 and APD90 were calculated as the time of 50% and 90% repolarisation to baseline, respectively (table 1).

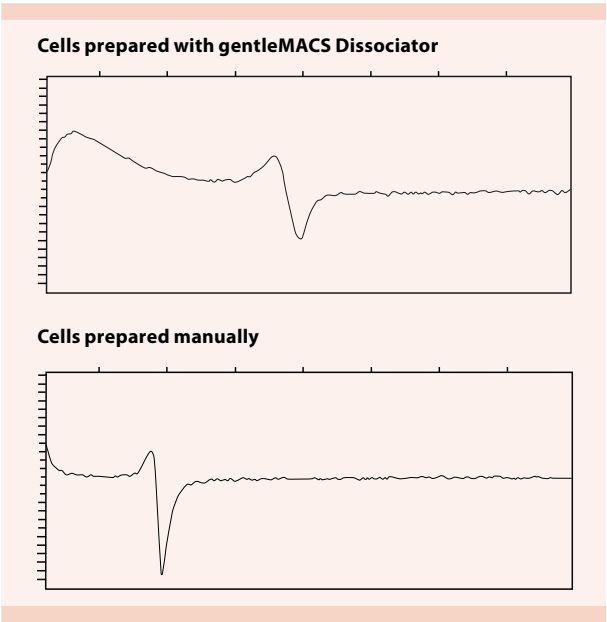

Figure 1: Electrogram traces from a single electrode from cells isolated automatically or manually.

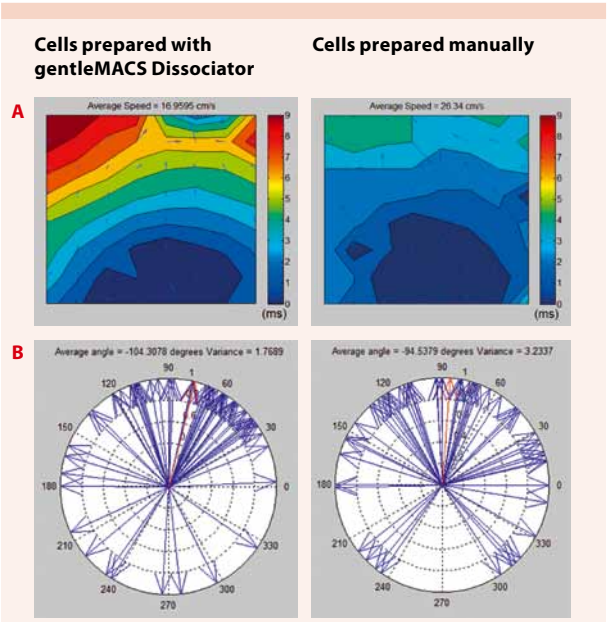

Figure 2: (A) Activation maps and (B) plots to show direction of propagation of cells isolated automatically or manually.

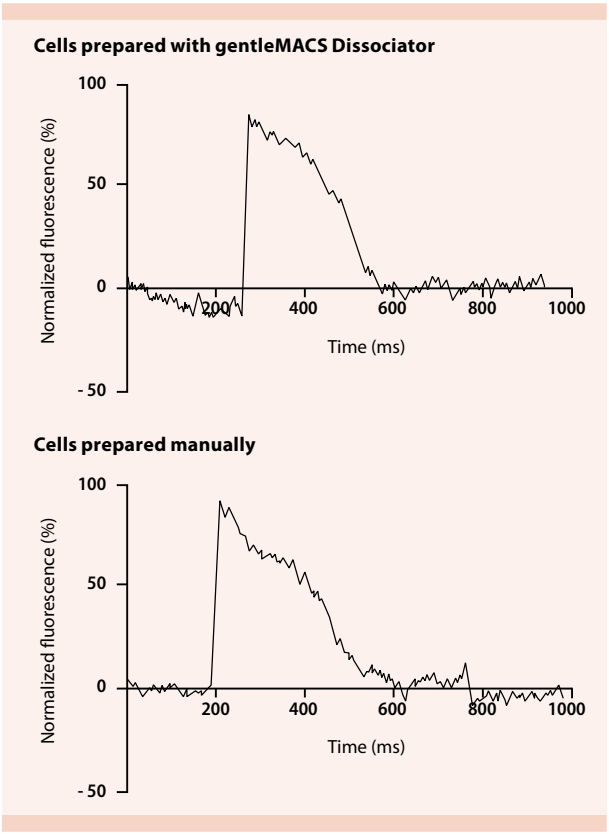

Figure 3: Optical mapping of action potentials for cells isolated automatically or manually.

Parameter	Cells prepared with gentleMACS Dissociator	Cells prepared manually
Rise time (ms)	10±3	11.89±1.342
APD 50 (ms)	209.3±28.15	198±21.56
APD 90 (ms)	282±38.22	296.1±24.52

Table 1: Functional parameters of cells prepared automatically or manually. Data are means±sd (n=3).

Conclusion

The presented data show that cells isolated automatically using the gentleMACS Dissociator are viable and fully functional to use for electrophysiology, and their parameters are similar to cells isolated manually.

Reference

1. Miragoli, M. *et al.* (2006) Electrotonic modulation of cardiac impulse conduction by myofibroblasts. Circulation research 98: 801–810.

Miltenyi Biotec

Miltenyi Biotec GmbH | Friedrich-Ebert-Straße 68 | 51429 Bergisch Gladbach | Germany | Phone +49 2204 8306-0 | Fax +49 2204 85197
macs@miltenyibiotec.de | **www.miltenyibiotec.com**

Miltenyi Biotec provides products and services worldwide. Visit **www.miltenyibiotec.com/local** to find your nearest Miltenyi Biotec contact.