Cor.At® Cardiomyocytes: Primary-like Cardiomyocytes for Manual and Automated Electrophysiological Screening

By Ralf Kettenhofen[1] and Sonja Stoelzle[2]
[1]Axiogenesis AG, Cologne, Germany, [2]Nanion Technologies, Munich, Germany

INTRODUCTION

Cor.At® Cardiomyocytes are derived from mouse embryonic stem cells (mESC). During differentiation of the mESC, about 5% of all cells develop into cardiomyocytes. Using transgenic mESC with the puromycin resistance cassette under the control of the cardiac α-myosin heavy chain-(MHC) promoter, 99.9% pure Cor.At® Cardiomyocytes can be selected from the large amount of non-cardiac myocyte cell population by the application of puromycin. For long-term storage, Cor.At® Cells are deep frozen as single cell suspensions in liquid nitrogen or -150°C deep freezers. Quality control strategies are implemented to guarantee lot-to-lot reproducibility and uniformity of functional properties of Cor.At® Cardiomyocytes for a storage period of at least 12 months. Thawed Cor.At® Cardiomyocytes readily form spontaneously and synchronously contracting monolayers overnight.

Seeded in low density on cover slips, Cor.At® Cardiomyocytes can be applied to manual patch clamp for the recording of action potentials as well as all three typical cardiac ion currents I_{Na}, $I_{Ca,L}$ and I_K (data not shown). Additionally, single cell suspensions of pre-cultured Cor.At® Cardiomyocytes can be readily analyzed with very high success rates in automated patch clamp systems like the Port-a-Patch® and Patchliner® from Nanion Technologies GmbH, Munich, Germany, as well as in other automated patch clamp systems (data not shown). The uniqueness of both Nanion systems is their capability to record action potentials in the current clamp mode and the possibility to perform the recordings at physiological temperature in addition to the standard measurements of ion currents in the voltage clamp mode.

METHODS AND MATERIALS

Cell culture and dissociation

A vial of Cor.At® Cardiomyocytes is thawed according to the Technical Manual.[1] For analysis in the automated patch clamp devices, Cor.At® Cardiomycoytes are seeded at a density of 10^5 viable cells per cm^2 culture area and pre-cultured for 4 days, then washed twice with ice cold PBS with Ca^{2+}/Mg^{2+} and incubated in the buffer at 4°C for 15 minutes. Afterwards, the Cor.At® Cardiomyocytes are washed once with PBS with Ca^{2+}/Mg^{2+} and dissociated with pre-warmed 1x trypsin/EDTA solution for 2 - 3 minutes in a humidified incubator at 37°C and 7% CO_2. The cell suspension is then transferred to Cor.At® Complete Culture Medium, centrifuged for 2 minutes at 200 xg. After discarding the supernatant, the cell pellet is resuspended to make a suspension of 1 million Cor.At® Cardiomyocytes per ml in the external patch clamp solution.

Immunostaining

A monolayer of Cor.At® Cardiomyocytes was fixed with 0.4% paraformaldehyde. Labeling was done using primary antibodies against α-sarcomeric actinin (Sigma-Aldrich, Taufkirchen, Germany) and Connexin 43 (Biotrend, Cologne, Germany). Primary antibodies were visualized by secondary antibodies conjugated to Cy3 and Cy5 (Dianova, Hamburg, Germany). Immunostainings were documented with an inverted microscope (Axiovert 200; Carl Zeiss, Jena, Germany).

Patch Clamp Solutions

External solution for Ca^{2+}-channel recordings: 80 mM NaCl, 3 mM KCl, 10 mM $MgCl_2$, 35 mM $CaCl_2$, 10 mM HEPES (Na^+-salt)/HCl, pH 7.4. External solution in voltage clamp and current clamp recordings: 140 mM NaCl, 4 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 5 mM D-Glucose monohydrate, 10 mM HEPES /NaOH pH 7.4. Internal solution: 50 mM KCl, 10 mM NaCl, 60 mM KF, 20 mM EGTA, 10 mM HEPES /KOH, pH 7.2.

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the Patchliner®.[2]

RESULTS

Cor.At® Cardiomyocytes display typical cardiac protein expression (cardiac α-actinin and Connexin 43) and structure when cultured in monolayers (Fig. 1). Suspensions of Cor.At® Cardiomyocytes show

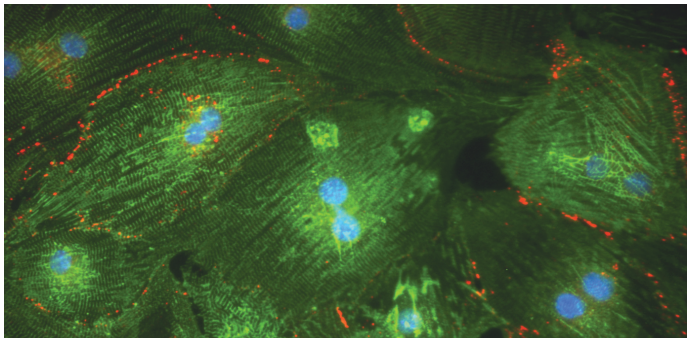

Fig. 1. Immunostaining of monolayers of Cor.At® Cardiomyocytes for cardiac actinin reveals typical cross striation of cardiac myocytes (green). Connexin 43 staining (red) marks foci of gap junctions between cells that are in contact. Nuclei are visualized by DAPI staining.

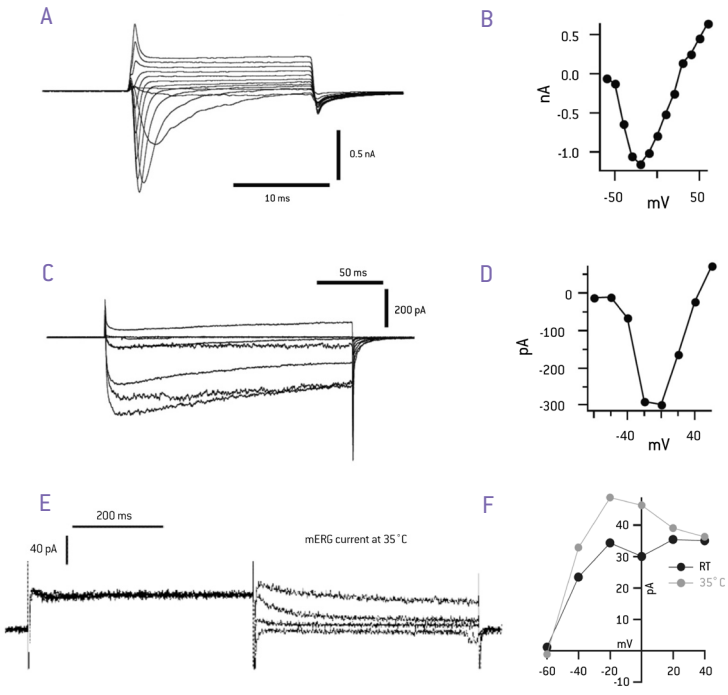

Fig. 2. Parallel voltage clamp recordings of Cor.At® Cardiomyocyte Ion Channel Currents in the automated clamp system Patchliner® from Nanion. I_{Na} (**A**), $I_{Ca,L}$ (**C**) and I_{Kr} (**E**) currents and corresponding I/V diagrams are shown in (**B**), (**D**) or (**F**) respectively. For the recording of I_{Kr} currents, physiological temperature is advantageous.

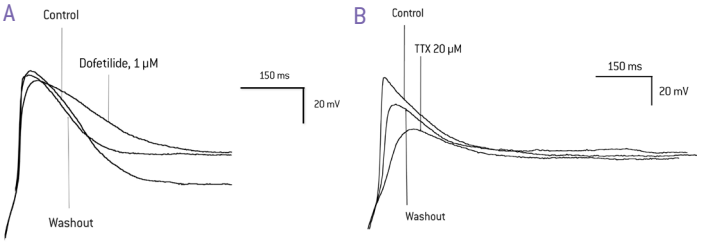

Fig. 3. Current clamp recording of action potentials from Cor.At® Cardiomyocytes in the Port-a-Patch® from Nanion. (**A**) The hERG blocker dofetilide (1 µM) induced reversible action potential duration prolongation. (**B**) 20 µM of the sodium channel blocker tetrodotoxin (TTX) induced slowing of V_{max} of the fast rise of the action potential and reduced its amplitude.

very high success rates for high quality sealing in the automated patch clamp devices Port-a-Patch® and Patchliner® from Nanion (Fig. 4) and reveal essential cardiac ion currents including I_{Na}, $I_{Ca,L}$ and I_{Kr} (Fig. 2). The hERG blocker dofetilide reversibly prolongs the action potential duration of Cor.At® Cardiomyocytes. An application of the Na-channel blocker TTX reversibly decreased the maximum of the action potential. Furthermore, depolarization was slowed.

CONCLUSION

Cor.At® Cardiomyocytes display the electrophysiological properties of primary cardiac myocytes and are suitable for application in both manual and automated patch clamp systems for screening of pharmacological and safety pharmacological drug effects.

REFERENCES

[1] Cor.At® Technical Manual. www.axiogenesis.com

[2] Recordings of Action Potentials in Cor.At® Cardiomyocytes on Nanion's Port-a-Patch® and Patchliner®. www.nanion.de

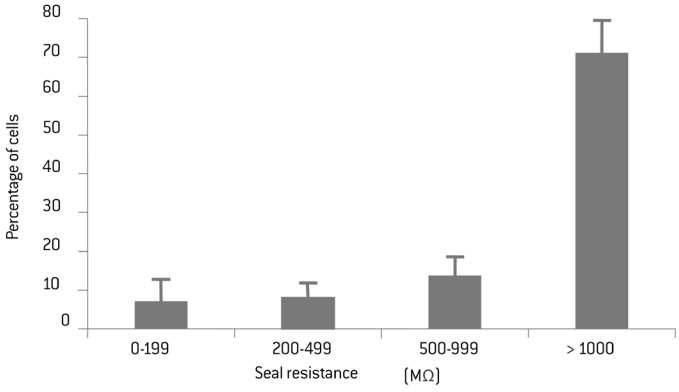

Fig. 4. Success rates to obtain high quality sealing in the Patchliner® from Nanion is more than 80% for high quality seal resistances of more than 500 MΩ.

ORDERING INFORMATION

Cat. No.	Description	Size
Cor.At® Cardiomyocyte Bundles		
XCAC-1001E	Cor.At® GFP mESC-Cardiomyocyte Bundle, EU (includes 100 ml culture media and puromycin)	100,000 Cells
XCAC-1005E	Cor.At® GFP mESC-Cardiomyocyte Bundle, EU (includes 100 ml culture media and puromycin)	500,000 Cells
XCAC-1010E	Cor.At® GFP mESC-Cardiomyocyte Bundle, EU, (includes 100 ml culture media and puromycin)	1 Million Cells
XCAC-1050E	Cor.At® GFP mESC-Cardiomyocyte Bundle, EU, (includes 250 ml media and puromycin)	5 Million Cells
XCAC-2010E	Cor.At® Colorless mESC-Cardiomyocyte Bundle, EU, (includes 100 ml media and puromycin)	1 Million Cells
XCAC-1P60E	Cor.At® GFP mESC-Cardiomyocyte Bundle, EU (includes 100 ml culture media, thawing media, and puromycin)	96-well plate
Cor.At® Cardiomyocyte Cells		
XCAC-1001	Cor.At® GFP mESC-Cardiomyocyte cryoamp plus puromycin	100,000 Cells
XCAC-1005	Cor.At® GFP mESC-Cardiomyocyte cryoamp plus puromycin	500,000 Cells
XCAC-1010	Cor.At® GFP mESC-Cardiomyocyte cryoamp plus puromycin	1 Million Cells
XCAC-1050	Cor.At® 5M GFP mESC-Cardiomyocyte cryoamp plus puromycin	5 Million Cells
XCAC-2010	Cor.At® Colorless mESC-Cardiomyocyte cryoamp plus puromycin	1 Million Cells