ELECTROPHYSIOLOGICAL PROPERTIES OF THE EFFECT OF USPIO-LABELING ON EMBRYONIC STEM CELLS

A. M. Chow[1,2], Y-C. Chan[3], Y-M. Lau[3], J. S. Cheung[1,2], T. Y. Chow[1], H-F. Tse[3], and E. X. Wu[1,2]

[1]Laboratory of Biomedical Imaging and Signal Processing, The University of Hong Kong, Pokfulam, Hong Kong, [2]Department of Electrical and Electronic Engineering, The University of Hong Kong, Pokfulam, Hong Kong, [3]Department of Medicine, The University of Hong Kong, Hong Kong

INTRODUCTION

MRI cell tracking using (ultrasmall) superparamagnetic iron oxide ((U)SPIO) nanoparticles have been used to monitor the migration of stem cells in different disease models[1]. Embryonic stem cells (ESCs) can maintain their pluripotency to differentiate into all cell types while propagating in culture, they may therefore provide an supply of specialized cells for cell-based therapies[2]. Direct injection of pluripotent ESCs after myocardial infarction has been suggested as a means to repair the myocardial infarct area[3]. However, cellular toxicity or inhibition of functionality following SPIO labeling is still under investigation. Furthermore, transplantation of cells with undesirable electrical properties into the heart can lead to arrhythmias[4]. Mesenchymal stem cells labeled with poly-L-lysine (PLL) coated SPIO has been reported of inhibiting chondrogenesis[5] and transient change in gene expression following SPIO labeling[6] was reported. With the application of imaging stem cells for cellular cardiomyoplasty, the potential functionality change in electrophysiology of (U)SPIO-labeled ESCs should be considered. In this study, we use monocrystalline iron oxide nanoparticles (MION), one kind of USPIO, and Poly-L-lysine (PLL) for magnetic labeling of mouse ESCs to report on influences of electrophysiological properties of USPIO-labeled mouse ESCs.

METHODS

Magnetic cell Labeling: Mouse ESCs R1 (SCRC-1011, ATCC, Manassas, VA) were labeled with MION-47 (CMIR, MGH, MA) and transfection agent Poly-L-lysine (PLL; Sigma, St Louis, MO). PLL of concentration 2.0 μg/mL was mixed with MION of concentration 50.0 μg/mL for 60 min in serum-free cell culture medium at room temperature on a rotating shaker. These culture media were then added to the cells and kept overnight for 24 hours at 37 C in a 95% air per 5% CO_2 atmosphere. Cells were washed twice with phosphate-buffered saline (PBS, pH = 7.4) to remove MION–PLL complex, trypsinized, washed, and re-plated on a 0.2% gelatin-coated plate for further investigation. Control cells were cultured without neither MION nor PLL. Viabilities of mouse ESCs after labeling were determined using Trypan blue exclusion and the result was expressed as percentage live cells of total cells. For Prussian blue staining, labeled cells were transferred to a microscope slide and fixed with 4% glutaraldehyde, washed, incubated for 30 minutes with 2% potassium ferrocyanide (Perls reagent) in 6% hydrochloric acid, washed again.

Electrophysiology: Electrophysiological recordings were performed from sampling of two batches of labeled cells (n = 5) and control cells (n = 4) at Day 1 at room temperature using whole-cell patch-clamp techniques established[7,8]. Pipette electrodes (TW120F-6; World Precision Instruments, Sarasota, FL) were fabricated using a Sutter P-87 horizontal puller and fire-polished and had final tip resistances of 3 – 4 MΩ. All recordings were performed at room temperature in a bath solution containing (in mM) NaCl 110, KCl 30, $CaCl_2$ 1.8, $MgCl_2$ 0.5, HEPES 5, and glucose 10, pH adjusted to 7.4 with NaOH. The internal solution for patch recordings contained (in mM) NaCl 10, KCl 130, $MgCl_2$ 0.5, HEPES 5, EGTA 1, and MgATP 5, pH adjusted to 7.3 with KOH. Cells were initially held at -80 mV and pulsed from -70 to 70 mV with 10 mV increments for 300 ms, followed by a -40 mV pulse for 50 ms. Current densities, steady-state currents normalized with its capacitance (specific capacitance of biological membranes is fairly constant with 1 μF/cm^2 membrane area typically), are plotted against the applied potential, with values presenting as mean ± SEM. Statistical significance was determined using unpaired Student's *t* test with $p < 0.05$ representing significance.

RESULTS

Labeled mouse ESCs viability in culture after labeling, as determined by absence of uptake of Trypan blue stain, was 92±4%. Prussian blue staining of mouse ESCs labeled with MION-47 using PLL for 24 hours showed intracytoplasmic iron inclusions as dense blue-stained vesicles (Figure 1(a)). A labeling efficiency of above 90% was observed, indicating that the MION-PLL labeled cells investigated are nearly all being loaded with USPIO. In contrast, no iron was detected in the control (Figure 1(b)). Depolarization-activated time-dependent noninactivating outward currents that increased progressively with positive voltages were recorded (Figure 2). Currents recoding normalized with its capacitances were plotted against applied voltage and illustrated in Figure 3 for control and MION-PLL labeled mouse ESCs, observing that the current densities increased with applied voltage in general for both control and MION-PLL labeled cells. Result of unpaired Student's *t* test showing no significant difference of current densities.

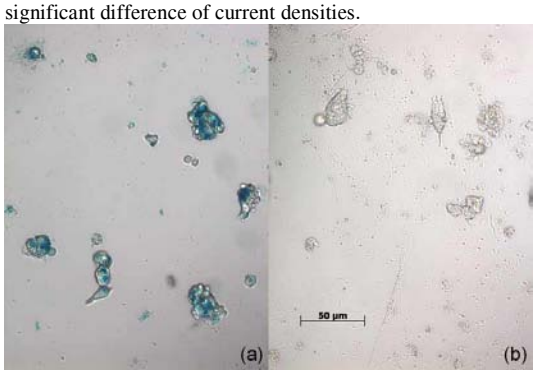

Figure 1 Micrographs show (a) clumps of mouse ESCs stained with Prussian blue to demonstrate the uptake of USPIO particles. USPIO particles are visible as blue iron deposits; (b) mouse ESCs without labeling of MION-PLL showing no apparent USPIO uptake.

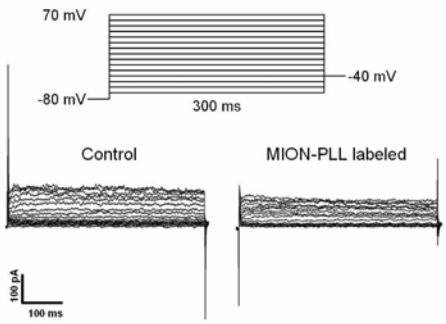

Figure 2 Representative current tracings recorded from control and MION-PLL labeled mouse ESCs. The electrophysiological protocol used for eliciting currents is also given.

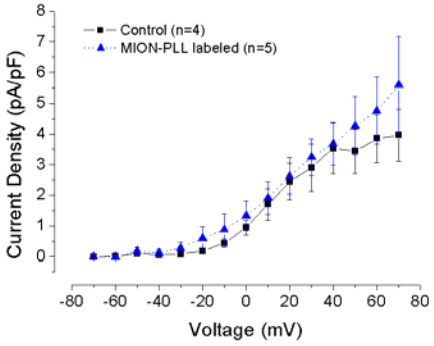

Figure 3 Steady-state current-voltage relationships of control and MION-PLL mouse ESCs. Result of unpaired Student's *t* test showing no significant difference (p = 0.55).

DISCUSSIONS AND CONCLUSIONS

USPIO labeling did not modify mouse ESCs' steady-state current-voltage relationship, presence of ionic currents demonstrates that ion channels were functionally expressed[9] in both control and USPIO-labeled cells, however, whether USPIO labeling changes the kinetics properties is yet to elucidate. Further studies on investigating the changes in current activation and inactivation kinetics should be carried out. The results are important for cellular MRI for tracking ESCs in treatment of myocardial infarction using USPIO labeling, with the anticipation of differentiation of pluripotent ESCs into cardiomyocytes for repairing purpose. Previous work showing transient gene expression alteration in SPIO-labeled neural stem cells[6] should be taken into account, effects of different amount and type of iron loading from SPIO labeling on cells should also be investigated. Further studies of cell proliferation and differentiation, cell metabolism, cell fate processes after magnetic labeling should be warranted.

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