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Easy and Efficient Nucleofection® of Cryopreserved Dissociated Adult Rat Vascular Smooth Muscle Cells

New Opportunities for Vascular Research

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Batch tested, ready-to-use, dissociated Adult Rat Aortic Smooth Muscle Cells (AoSMCs), combined with high quality and efficient transfection by Nucleofection®, offer new opportunities for laboratories employing genetically modified strategies to study vascular pathologies *in vitro*.

Introduction

Maintained in culture, vascular smooth muscle cells (VSMCs) develop a proliferative phenotype and offer an excellent model for the study of vascular remodeling.[1] Cultured aortic smooth muscle cells (AoSMCs) lose contractile capacity within 5 – 7 days. However, they continue to maintain their phenotypic characteristics including expression of vascular smooth muscle-specific proteins.[2] This is consistent with what occurs in atherosclerosis, which involves the proliferation of VSMCs and morphogenesis from the contractile smooth muscle state to an active synthetic phenotype.

These cells migrate/relocate from the medial layer to the intima where they produce extracellular matrix proteins necessary to generate the fibrous cap that stabilizes the plaque.[3] VSMC cultures offer the experimental advantage of being able to assess molecular and cellular mechanisms of cellular proliferation as well as the opportunity to screen for anti-proliferative therapeutics. The application of these cells *in vitro* has been advanced by the commercial availability of cryopreserved dissociated adult rat AoSMCs (distributed by Lonza), that offer the researcher batch tested and guaranteed, standardized primary cells which can be simply thawed, cultured and assayed. This represents a significant advantage for the laboratory, eliminating animal handling and dissection and difficult vascular tissue preparation. In addition, the ability to control when and where the VSMC culture can be undertaken streamlines the research workflow and speeds R&D programs.

In this study, we show the quality of commercially available cryopreserved dissociated adult rat AoSMCs developed by QBM Cell Science, Inc. (available from Lonza), cultured in multi-well plates. Furthermore, we show their suitability for transfection using Nucleofection® either in suspension or adherent formats. This electroporation-based transfection technique[4,5] is a powerful, high efficiency and high-throughput technique for transfecting plasmids directly into the nucleus to genetically modify dissociated primary cells. With this technique, the combination of specific programming of electrical parameters and specific transfection solutions optimizes the physiological environment and viability of the cells post transfection.[6]

Materials and Methods

Vials of frozen cells dissociated from adult Sprague Dawley rat aortic smooth muscle **(Lonza Cat. No.: R-ASM-580)** were thawed and prepared for cell culture and for transfection using the **4D-Nucleofector® X Unit or Y Unit.**

X Unit Transfection

AoSMCs were thawed and cultured in flasks using DMEM/F12 and supplements. After the 4th passage, the cells were harvested for transfection. Optimal Nucleofection® Conditions were determined in the 16-well Nucleocuvette® Strip format following the recommendations of the Basic Protocol for Primary Smooth Muscle Cells using P1 4D-Nucleofector® Solution and six different programs. The optimal conditions were transferable to the 100 µL single Nucleocuvette® Vessel. Per sample, 1×10^5 cells (in 20 µL strips) or 1×10^6 cells (in 100 µL cuvettes) were transfected with 2 µg/100 µL pmaxGFP™ Vector. After Nucleofection®, prewarmed media was added and 100 µL of cells from each cuvette were transferred to a microtube containing 800 µL of warm media. Cells were plated in 96-well plates ($1 - 8 \times 10^4$ cells/well), cultured 48 hours and then processed for morphological assessment.

Y Unit Transfection

The cells were thawed and cultured in 24-well plates, provided with the Y Unit Kits, for up to 7 days. Following the Primary Cell Optimization Protocol for adherent Nucleofection®, optimal Nucleofection® Conditions were determined by testing two solutions (AD1 and AD2) with 11 programs and 17.5 µg pmaxGFP™ Vector per well. Post Nucleofection®, the solutions were removed from the wells and replaced with fresh 37°C culture media. The cells were fixed at 24 hours post Nucleofection® using our standard procedure.

Morphological Assessment

Fluorescent micrographs were used to assess viability, transfection efficiency, and morphology of the smooth muscle cells. Cultures were fixed and stained for immunohistochemical examination directly in the wells and examined using inverted microscopy. Expression of maxGFP™ Fluorescent Protein was examined together with immunohistochemical identification of the cell cultures using commercially available antibodies for α-smooth muscle actin (Chemicon Cat. No.: CBL171) and VE cadherin (Santa Cruz Cat. No.: sc28644) as negative control for endothelial cells. Cell nuclei were stained using Hoechst.

Results and Discussion

Following thaw and culture, the dissociated adult rat AoSMCs displayed excellent morphology. Figure 1 shows the quality of cryopreserved cells compared to freshly cultured cells. In this example, cells were cultured, harvested and either used for immediate culture or cryopreserved and stored frozen. Sample vials of frozen cells were then thawed and cultured. The cryopreserved and fresh cells (from the same batch) show equivalent morphology and viability in culture.

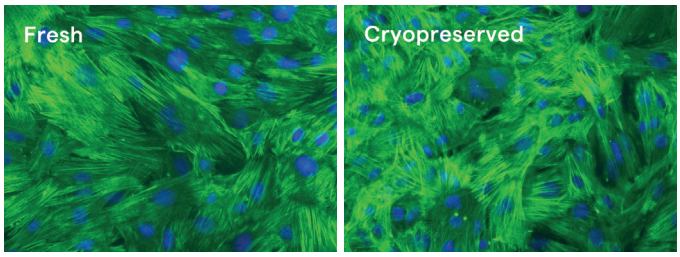

Figure 1.
Morphological study of adult rat AoSMCs from the same dissection-dissociation batch. Cells were used for immediate culture in 96-well plates (7 days) or cryopreserved, then thawed and cultured for 7 days. Green = anti α-actinin; Blue = Hoechst.

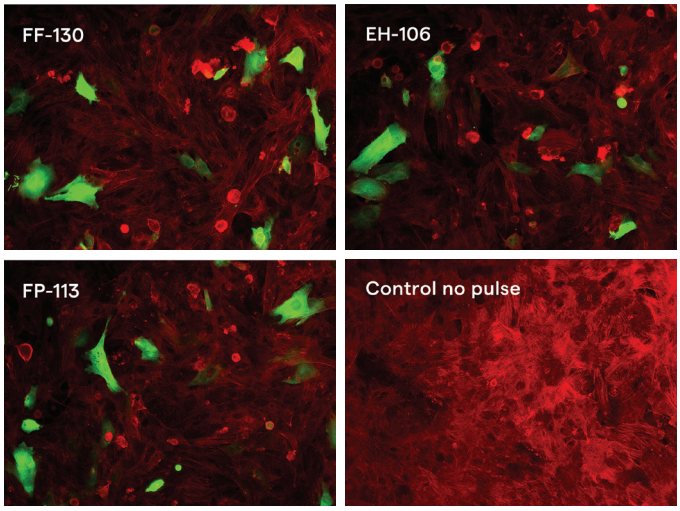

Figure 2.
Transfection of rat AoSMCs using the 4D-Nucleofector® X Unit with different programs. Cryopreserved cells were thawed and cultured for four passages. Cells were transfected in suspension using P1 Primary Cell Solution and six different programs (three shown here). Transfected cells were plated and fixed 48 hours post transfection. FP-113 was determined as the optimal program. Red = anti α-actinin; Green = maxGFP™ Protein.

Suspension Nucleofection® Using the 4D-Nucleofector® X Unit

Using the 4D-Nucleofector® X Unit, we found that the cryopreserved dissociated adult rat AoSMCs can be easily transfected and then cultured. Nucleofector® Programming Conditions were critical. From the six transfection program parameters tested, program FP-113 was assessed as giving the optimal results (Figure 2, showing three of six programs). The cultures displayed cell morphology comparable to the non-transfected controls. The quality of the transfection can be further appreciated in Figure 3. From observer-based cell counts, X Unit transfection efficiency was calculated to be 30 – 40% of the surviving α-actinin in positive cells.

Transfected cells could be easily visualized in cell plating densities as low as 1 × 104 cells. For comparison, Figure 4 shows the distribution of GFP-positive cells across two plating densities.

Adherent Nucleofection® Using the 4D-Nucleofector® Y Unit

Nucleofection® of rat AoSMCs can also be performed in adherence. Transfection in adherence, using the 4D-Nucleofector® Y Unit, gave comparable results (Figure 5) to transfection in suspension. All 11 programs tested (both in combination with AD1 and AD2 4D-Nucleofector® Y Solution) were effective in transfecting the cells. Transfection in AD1 Solution in combination with programs EH-158 or FB-166 gave the highest quality transfection and cell morphology. From observer-based cell counts, the best Y Unit transfection efficiency was calculated to be 20 – 30% of the surviving α-actinin positive cells.

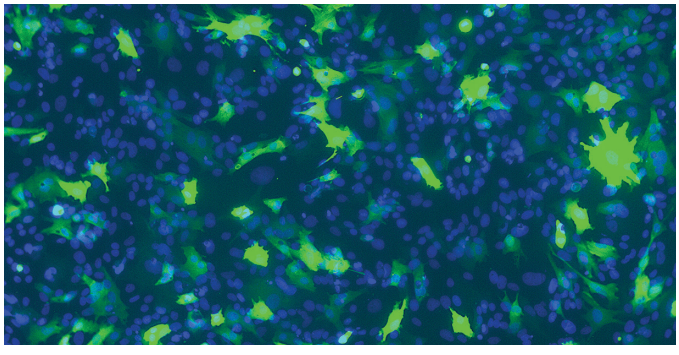

Figure 3.
High quality transfection of rat AoSMCs using the 4D-Nucleofector® X Unit. Cells were transfected in suspension using P1 Primary Cell Solution and program FP-113. Transfected cells were plated and fixed 48 hours post transfection. Green = maxGFP™ Protein; Blue = Hoechst.

Taken together, these data show high quality transfection by Nucleofection® of dissociated adult rat VSMCs. The ready-to-use advantage of batch tested, cryopreserved dissociated rodent neuronal cells, combined with the high quality, high-throughput transfection of these cells using the 4D-Nucleofector® System, offers a convenient, effective and time-saving approach for researchers employing genetically modified strategies for their R&D programs.

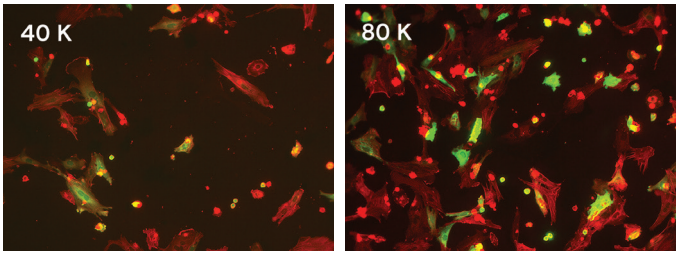

Figure 4.
Result of different plating densities after transfection with the 4D-Nucleofector® X Unit. Rat AoSMCs were transfected in passage 4 using P1 Primary Cell Solution and program FP-113. Post transfection, cells were plated at two different densities and fixed for analysis after 48 hours. Red = anti α-actinin; Green = maxGFP™ Protein.

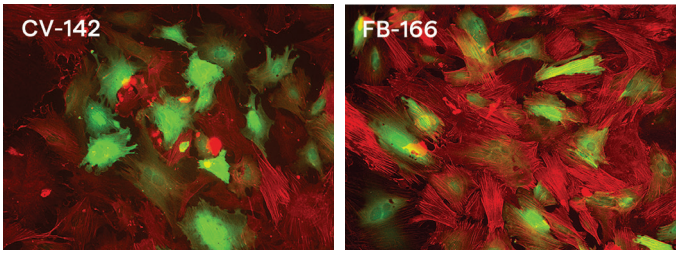

Figure 5.
Nucleofection® of rat AoSMCs in the 4D-Nucleofector® Y Unit. Cryopreserved rat AoSMCs were thawed and cultured for 7 days in 24-well culture plates. Cells were transfected in adherence using AD1 4D-Nucleofector® Y Solution and program FB-166 (optimal program) or CV-142. 24 hours post transfection, cells were fixed and analyzed. Red = anti α-actinin; Green = maxGFP™ Protein.

References:

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