Application Note

Thermo Scientific Nunclon Vita Surface

Feeder Cell- and Extracellular Matrix-Free Cultivation of Human Pluripotent Stem Cells Using Thermo Scientific Nunclon Vita Surface and Rho-Kinase Inhibition

Introduction

The promise of pluripotent stem cells lies in their ability to form any cell or tissue in the body. However, this promise requires a stable and reproducible method to grow the cells. Current methods rely on feeder cells or extracellular matrix proteins to cover the cultureware growth surface, and either manual selection or enzymatic dissociation in cell passaging and harvesting. This technical note describes a novel and simple method to grow pluripotent stem cells without the use of feeder cells or extracellular matrix proteins.

Methods

Human ESC cultivation

Cells. Passage-49 human ESC (H1 line from WiCELL, USA) were maintained in mouse embryonic fibroblast (MEF)-conditioned medium on Nunclon™ surface (Thermo Fisher Scientific, Denmark) coated with a 1:30 dilution of growth-factor reduced Matrigel™ (Becton Dickinson, USA). Cells were dissociated from the surface for passage by treatment with 1 mg/ml collagenase, and then seeded onto Nunclon™ Vita™ surface with or without Rho-kinase inhibitor in the medium, as described below.

Cultivation without Rho-kinase inhibition. H1 ESC were grown for 4 passages in MEF-conditioned medium on Nunclon Vita surface. Cells were dissociated from the surface for passage by treatment with 1 mg/ml collagenase. Normal passage time for H1 ESC was 3-4 days on Matrigel™. However, cells plated on the Nunclon Vita surface took 7 days of culturing before they were ready for passage, and a spontaneous decrease in growth rate over the passages was observed.

Cultivation with Rho-kinase inhibition. H1 ESC were grown in MEF-conditioned medium supplemented with Rho-kinase inhibitor, Y-27632 (10 µM unless otherwise indicated; Sigma-Aldrich, USA). Cells were dissociated from the surface for passage by treatment with 1 mg/ml collagenase. Cells plated in medium with 10 µM Y-27632 on the Nunclon Vita surface were ready for passage 4 days after plating. Cells were grown for the number of passages indicated.

Human ESC characterization

Colony presence and morphology were determined using phase-contrast microscopy, and by the naked eye after staining colonies with 0.5% crystal violet.

Pluripotency was determined by the presence of pluripotency markers through the use of qRT-PCR for gene expression, flow cytometry for cell-surface marker expression, and immunofluorescence for cell-surface and nuclear proteins.

Karyotypic stability was determined by cytogenetic analysis of 20 G-banded metaphase cells, and by fluorescent in situ hybridization (FISH) on 200 interphase nuclei using probes for the ETV6 BAP (TEL) gene located on chromosome 12 and for chromosome 17 centromere.

Ability to form embryoid bodies was determined by growing ESC in a low-binding plate for 10 days in DMEM/F12 containing 10% FBS.

Human ESC can be passaged a few times on the Nunclon Vita surface before the growth rate spontaneously declines

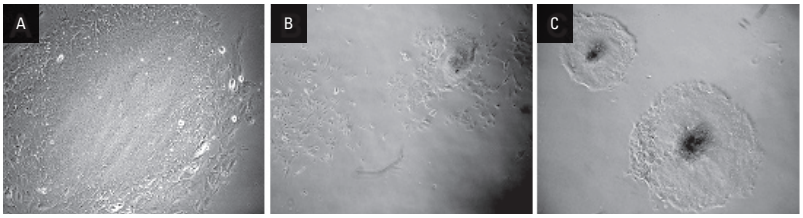


FIGURE 1.
Phase-contrast micrographs of human ESC passaged twice on 1:30 dilution of Matrigel™ (A), a standard tissue culture-treated surface (B), or the Nunclon Vita surface (C).

Thermo SCIENTIFIC

The decline in growth rate of human ESC on the Nunclon Vita surface is not observed if the culture medium is supplemented with Rho-kinase inhibitor Y-27632

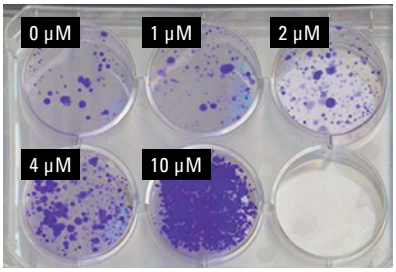


FIGURE 2.
Dose-response effect of Rho-kinase inhibitor on the attachment of human ESC to the Nunclon Vita surface. Y-27632 was added to the cultures at a specified concentration (0, 1, 2, 4, or 10 µM) at seeding. The cells were then maintained from day 2 onward in media containing 10 µM Y-27632 with daily media changes for five days after which cells were stained with crystal violet.

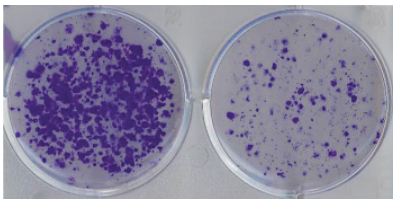


FIGURE 3.
Detachment of human ESC from the Nunclon Vita surface upon withdrawal of Rho-kinase inhibitor. Cells were seeded and maintained for 4 days in medium containing 10 µM Y-27632 (left well) or Y-27632 was removed from medium for 24 hours on the 3rd day (right well). After 4 days in culture, the cells were stained with crystal violet.

Human ESC grown on the Nunclon Vita surface in the presence of Y-27632 have normal karyotype, express pluripotency markers, and can be differentiated into embryoid bodies

A B

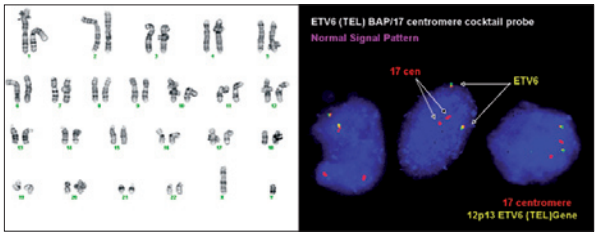


FIGURE 4.
Normal karyotype (A) and FISH pattern (B) for human ESC after 11 passages on the Nunclon Vita surface.

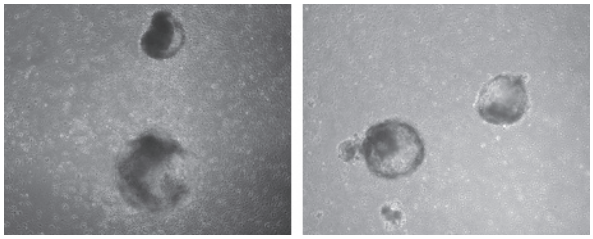
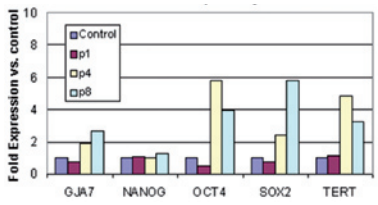


FIGURE 5.
Human ESC can form embryoid bodies after 11 passages on the Nunclon Vita surface.

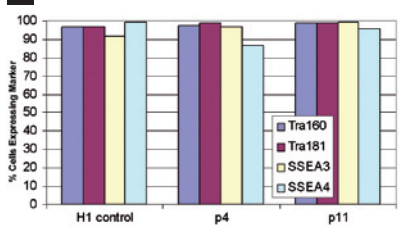
FIGURE 6.

A



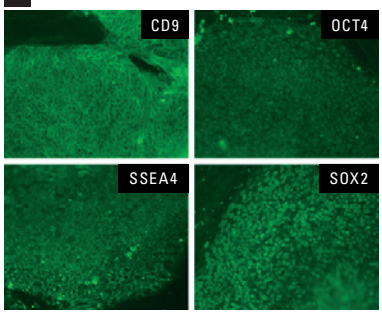
A. Expression of pluripotency markers in human ESC as determined by qRT-PCR after 1 passage (p1), 4 passages (p4), and 8 passages (p8) on the Nunclon Vita surface.

B



B. Expression of pluripotency markers in human ESC as determined by flow cytometry after 4 passages (p4) and 11 passages (p11) on the Nunclon Vita surface.

C



C. Expression of pluripotency markers in human ESC as determined by immunofluorescence staining after 11 passages on the Nunclon Vita surface.

Non-enzymatic passaging of human ESC by Y-27632 withdrawal

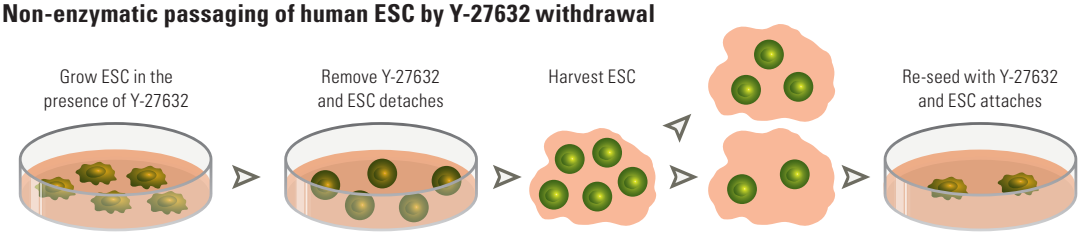


FIGURE 7.
Human ESC grown in the presence of Rho-kinase inhibitor can be dissociated from the Nunclon Vita surface by incubating the plate with fresh growth medium without Rho-kinase inhibitor for 15-30 min. Cells can then be mechanically dissociated from the plate by gentle pipetting or scraping, briefly centrifuged, and resuspended in growth medium containing Rho-kinase inhibitor. The ESC can finally be mechanically dissociated by gentle trituration and re-seeded.

Conclusions

The Nunclon Vita surface supported feeder cell- and extracellular matrix-free attachment, colony formation and growth of human ESC:

- for a few passages in medium conditioned by mouse embryonic fibroblasts
- for several passages in medium conditioned by mouse embryonic fibroblasts and supplemented with Rho-kinase inhibitor Y-27632

Human ESC grown 11 passages on the Nunclon Vita surface in medium with Y-27632 had normal karyotype, expressed pluripotency markers, and could be differentiated into embryoid bodies.

Human ESC could be passaged without the use of enzymes or manual selection by withdrawing the Rho-kinase inhibitor from the culture in order to lift the cells from the Nunclon Vita surface, followed by re-plating cells in the presence of the Rho-kinase inhibitor.

For research use only

Important information about patents:

Attachment, cultivation and detachment of cells using methods described herein are covered by patent applications WO 2009/105570 and US 12/388,930. A license to use these methods with Nunclon Vita Surface cultureware solely in connection with research is granted with the purchase of Nunclon Vita cultureware.

Inquiries for a license to use these methods for commercial purposes, except for those purposes relating to amelioration of diabetes mellitus, should be sent to: Thermo Fisher Scientific, 81 Wyman Street, Waltham, MA 02451, Attn: Legal Dept.

Inquiries for a license to use these methods directly or indirectly in the amelioration of diabetes mellitus should be sent to: Att. Vice President of BetaLogics Centocor Research & Development, Inc, 145 King of Prussia Road, Radnor, PA 19087, USA.

Particular types of cells, as well as methods for manipulating cells, may be covered by one or more patents held by others. Use of Nunclon Vita cultureware is recommended only for applications which do not violate proprietary rights of others or for which the user has a license or other permission under such proprietary rights.

www.thermoscientific.com

Austria
+43 1 801 40 0
Belgium
+32 53 73 42 41
China
+86 21 68654588
Denmark
+45 4631 2000
France
+33 2 2803 2180
Germany
+49 6184 90 6940
India
+91 22 6716 2200
Italy
+39 02 02 95059 or 434-254-375
Japan
+81 3 3816 3355
Netherlands
+31 76 571 4440
Nordic/Baltic countries
+358 9 329 100
North America
+1 585-586-8800
Russia/CIS
+7 (812) 703 42 15
Spain/Portugal
+34 93 223 09 18
South America
+1 585 899 7298
Switzerland
+41 44 454 12 12
UK/Ireland
+44 870 609 9203
Other Asian countries
+852 2885 4613
Countries not listed
+49 6184 90 6940 or
+33 2 2803 2180

TILSPVITA 0910
77033

nunc

Thermo SCIENTIFIC