Video Article

From MEFs to Matrigel 2: Splitting hESCs from MEFs onto Matrigel

Ivan Khvorostov, Jin Zhang, Michael Teitell
David Geffen School of Medicine, University of California, Los Angeles

Correspondence to: Michael Teitell at MTeitell@mednet.ucla.edu

URL: http://www.jove.com/index/Details.stp?ID=831

DOI: 10.3791/831

Citation: Khvorostov I., Zhang J., Teitell M. (2008). From MEFs to Matrigel 2: Splitting hESCs from MEFs onto Matrigel. JoVE. 16. http://www.jove.com/index/Details.stp?ID=831, doi: 10.3791/831

Abstract

This video demonstrates how to grow human embryonic stem cells (hESCs) on mouse embryonic fibroblast (MEF) feeder cells, how to passage hESCs from MEF plates to feeder cell-free Matrigel plates.

Protocol

hESC culture daily maintenance

1. hESC culture media must be changed every 24 hrs. From the ES media stock bottle stored at 4°C, take out the amount of solution needed (~20ml per 6-well plate), place in a 50ml falcon tube, and warm to 37°C in a water bath. Once the media is warmed, add bFGF stored at 4°C to a final concentration of 10ng/ml. Put the bFGF stock back at 4°C immediately after use!
2. Remove from the 6-well plates all but ~500µl of media. Make sure to swirl the culture dish to suspend debris for removal. Make sure the bFGF in the hESC culture media is thoroughly mixed and add 3ml of fresh media back to each well of a 6-well plate. Put the plate back in the incubator.

Splitting hESCs from MEFs onto Matrigel

Usually a confluent 6-well plate of hESCs on MEFs can be split 1:2 to 1:3 onto Matrigel 6-well plates, with the wells becoming confluent again 4-5 days after splitting.

1. When splitting hESCs onto Matrigel, MEF-conditioned media (CM) is used to maintain pluripotency. MEF-conditioned media is made in advance. On the first day, 1.5×10^7 γ-irradiated MEFs are seeded into a T75 flask. On the second day, MEFs are washed once with room temperature 1×PBS, pH 7.4, and then 15ml of ES media supplemented with 5ng/ml bFGF is added to the flask. (NOTE: MEFs can be plated into smaller or bigger flasks but proportion between cell density and volume of ES media should be constant.) The flask is incubated at 37°C for another 24 hrs. On the third day, the CM is collected and replaced with 15ml of fresh ES media supplemented with 5ng/ml bFGF. The collected CM is stored at 4°C. MEF-conditioned media from one plating of MEFs are harvested over seven consecutive days to generate 15 × 7 = 105ml. After seven days, all CM fractions are combined, sterile filtered, aliquoted and stored at -20°C. CM media prepared as described can be stored and used for 1 month.
2. One day before hESC splitting, put Matrigel aliquots in eppendorf tubes at 4°C to thaw overnight (one aliquot contains 76.2mg of Matrigel, and this is the amount needed for one 6-well plate). On the day of splitting, take one vial of the thawed Matrigel for one 6-well plate, put the vial and 6ml of cold DMEM/F12 media on ice. Transfer the Matrigel into the DMEM/F12 media and mix well. Add 1ml of the solution to each well of a 6-well plate. Swirl the plate to distribute Matrigel on surface and incubate the Matrigel-covered plate at room temperature for at least 1 hr before splitting the hESCs.
3. hESCs on MEFs are washed once with 1×PBS, pH 7.4. Subsequently 1ml of warm collagenase IV (1mg/ml) is added to each well of the 6-well plate and then the plate is incubated at 37°C for 5-10 min.
4. Add 1ml of ES media without bFGF to each well. Use a 1ml pipette to blow the stem cells off the plate. Collect all the media containing clumps of hESCs and dead MEFs into a 50ml falcon tube. Let big clumps of cells settle for 5-10 min so that the hESC clumps form a pellet at the bottom of the tube while the MEFs remain suspended in the supernatant. Discard the supernatant, and wash by resuspending the hESC pellet using ES media lacking bFGF, followed by centrifugation at room temperature at 200g for 5 min.
5. To plate the stem cells on Matrigel plates, first wash the Matrigel plates with room temperature DMEM/F12 (1ml per well) and adding 2.5ml of CM supplemented with 10ng/ml bFGF per well. Resuspend the pellet in an appropriate volume of CM supplemented with 10ng/ml bFGF, pipette the cell suspension up and down several times until the clumps become uniform and small in size. Aliquot the suspension at 0.5ml per well on the Matrigel plate. Note that the colony density on Matrigel is higher than the density of colonies by usual splitting on MEFs. Place the plate in a 37°C tissue culture incubator.
6. To maintain the hESCs on Matrigel, CM media supplemented with 10ng/ml bFGF is changed every 24 hrs for up to 4-5 days.

Discussion

This video demonstrates how to passage hESCs from MEF plates to feeder cell-free Matrigel plates. Note that the colony density on Matrigel is higher than the density of colonies by usual splitting on MEFs. Immunofluorescence staining and microscopy or flow cytometry for hESC pluripotency markers, such as Oct-4 and SSEA-4, are needed to confirm maintenance of hESCs in an undifferentiated state in feeder-free culture conditions.

Acknowledgements

Human embryonic stem cell studies in the Teitell lab are supported by a California Institute for Regenerative Medicine (CIRM) seed grant RS1-00313. We thank members of the Broad Center of Regenerative Medicine and Stem Cell Research at UCLA, especially Dr. Amander Clark, Dr. Jerome Zack, and members of the UCLA Broad Institute Stem Cell Core Facility for their support of our studies.

References

1. James A. Thomson, Joseph Itskovitz-Eldor, Sander S. Shapiro, Michelle A. Waknitz, Jennifer J. Swiergiel, Vivienne S. Marshall, and Jeffrey M. Jones Embryonic Stem Cell Lines Derived from Human Blastocysts Science 282, 1145-1147 (1998).

2. Chunhui Xu, Margaret S. Inokuma, Jerrod Denham, Kathaleen Golds, Pratima Kundu, Joseph D. Gold & Melissa K. Carpenter Feeder-free growth of undifferentiated human embryonic stem cells Nature Biotechnology 19, 971-974 (2001).

